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Influence of Yeast-treated Rice By-products on Growth, Yield and Grain Quality of Rice

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ABSTRACT The use of agricultural by-products as alternative nutrient sources in crop production had gained popularity in order to reducing the rate of chemical fertilizer application in the field. This study was conducted to determine whether the application of rice milling by-products treated with yeast inoculants could substitute, or reduce the rate of chemical fertilizer application. The results of agronomic measurements showed that the effect of incorporated materials was not immediate, as compared to 100% chemical fertilizer application. However, grain yield and quality was either the same or greater than 100% chemical fertilizer application. It was found out that expanded rice hull (treated with yeast or not) could reduce the rate of applying chemical fertilizers by half. Also, yeast treatment was only favorable only to expanded rice hull and not with rice bran, and was already found to be a potential material in reducing chemical fertilizer application in rice production.

Keywords : expanded rice hull, rice bran, yeast, grain quality

Without the use of chemicals in agriculture, productivity would have not been able to support the needs of human population in the past. However, farmers became irrationally-dependent to these agro-chemicals, especially fertilizers that are easy to apply in crop fields. The environmental impacts of intensive agricultural activities are just being realized now. Nitrate pollution of groundwater, and importantly drinking water, has been evident in numerous areas (Almasri & Kaluarachchi, 2004; Jalali, 2005; Chen *et al.*, 2005) which was attributed to continuous and over application of Nitrogen fertilizer. In other to prevent this, we have to find alternatives that will substitute chemical fertilizers as the main nutrient source in crop production.

Currently, wastes or by-products in agriculture are being exploited for its potential use as alternative nutrient sources. Shaviv & Mikkelsen (1993) emphasized the importance of slow- or controlled-release fertilizers in reducing nutrient losses and environmental degradation, and increasing the nutrient-use efficiency of crops. Most of the researches involving the use of agricultural wastes though, are focused on its processing into "activated carbon" for absorption of heavy metals (Ahmedna, Marshall, & Rao, 2000; Kadirvelu *et al.*, 2003; Teixeira Tarley & Zezzi Arruda, 2004). The use of agricultural by-products (specifically, rice milling by-products) in crop allelopathic research has been demonstrated by Xuan *et al.* (2003) and Khanh *et al.* (2005), which indirectly influenced rice yield through weed control. It would be appropriate to verify the effect of such materials into the growth, yield, and quality of crop produce in order to have a projection of its outcome and if to be used in farmer fields.

Rice is an important food crop and currently, most countries in Asia still rely on chemical fertilizers for its production. In the pursuit of reducing the rate of chemical fertilizer used, the study will examine the possibility (-ies) of using rice milling by-products as alternative nutrient source in rice crop production. Huge quantities of rice bran and rice hull are left after rice milling, and these two by-products have also been extensively used for hot pepper crop production research of hot pepper in Korea (J. W. Lee *et al.*, 2000; Y. J. Suh, 2011).

Fermentation is a process by which facultative microorganisms like yeast transform complex organic compounds into simple organic molecules that could be easily accessible for plant uptake and utilization (Higa & Parr, 1994). This could be used to enhance the effectiveness of applying such low-nutrient materials e.g. expanded rice hull into soil amendments

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that could have low impact on nutrient immobilization especially for N and P due to its inherently high C/N ratio. Rice bran has been found to successfully reduced the rate of chemical fertilizer in rice production (Kang *et al.*, 2008) but it will still be used in this experiment to check whether yeast treatment will have a synergistic effect on the material. Hence, the following were verified whether: a) yeast treatment of rice bran or expanded rice hull was effective, b) or integrated use of expanded rice hull (treated with yeast or not) could reduced the rate of chemical fertilizer application, and lastly c) checked the overall effects of the treatments to yield and quality of rice.

MATERIALS & METHODS

Site Description

A field experiment was conducted at Gyeongsangbuk-do Agricultural Research & Extension Services (35°57'17"N and 128°33'39"E) located in Daegu, South Korea from May 1 to October 15, 2009 to determine the effects of various alternative nutrient sources on the growth, yield and grain quality of the Japonica cultivar *Ilpum*. The soil (Table 1) was characterized to be moderately acidic, containing moderate to high levels of organic matter and high amounts of exchangeable calcium.

Treatment description and crop establishment

The experiment includes 9 treatments composed of 2 rice by-products: Rice Bran (RB) and Expanded Rice Hull (RH) treated with yeast. Rice bran was found to be highly acidic but contains very high amount of organic matter (OM), available phosphorus, and exchangeable potassium (K) while expanded rice hull is only slightly acidic, contains high amount of OM and low exchangeable K compared to rice bran (Table 1). Prepared yeast cultures (in liquid form) were supplied by the Korea Inkal (Yeast) Research Institute, Yeongcheon-si, Gyeongsangbuk-do, Korea. Yeast cultures were allowed to multiply in calcium-phosphate buffer before inoculation to the rice by-products. The materials were incubated 3~5 days after inoculation prior to field application. The treatment description and the amount of each material applied into the soil was based on company/farmer recommendations as following: T1-Conventional Practice, T2-Rice Bran (RB, 3600 kg ha^{-1}), T3-Expanded Rice Hull (RH, 3600 kg ha^{-1}), T4-Rice Bran+Yeast (RBY, 3600 kg ha^{-1}), T5-Expanded Rice Hull+Yeast (RHY, 3600 kg ha^{-1}), T6-Rice Bran (50% RB, 1800 kg ha^{-1})+Chem. Fertilizer (45-28-24, kg ha^{-1}), T7-Expanded Rice Hull (50% RH, 1800 kg ha^{-1})+Chem. Fertilizer (45-28-24, kg ha^{-1}), T8-Rice Bran+Yeast (50% RBY, 1800 kg ha^{-1})+ Chem. Fertilizer (45-28-24, kg ha^{-1}), T9-Expanded Rice Hull+Yeast (50% RHY, 1800 kg ha^{-1})+Chem. Fertilizer (45-28-24, kg ha^{-1}).

Chemical fertilizers were applied in the Conventional Practice (CP), with N-P_2O_5-K_2O (90-55-48, kg ha^{-1}, respectively) requirements. All alternate nutrient sources were applied 10 days before transplanting. For the CP treatment, nitrogen (50%), phosphate (70%) and potash (100%) requirements were also basally, using chemical fertilizers (Urea-N, Rock Phosphate-P_2O_5, Muriate of Potash-K_2O). Thirty-day old seedlings were hand-transplanted on June 5, 2009 with planting distance of 30×15 cm. Weed control was done manually at 14 days after transplanting. At maximum tillering stage, additional 25% Nitrogen and 30% phosphate (30%) was applied in the CP treatment while the remaining nitrogen (25%) requirement were applied at heading stage. The experiment followed a Randomized Complete Block (RCB) design with three replicates.

Table 1. Chemical properties of soil and raw materials before the experiment.

Soil type	$pH_{1:5}$	$EC_{1:5}$ (dS m^{-1})	OM (g kg^{-1})	Total N (%)	P_2O_5 (%)	Ex. Cations (%)		
						K	Ca	Mg
Sandy loam	5.8	0.33	35	nd	0.01	0.02	0.07	0.02
Rice Bran	3.31	1.46	81.24	2.15	4.87	8.11	0.34	1.68
Expanded Rice Hull	6.60	0.57	67.97	0.44	0.20	1.48	0.56	0.21

nd-not detected/measured.

Plant, yield and grain measurements

Plant height (cm), tiller number and chlorophyll-SPAD values (using SPAD-502 chlorophyll meter, Konica-Minolta, Japan) of 5 randomly selected plants were routinely checked at tillering and heading stages. Grain yield (kg ha^{-1}) and yield components of plants in 5 randomly selected hills were measured at crop maturity. At harvest, plants in 100 randomly selected hills were gathered and processed for the analysis of grain quality. Dehulled and polished grains were analyzed of percent (%) whole grain, broken grain, damaged grain and chalkiness (%) using a Whole Grain Analyzer (Cervitec 1625 Grain Inspector, Foss Tecator, Sweden). Protein and amylose contents (%) were checked using a Grain Value Analyzer (Foss Infratec 1241 Grain Analyzer, Sweden) while palatability was measured in 30g-portion of milled rice per treatment using a TOYO-taste meter (TOYO MB-90A, Japan).

Data analysis

Data were analyzed based on ANOVA using the Statistical Analysis System© (Cary NC, 2002) and the mean separation procedure used was Tukey's Honesty Significant Difference (HSD) Test at 1% level of significance.

RESULTS & DISCUSSION

Effect on Agronomic Characters at Tillering and Heading Stages

In this experiment, we used indirect methods through agronomic data measurements was used to check whether rice by-products treated with yeast could substitute to the effect of chemical fertilizers. At tillering stage, the height and tiller count of plants grown under the conventional practice (CP) remained superior (71.10 cm and 42.45 tillers) among other treatments. Rice bran (100% RB) application did not showed effects on growth during this stage and resulted to low values compared to other treatments (Table 2). Yeast treatment of rice bran (100% RBY) apparently resulted to significantly higher plant heights compared to applying rice bran only. In this case, yeast may have influenced nutrient mineralization and release from rice bran. Expanded rice hull (100% RH) application resulted to relatively good plant growth (as reflected by plant height) and vigorous tiller formation at tillering stage (41.90) which was not significantly different from CP. Yeast treatment of RH (100% RHY) even produced higher plants at tillering stage (68.50 cm), which were not significantly different from the CP (71.10 cm). This was due to the glucose and xylose content in rice hull (Juliano, Maningat, & Pascual, 1987); that was utilized by yeast as

Table 2. Effect of alternative nutrient sources to agronomic characters of rice. cv. *Ilpum* at Tillering and Heading stages.

Treatments	Plant Height (cm)		Tiller Number		Chlorophyll Content (SPAD value)	
Treatments	Tillering Stage	Heading Stage	Tillering Stage	Heading Stage	Tillering Stage	Heading Stage
Conventional Practice	71.10 a	113.50 bcd	42.45 a	23.45 a	24.93 ab	27.25 fg
100% RB	56.30 e	108.10 e	33.90 d	19.40 c	23.35 b	29.23 d
100% RH	65.40 bc	118.00 a	41.90 a	21.10 bc	24.80 ab	36.20 a
100% RBY	63.90 bcd	106.30 e	33.40 d	21.80 ab	24.53 ab	28.15 ef
100% RHY	68.50 ab	115.70 ab	36.50 c	20.60 bc	25.43 ab	27.80 fg
§50% RB+CF	63.20 cd	106.20 e	36.50 c	21.20 bc	26.93 a	30.35 c
50% RH+CF	62.70 cd	113.00 cd	34.80 cd	21.90 ab	24.70 ab	32.40 b
50% RBY+CF	60.40 de	112.50 d	34.20 d	21.50 ab	25.53 ab	27.00 g
50% RHY+CF	66.70 abc	115.00 bc	38.90 b	21.30 bc	23.68 b	29.10 de

Means within the same column having the same letter are not significantly different based on Tukey's $HSD_{0.01}$
§Supplemented with Chemical Fertilizer: Total of 45 kg N ha^{-1}, 28 kg P_2O_5 ha^{-1}, 24 kg K_2O ha^{-1}

energy source. Moreover, rice hull contains considerable amounts of cellulose and hemi-cellulose which was probably broken-down by yeast into simple sugars. On the other hand, the chlorophyll-SPAD values of alternative treatments were low and not significantly different from CP at tillering stage. At heading stage, 100% RH retained significantly highest values of plant height and chlorophyll-SPAD (118.0 cm and 36.20, respectively) among other treatments. Tiller count however, decreased at this stage and 100% RH value (21.10) was significantly lower than the CP value (23.45) while 100% RBY (21.80) was not significantly different from CP. Among treatments with chemical fertilizer supplements, only 50% RH and 50% RBY were not significantly different from the CP in terms of tiller number at heading stage (Table 2). Except for 50% RBY, this group of treatments had significantly higher chlorophyll-SPAD values than CP at heading stage.

Plant elongation and vigorous tillering was accompanied by a low leaf-N status as seen through chlorophyll-SPAD values at tillering stage. Nitrogen may have been immobilized in the soil at this stage, and apparently resulted to N deficiency. There is a possibility that nutrients especially nitrogen is supplied and remobilized from young leaves, with the formation of tissues which will soon act as “sinks” (Mae and Ohira, 1981; Feller and Fischer, 1994) and thus, explains the low chlorophyll-SPAD values. The increase in SPAD values at heading stage on the other hand, is attributed either to nutrient release from the incorporated rice by-products or, application of chemical fertilizer supplements. Overall, it seems that yeast treatment of RB or RH resulted to better plant heights and tiller number than untreated rice by-products.

Effect on Yield and Yield Components

Chemical fertilizer application (or CP) fairly resulted to numerous panicles, filled spikelets and heavy grains which explain for its grain yield value, 6,483.8 kg ha^{-1} (Table 3). CP produced an average of 23.05 panicles. Only 100% RH (22.50) and 50% RH (with supplemental chemical fertilizer application, 21.60) treatments were not significantly different from the control. Applying 100% RB (94.88) resulted to significantly higher average spikelet number than CP (72.39) while the 100% RH treatment resulted to fewest spikelets (57.0). When 100% RH was applied into the soil, it resulted to a good vegetative growth as exemplified by the agronomic measurements (Table 2). This could be the reason for fewer spikelets in the 100% RH treatment as Hasegawa *et al.* (1994) also stated that vigorous growth with high plant N concentration (observed through the plants chlorophyll SPAD value, 100% RH) up to the panicle formation stage could result in inefficient use of N for spikelet formation. Other treatments were not significantly different from CP in terms of spikelet number. Interestingly in terms of grain ripening/percentage filled-spikelet, all

Table 3. Effect of alternative nutrient sources to yield and yield components of rice cv. *Ilpum.*

Treatments	Panicle per Hill	Spikelet per Panicle	Percentage Filled-Spikelets (%)	1,000-grain weight (g)	Grain Yield (kg ha^{-1})
ConventionalPractice	23.05 a	72.39 bc	87.89 a	23.44 ab	6,483.8 abc
100% RB	19.50 de	94.88 a	91.32 a	24.23 a	6,512.7 abc
100% RH	22.50 ab	57.00 c	90.03 a	21.05 c	6,088.3 cd
100% RBY	19.10 e	74.44 b	91.13 a	22.60 b	5,621.7 d
100% RHY	20.90 cd	67.67 bc	90.26 a	23.73 ab	6,341.6 bcd
§50% RB+CF	20.80 cd	71.31 bc	90.42 a	23.75 ab	7,163.7 a
50% RH+CF	21.60 abc	71.64 bc	82.29 b	24.47 a	6,930.4 ab
50% RBY+CF	21.40 bc	72.20 bc	89.35 a	23.71 ab	6,921.5 ab
50% RHY+CF	20.80 cd	73.72 b	88.36 a	24.77 a	6,606.0 abc

Means within the same column having the same letter are not significantly different based on Tukey’s $HSD_{0.01}$

§Supplemented with chemical fertilizer: Total of 45 kg N ha^{-1}, 28 kg P_2O_5 ha^{-1}, 24 kg K_2O ha^{-1}

treatments were not statistically different from CP (87.89%) except the 50% RH treatment (82.29%). This may be due to the reduced rate of RH application in which this material happens to have a low N content also. Fageria and Baligar (1999) previously confirmed the highly significant effect of N application on spikelet filling in rice. The grain weight of plants grown with 100% RB (24.23 g) was not significantly different from CP (23.44 g). The high amount of total N of rice bran is the reason for its positive effect on spikelet formation and filling. Applying 100% RH resulted to lower grain weight than CP (21.05 g) but yeast treatment (100% RHY) resulted to significantly increased grain weight (23.73 g). Treatments with chemical fertilizer supplements resulted to grain weights not significantly different from CP. And lastly, only 100% RB was able to surpass the grain yield of CP, though both values were statistically similar (Table 3). Yeast treatment was apparently ineffective to RB (100%) while to RH (100%), it resulted to a slight increase in yield value (6,088 à 6,342 kg ha^{-1}). This is due to the fact that there are no evidences yet of protein fermentation by yeast. Possibly, *"putrefaction"* could have occurred too, as suggested by Higa & Parr (1994) in which the anaerobic decomposition of protein (in this case protein-rich material like rice bran) resulted to incompletely oxidized metabolites that are often toxic to plants or animals. There is a study on fermentation of protein-rich materials such as soybean using rather the fungi *Aspergillus oryzae* (Hong *et al.*, 2004). Expanded rice hull which is a cellulosic material predominantly contains carbohydrates, and is very likely to be receptive to fermentation by yeast and hence explains the yield response of 100% RHY over 100% RH. On the other hand, treatments with supplemental chemical fertilizer application resulted to appreciably higher grain yields compared to the CP, among which 50% RB was the highest (7, 164 kg ha^{-1}). This result was due to roughly high and uniform yield component values, and mainly because of chemical fertilizer (supplements) application. Kang *et al*. (2008) previously reported the possibility of reducing the rate of chemical fertilizer application in rice fields using rice bran. In addition, these results also suggest that the rate of chemical fertilizer application can be reduced into half using another rice by-product, which is expanded rice hull.

Effect on Grain Quality Indexes

Aside from grain yield, the quality of produce is an important factor in deciding if alternative nutrient sources are practically applicable for field use. Applying 100% RB and RH resulted to significantly higher whole grain ratio (90.05% and 89.00%, respectively) than CP (84.98%). This is probably due to slow and late decomposition of OM, which lead to late nutrient release and juvenility of grains. Yeast treatment of these materials did not have a favorable effect to the milling quality index of rice, as it even increased the percentage of broken grains compared to without yeast treatment (Table 4a). Treatments with chemical fertilizer supplements resulted to higher whole grain and lower broken grain percentages compared to CP but not

Table 4a. Effect of alternative nutrient sources to the grain quality (milling characteristics) of rice cv. *Ilpum.*

Treatments	Whole grain (%)	Broken grain (%)	Chalkiness (%)	Damaged grain (%)
Conventional Practice	84.98 cd	6.95 bc	2.58 bcd	1.70 b
100% RB	90.05 ab	7.00 bc	2.90 bc	0.55 cd
100% RH	89.00 ab	7.15 bc	3.50 ab	0.85 c
100% RBY	83.60 d	12.70 a	2.35 cd	0.40 cd
100% RHY	85.50 cd	11.35 ab	3.40 abc	0.20 d
§50% RB+CF	87.65 abc	4.00 c	2.70 bcd	0.70 cd
50% RH+CF	90.35 a	5.65 c	1.75 d	0.55 cd
50% RBY+CF	87.35 bc	5.70 c	4.35 a	2.95 a
50% RHY+CF	89.05 ab	6.80 bc	2.75 bcd	1.75 b

Means within the same column having the same letter are not significantly different based on Tukey's $HSD_{0.01}$
§Supplemented with chemical fertilizer: Total of 45 kg N ha^{-1}, 28 kg P_2O_5 ha^{-1}, 24 kg K_2O ha^{-1}

statistically different from this control. Wopereis-Pura *et al.* (2002) offered an explanation for less grain cracking during milling; the juvenility of grains as a result of late N application. This scenario was the same with 50% of the treatments having chemical fertilizer supplements. Almost all treatments did not significantly differ from CP in terms of percentage chalky grains except 50% RBY, which showed the significantly highest incidence of chalkiness (4.35%). The ratio of damaged grains was significantly reduced in alternative nutrient sources, except for 50% RBY which was higher (2.95%) than CP, and 50% RHY (1.75%) which was statistically not different from CP (1.70%). With regards to these results, it may still be favorable to apply chemical fertilizer supplements instead of applying only 100% of alternative nutrient sources. This is due to the fact that 100% alternative sources resulted to higher broken grain and chalkiness percentages than the CP.

Grain palatability determined through TOYO-taste values, was significantly increased by alternative nutrient sources compared to CP (Table 4b). Treatments with supplemental chemical fertilizer application also had significantly higher TOYO values compared to CP except 50% RBY (68.65) and 50% RHY (69.45) treatments, which were not significantly different from CP (68.88). Protein contents generally decreased in alternative nutrient sources even if 100% RBY (7.50 %) was not significantly different from CP (7.78 %). This is the main reason for improved palatability (TOYO) values, as also noted by Choi, Y.H. *et al.* (2006). Applying chemical fertilizer supplements resulted to protein contents not significantly different from CP except 50% RB (7.10 %), which was lower than CP. Only 100% RHY (19.15 %) and 50% RB (20.10 %) treatments were significantly different from CP (19.58 %) in terms of amylose content. This change in grain amylose content does not have much implication/s to grain quality since it still remains in the low to intermediate grade.

SUMMARY & CONCLUSIONS

To check the potential of rice by-products as alternative nutrient sources in rice crop production, a field experiment was conducted by soil application of untreated and yeast-treated rice bran and expanded rice hull. Prepared yeast cultures were allowed to multiply in calcium-phosphate buffer before inoculation to the rice by-products. These materials were incubated 3~5 days after inoculation prior to field application. Agronomic data measurements at maximum tillering stage revealed that the conventional practice (or CP) of chemical fertilizer application (100%) still remained superior over alternative treatments in terms of plant height and tiller number. The chlorophyll-SPAD values of alternative treatments however, were statistically similar to CP. At heading stage, most of the agronomic values from alternative treatments were not significantly different from CP, or in some cases exceeded its value. This may imply that nutrient release from the materials were slow or delayed. Apparently, yeast treatment of RB

Table 4b. Effect of alternative nutrient sources to palatability and cooking quality indexes of rice cv. *Ilpum.*

Treatments	TOYO-taste Value	Protein content (%)	Amylose content (%)
Conventional Practice	68.88 e	7.78 a	19.58 bc
100% RB	77.25 b	6.75 cd	19.30 cd
100% RH	78.80 a	6.45 d	19.80 ab
100% RBY	73.60 c	7.50 ab	19.55 bc
100% RHY	76.55 b	5.75 e	19.15 d
§50% RB+CF	72.40 d	7.10 bc	20.10 a
50% RH+CF	74.10 c	7.75 a	19.70 b
50% RBY+CF	68.65 e	7.60 a	19.25 cd
50% RHY+CF	69.45 e	7.70 a	19.65 b

Means within the same column having the same letter are not significantly different based on Tukey's $HSD_{0.01}$.
§Supplemented with chemical fertilizer: Total of 45 kg N ha^{-1}, 28 kg P_2O_5 ha^{-1}, 24 kg K_2O ha^{-1}.

or RH resulted to better plant heights and tiller number than untreated rice by-products, indicating that nutrient mineralization from rice milling by-products occurred during tillering stage. Hoever, we were not able to obtain actual soil data in these stages that could give us an idea of the nutrient dynamics, as brought about by the application of the said materials.

Looking into the effects on grain yield, yeast treatment has a slight-positive influence on expanded rice hull, while it was apparently unfavorable to rice bran. Yield results also suggest the possibility of integrating expanded rice hull (treated or untreated with yeast) with chemical fertilizers to reduce the rate of application into half. The percentage of whole grains obtained after milling was generally more in alternative treatments than those from CP. Damaged grain ratio was also generally reduced. Grain palatability through TOYO values were improved by alternative treatments due to their "lowering" effect on grain protein content. As a conclusion, it was suggest that even expanded rice hull, a low-nutrient but high OM-containing material, can be used in integration with chemical fertilizers to reduce the rate into 50% aiming for a yield and grain quality that is more or less similar compared to using 100% chemical fertilizers. Yeast treatment was effective with expanded rice hull but treating rice bran should be avoided. With the cost and ease of yeast treatment, this can be recommended to be used in rice production, however, but if the technology or microorganism is unavailable, untreated by-products can be opted as alternatives.

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