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Effects of Microbe-inoculated Expanded Rice Hull on Growth, Yield and Grain Quality of Rice

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ABSTRACT The excessive and indiscriminate use of chemical fertilizers in the past has brought serious soil and other environmental problems so alternatives over this agrochemical are being searched. Our study focuses on the effects of expanded rice hull inoculated with selected beneficial microorganisms on growth (through agronomic characters), yield and yield components, and grain quality indices of rice. Results showed that favorable effects of different expanded rice hull preparations were not readily apparent at vegetative stage and only treatments with supplemental chemical fertilizer application were comparable with the conventional practice. Expanded rice hull combined with 50% rate of chemical fertilizer exhibited a significantly higher yield (6,471 kg ha^{-1}) over conventional practice (5,719 kg ha^{-1}). Good milling quality indices were observed in treatments having 50% chemical fertilizers plus alternatives from expanded rice hull. Finally, we demonstrated that chemical fertilizer rate can potentially be reduced into 50% if combined with expanded rice hull, and show even better output than chemical fertilizer alone.

Keywords : expanded rice hull, beneficial microorganisms, yield components, rice grain quality

Chemical fertilizers have undoubtedly increased crop yields in the past but the drawbacks of its indiscriminate use are now apparent. Due to excessive chemical fertilizer application in vegetable greenhouses at Northeast China and elsewhere, Ju *et al.* (2007) predicted a decrease in quality of soils in these areas as a result of nitrate and salt accumulation. The increasing concentration of nitrate-N in drinking and groundwater is also becoming a problem in Japan and Korea (Kumazawa, 2002; Kim, 2010). If these scenarios will continue, it might have serious implications to human health too, in the future. Controlled or slow-release fertilizers promise plant growth stimulation and high yields as results of increased nutrient-use efficiency and/or reduced nutrient leaching (Shaviv, 2001; Mikkelsen *et al.*, 1994). Organic-based fertilizers and crop residues have an unpredictable behavior in supplying nutrients to the plants. OM decomposition, which is a slow process, is the key factor that determines the release of nutrients into the soil. Currently, a number of agricultural waste or by-products are being exploited of its potential as alternative to chemical fertilizers. Rice hull is a by-product of rice milling and an abundant agricultural waste facing problems of disposal. In Korea, it is processed under high pressure to turn into "*expanded rice hull*". Lee *et al.* (2000a,b,c) conducted a number of experiments and noted that decomposed rice hull has an improved water retentive capacity over fresh rice hulls. Also, they were able to demonstrate the advantages of using decomposed over fresh rice hull in terms of the growth of hot pepper seedlings.

The concept of *"effective microorganisms"* (EM) in agriculture was coined and developed by Higa (1994) as inoculants containing a mixture of beneficial and naturally-occurring microorganisms. EM differs from *"beneficial microorganisms"* (BM) as the latter has not yet been identified or classified as such 'effective'; and that there is a continuous pursuit to find BM and select species that will have synergistic effects with each other when put in a mixed culture. Yoon *et al.* (2012) used 3 different single species and 1 complex microorganism treatment to observe its effects to growth, fruit yield, and nutrient contents of hot pepper. By injecting bacterial cultures into the stem of pepper plants, they were able to demonstrate that single

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species microorganism is better than the complex microorganism treatment. However, this procedure might not be applicable to cereal and other field crops. EM was found to increase the efficiency of organic and mineral nutrient sources but if applied alone, was not able to increase the yield of cotton (Khaliq *et al.*, 2006).

Hence, we decided to conduct an experiment comparing the effects of single species of microorganisms if used as inoculants to promote decomposition of expanded rice hull and its subsequent effect on nutrient availability as could be reflected on the growth and yield of rice. The raw material will serve as an energy source and it also contains ample amounts of carbohydrates, which may be broken down through microbial action in the soil. Our experiment objectives include: a) checking whether beneficial microorganisms will increase the efficiency of applying expanded rice hull in paddy rice fields, b) identifying which microorganism has the best synergistic effect with expanded rice hull, and c) to reduce the rate of chemical fertilizer application through alternative nutrient sources from this raw material.

MATERIALS AND METHODS

Site Description

The 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, 2010 to determine the effects of various alternative nutrient sources on the growth, yield and grain quality of the Japonica cultivar Ilpum. The soil and raw material chemical properties were presented in Table 1. The soil is characterized to be slightly acidic; contains moderate levels of nutrients and OM typical to most paddy soil in South Korea.

Treatment description and crop establishment

The experiment includes 10 treatments composed of expanded rice hull inoculated with 4 different microorganisms, compared to (conventional) farmers' practice using chemical fertilizers. The raw material, expanded rice hull (ERH), is slightly acidic, contains high amount of OM, and very high available silica (Table 1). ERH was inoculated with *Bacillus licheniformis*, *Trichoderma reesei*, *Enterobacter cloaceae*, and *Bacillus subtilis* with the assumption that these microorganisms will aid in its degradation. *Bacillus licheniformis* and *Trichoderma reesei* were chosen specifically due to their cellulase activity while *Enterobacter cloaceae* and *Bacillus subtilis* were isolated from naturally decomposing organic materials like spent mushroom compost and rice bran, respectively. Microbial cultures were allowed to multiply first in agar plates then, in calcium-phosphate buffer before inoculation to the expanded rice hull. Prior to field application, the materials were incubated within 3~5 days after inoculation. The treatment description and the amount of each material used in the experiment was based on company/farmer recommendations and listed below:

Treatment No.	Fertilizer rate (N-P_2O_5-K_2O)	ERH rate	Microorganism present
1	90-55-48 kg ha^{-1}	-	-
2	-	3600 kg ha^{-1}	-
3	-	3600 kg ha^{-1}	*Bacillus licheniformis*
4	-	3600 kg ha^{-1}	*Trichoderma reesei*
5	-	3600 kg ha^{-1}	*Enterobacter cloaceae*
6	-	3600 kg ha^{-1}	*Bacillus subtilis*
7	45-28-24, kg ha^{-1}	1800 kg ha^{-1}	-
8	45-28-24, kg ha^{-1}	1800 kg ha^{-1}	*Bacillus licheniformis*
9	45-28-24, kg ha^{-1}	1800 kg ha^{-1}	*Trichoderma reesei*
10	45-28-24, kg ha^{-1}	1800 kg ha^{-1}	*Enterobacter cloaceae*
11	45-28-24, kg ha^{-1}	1800 kg ha^{-1}	*Bacillus subtilis*

Table 1. Soil and expanded rice hull chemical properties before the experiment.

Soil type	pH 1 : 5	EC 1 : 5 (dS m^{-1})	OM (g kg^{-1})	Avail. P_2O_5 (mg kg^{-1})	Avail. SiO_2 (mg kg^{-1})	Ex. Cations (cmol+kg^{-1})		
						K	Ca	Mg
Sandy loam	6.6	0.38	20.00	36.00	312	0.37	3.94	1.37
Expanded Rice Hull	6.6	0.57	67.97	0.20	81,500	0.38	nd	nd

nd – not detected/measured

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 21, 2010 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.

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 30 g-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 AND DISCUSSION

Agronomic Effects

The effects of expanded rice hull (ERH), even with inoculation of microorganisms, were not readily observed at tillering stage (Table 2). Studies by Lee *et al.* (2000b) using hot pepper noted that fresh rice-hull based substrates were unable to promote vigorous seedling growth, even in the presence of nutrient supplements. The conventional practice (CP) was clearly superior in terms of plant height, tiller

Table 2. Effect of alternate nutrient sources to agronomic characters of Japonica cv. Ilpum.

Treatments		Plant Height (cm)		Tiller Number		Chlorophyll Content (SPAD value)	
		Tillering Stage	Heading Stage	Tillering Stage	Heading Stage	Tillering Stage	Heading Stage
Conventional Practice		49.47 a	75.77 ab	19.33 a	16.17 a	38.43 a	31.40 a
100%	ERH	42.00 bcd	70.60 de	12.70 cde	11.50 cd	35.72 abc	32.24 a
	ERHBL	44.40 bc	70.60 de	11.50 ef	10.90 d	36.80 ab	29.79 abc
	ERHTR	42.20 bcd	73.50 bc	15.90 b	11.40 cd	33.99 bcd	30.21 ab
	ERHEC	39.20 def	71.60 cd	16.20 b	13.30 bc	34.25 bcd	28.52 bcd
	ERHBS	37.90 ef	65.60 f	8.20 g	11.20 d	31.07 de	27.01 d
§50%	ERH	41.90 cd	73.20 bcd	13.70 cd	13.60 b	30.40 de	30.84 ab
	ERHBL	37.80 ef	68.60 e	14.70 bc	13.90 b	27.46 e	26.98 d
	ERHTR	36.70 f	65.70 f	10.30 f	13.50 b	32.08 cd	29.96 abc
	ERHEC	41.20 cde	77.90 a	12.00 def	12.20 bcd	34.28 abcd	30.81 ab
	ERHBS	45.40 c	74.90 b	16.20 b	13.70 b	30.31 de	27.36 cd

Means wihin 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}

count and chlorophyll-SPAD values at tillering stage which implies that nutrient sources other than chemical fertilizer only could not support plant needs for nutrients at vigorous vegetative growth. However, applying ERH inoculated with *Bacillus licheniformis* and *Trichoderma reesei* (100% ERHBL and 100% ERBTR, respectively) into the soil resulted to slightly improved plant height over ERH only (100% ERH) at tillering stage. Also, tillering was significantly improved by 100% ERBTR and expanded rice hull plus *Enterobacter cloaceae* (100% ERBEC) compared to 100% ERH alone, as seen at tillering stage. Only 100% ERHBL (36.80) had a higher chlorophyll-SPAD value than 100% ERH alone (35.75) at tillering stage, which was also not significantly different from CP (38.43). Treatments without supplementary applications of chemical fertilizer (or 100% materials) were significantly different to (generally lower than) CP based on plant height and tiller count. It was only in terms of chlorophyll-SPAD values where CP was not significantly different from 100% ERH alone, and to some treatments with microorganism inoculation (100% ERHBL, 100% ERHTR at heading stage). In general, the gap of values between CP and the alternative treatments were wide at tillering stage, and became narrow during heading stage probably due to the nutrients released upon decomposition of ERH or, due to the supplemental application of chemical fertilizers. It may be possible that ERH decreased the availability of essential elements like nitrogen and phosphorus as also observed by Kim *et al.* (2003), when they tried to use ERH as a nursery bed soil for rice.

Effects on Yield Components and Grain Yield

The application of different expanded rice hull preparation rather resulted to poor growth and tiller formation but nevertheless, microorganism-inoculation (using *Bacillus licheniformis*, *Trichoderma reesei*, and *Enterobacter cloaceae*) of ERH increased the average number of panicles per hill compared to applying 100% ERH alone and as such said treatments were not significantly different from CP (Table 3). Applying ERH inoculated with *Bacillus subtilis* (100% ERBBS) resulted to very poor panicle formation (10.10) and was significantly lower than CP. Spikelet formation was not significantly different from CP when applying ERH alone; values even became lower when microorganisms were inoculated to ERH. Supplemental application of chemical fertilizer resulted to significantly higher spikelet formation (except 50% ERHEC) than the CP. On the other hand, grain ripening or percentage filled-spikelet were not significantly different among treatments. Grain weight however, was generally improved by 100% materials. Grain weight is significantly affected by rice hull weight (Yoshida, 1981), and this maybe a result of high silicon availability in the raw material. Grain yield though, was not improved by

Table 3. Effect of alternate nutrient sources to yield and yield components of Japonica cv. Ilpum.

Treatments		Panicle per Hill	Spikelet per Panicle	Percentage Filled-Spikelets (%)	1,000-grain weight (g)	Grain Yield (kg ha^{-1})
Conventional Practice		12.77 ab	88.88 d	87.21 abcde	25.83 cd	5,719.3 b
100%	ERH	11.60 cde	90.23 d	88.76 abcd	27.39 bc	5,289.8 bcd
	ERHBL	12.10 abcd	63.27 h	85.95 cde	27.46 bc	3,901.6 f
	ERHTR	11.70 bcde	78.03 f	90.19 ab	25.94 cd	4,225.7 ef
	ERHEC	12.10 abcd	71.28 g	85.72 cde	29.80 a	4,879.4 d
	ERHBS	10.10 f	86.90 d	84.50 e	27.87 b	5,004.0 d
§50%	ERH	12.50 abc	96.09 c	86.46 bcde	25.29 d	6,471.3 a
	ERHBL	10.70 ef	94.40 c	85.71 cde	28.24 ab	5,042.1 cd
	ERHTR	9.90 f	104.20 b	89.51 abc	27.44 bc	4,712.8 de
	ERHEC	12.80 a	82.36 e	91.17 a	27.62 bc	5,644.5 bc
	ERHBS	11.40 de	109.53 a	84.83 de	26.81 bcd	6,422.2 a

Means wihin 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}

alternative nutrient sources. Treatments without chemical fertilizer supplement were not able to surpass CP in terms of grain yield. ERH alone was not significantly different from CP but still, the yield value was lower than the CP. The highest grain yield was obtained by applying supplemental chemical fertilizer in combination with 50% ERH (6,471 kg ha^{-1}), followed by 50% ERH inoculated with *Bacillus subtilis* (mainly because of its high average spikelet number) with 6,422 kg ha^{-1} grain yield. These treatments were significantly higher than CP. These results suggest that it would still be practical to have supplemental chemical fertilizer application since applying 100% of ERH-based alternatives cannot exceed CP in terms of grain yield.

Effect on Grain Quality Indexes

The ratio of whole grains was significantly increased by alternative nutrient sources except for the 100% ERHBL treatment, which only had approximately 50% whole grains from the sample analyzed (Table 4a). Applying ERH alone already resulted to high whole grain ratio (70.5 %) and inoculation with *Enterobacter cloaceae* (100% ERHEC) further increased the average percentage of whole grains (72.9 %). The treatments with supplemental addition of chemical fertilizer generally increased the whole grain percentage and were higher in values compared to CP (52.9 %). The percentage of broken grains in 100% of alternative nutrient sources was lower than CP except for the 100% ERHBL treatment (27.3 %). This may imply that nutrient release happened during the later parts of reproductive stage as an effect of additional fertilizer application. Nitrogen application could have facilitated OM decomposition and consequently N mineralization which favored spikelet filling. Moreover in the study by Wopereis - Pura *et al.* (2002), late N application resulted to higher milling recovery mainly because of delayed senescence in rice plants. It was the reason for less grain cracking and eventually higher head rice ratio. All treatments applied with supplemental chemical fertilizer resulted to significantly lower ratio of broken grains compared to CP. In terms of chalkiness, 100% ERH, 100% ERHBL, 100% ERHTR, and 100% ERHEC had significantly lower values compared to the CP (21.6 %). Supplemental application of chemical fertilizer also resulted to lower incidence of chalkiness compared to CP except for 50% ERHEC treatment (22.7 %). Treatments applying RH alone

Table 4a. Effect of alternate nutrient sources to the grain quality (milling characteristics) of Japonica cv. Ilpum.

Treatments		Whole grain (%)	Broken grain (%)	Chalkiness (%)	Damaged grain (%)
Conventional Practice		52.90 h	18.77 b	21.57 abc	7.04 b
100%	ERH	70.45 c	6.28 ef	13.90 f	8.33 a
	ERHBL	50.28 i	27.30 a	19.98 c	2.10 e
	ERHTR	69.23 d	7.10 e	15.40 ef	6.63 b
	ERHEC	72.88 ab	5.53 fg	15.70 e	5.30 c
	ERHBS	56.50 g	14.88 c	21.83 ab	7.85 a
§50%	ERH	72.50 b	4.10 h	20.38 bc	2.10 e
	ERHBL	71.00 c	12.35 d	13.95 f	3.20 d
	ERHTR	73.75 a	4.53 gh	17.55 d	5.23 c
	ERHEC	58.10 f	15.13 c	22.73 a	5.63 c
	ERHBS	62.35 e	14.40 c	16.40 de	5.76 c

Means wihin 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}

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		74.53 a	5.82 d	17.43 f
100%	ERH	75.45 a	5.98 b	17.53 f
	ERHBL	75.26 a	5.63 f	18.63 a
	ERHTR	71.58 b	6.05 a	17.73 e
	ERHEC	76.39 a	5.83 d	18.23 b
	ERHBS	75.40 a	5.90 c	17.45 f
§50%	ERH	74.39 a	5.55 g	18.15 bc
	ERHBL	74.99 a	5.75 e	17.85 d
	ERHTR	75.11 a	5.88 cd	18.58 a
	ERHEC	73.74 ab	5.68 f	17.30 g
	ERHBS	74.69 a	5.85 cd	18.08 c

Means wihin 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}

and inoculating it with *Bacillus subtilis* (100% ERHBS) were not better than CP in terms of the percentage of damaged grains while supplemental addition of chemical fertilizer significantly reduced the percentage of damaged grains, mainly due to the increase in whole grain percentage.

On the other hand, alternative treatments were not significantly different from CP in terms of TOYO-taste values (Table 4b). Only treatment with 100% ERHTR resulted to the lowest average TOYO value (71.6) but has the significantly highest protein content (6.1 %). This is due to an established fact that high protein content in grains is negatively correlated to TOYO-taste values or palatability (Choi *et al.*, 2006; Shin *et. al.*, 2006). However, we cannot fully conclude that the decrease in protein content may offer favorable results in terms of palatability (by TOYO-taste values) as we observed that protein content significantly varied almost among all treatments. Amylose content was slightly increased by 100% ERH, 100% ERHTR, and 100% ERHBS while significant increases were observed in 100% ERHBL and 100% ERHEC treatments. Only 50% ERHEC was significantly lower than CP in terms of average amylose content in grain.

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