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Effects of Combined Application of Rice Bran and Chemical Fertilizer on the Phytochemical Contents of Rice

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ABSTRACT The effect of rice bran (RB) applied alone or in combination with chemical fertilizer at different application rate on the phytochemical and antioxidant properties of rice was investigated. The treatments were 3 levels of RB namely: 200% RB (500 kg $10a^{-1}$), 100% RB (250 kg $10a^{-1}$), 50% RB (125 kg $10a^{-1}$), Recommended fertilizer dose (RF: N-P_2O_5-K_2O, 11-5.5-4.8 kg $10a^{-1}$) combined with each RB, Half-recommended fertilizer dose (HRF: N-P_2O_5-K_2O, 5.5-2.75-2.4 kg $10a^{-1}$) combined with each RB, RF and HRF applied at 1, 5, 10 days before rice transplanting (DBT). The parameters investigated were antioxidant, phytosterol and fatty acid contents. Results showed that the antioxidant property and phytosterol contents were high at 10 DBT HRF plus RB, 5 DBT RF plus RB, and 1 DBT 100 and 200% RB. However, total polyphenols increased from 10 to 1 DBT. In the case of fatty acids, no general trend was observed between treatments at different application times. Linoleic acid was high at 10 DBT HRF plus RB while linolenic acid was not affected at different application times. Palmitoeic and oleic acids were not also affected at 5 and 10 DBT. Saturated fatty acids were not also affected by any treatment at different application times except for palmitic acid. Most parameters obtained higher values at 100 and 200% RB treatments in 1 DBT.

Keywords : antioxidant, fatty acid, organic fertilizer, phenolic compounds, phytosterol, rice, rice bran

Organic farming has shown to significantly improve the nutritional quality of crops compared to conventional farming system. It increased the phytochemical and antioxidant contents of fruit crops (Kazimierczak *et al*., 2008, Wang *et al*., 2008), vegetable (Larson *et al*., 2000; Ren *et al*., 2001; Xu *et al*., 2003, Khan *et al*., 2007a) and cereals like oats and rice (Dimberg *et al*., 2005; Na *et al*., 2007; Rico *et al*., 2007; Saha *et al*., 2007). While these observations are not yet absolutely acceptable, there are increasing numbers of reports that organic farming system can increase nutritional quality of crops (Brandt & Molgaard, 2001; Benbrook, 2005).

Many studies have been conducted on the efficacy of organic farming on agronomic characters and yield of cereals, but studies on its effects on the nutritional quality of grains are lacking (Dimberg *et al*., 2005). Cereals contain unique phytochemicals that have been linked to decreased risk of cancer, and prevention of cardiovascular and age-related diseases (Dimberg *et al*., 2005; Fardet *et al*., 2008). Considering that rice is the staple food of over half of world's population, effect of organic fertilizer on the nutritional quality of rice can be investigated.

Long-term use of organic fertilizer has been shown to be more beneficial on soil microorganism and nutrition (Worthington, 1998; Mader *et al*., 2002; Kubat & Lipavsky, 2006; Khan *et al*., 2007b; Kazimierczak *et al*., 2008). While organic farming system may improve antioxidant property in agricultural produce and be more beneficial on sustainable soil management, whole dependence on it may not produce enough yield (Mader *et al*., 2002; Rembialkowska, 2007) for the increasing demand of an ever growing population. To meet the increasing demand for agricultural produce, chemical fertilizer is still indispensable. However, continuous application of chemical fertilizers poses problems and may also result in failed agricultural production in the long run (Nelson

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et al., 2004). Combined application of organic and reduced dose of chemical fertilizer can be beneficial for yield and soil productivity (Mamaril, 2004; Xu *et al*., 2008). In terms of nutritional quality, few studies have shown so far that mixed application of organic and reduced dose of chemical fertilizer performed better compared with application of recommended dose of chemical fertilizer alone (Mintah *et al*., 2007; Rico *et al*., 2007; Saha *et al*., 2007).

Therefore, the objective of this study is to investigate the effect of mixed application of rice bran and reduced dose of chemical fertilizer on the antioxidant property, and fatty acid and phytosterol contents of rice grain.

MATERIALS AND METHODS

The experiment was conducted at the Agricultural Research & Extension Services, Chilgok, Kyungpook Province. The Rural Development Administration (RDA), Gyeongbuk Province provided the rice variety Ilpumbyeo and rice bran (RB). The experiment was laid out in randomized complete block design using 3 replicates. The treatments were: 200% RB (500 kg 10^{-1}), 100% RB (250 kg 10^{-1}), 50% RB (125 kg 10^{-1}), RF+200% RB [Recommended fertilizer dose (RF): N-P_2O_5-K_2O, 11-5.5-4.8 kg $10a^{-1}$], RF+100% RB, RF+50% RB, HRF+200% RB [Half-recommended fertilizer dose (HRF): N-P_2O_5-K_2O, 5.5-2.75-2.4 kg $10a^{-1}$], HRF+100% RB, HRF+50% RB, RF and HRF. The application times were 1, 5 and 10 days before transplanting (DBT).

Antioxidant analysis

Brown rice was used for total phenol and electron donating capacity analysis. Rice sample (4 g) was dissolved in 40 mL 70% ethanol by continuous shaking for 24 hours at 40℃ ambient temperature. The supernatant was used for the analysis.

The electron donating capacity was analyzed using a method modified from the procedure described by Yen & Chen (1995). The reaction mixture contained 0.5 mL 0.05 mM DPPH and 0.01 mL brown rice supernatant. The solution was allowed to react for 30 min in room temperature and then absorbance reading at 517 nm was recorded.

Total phenolic compounds were determined using the procedure described by Zielinski & Kozlowska (2000). Rice sample (0.250 mL) was mixed with 0.250 mL of 50% Folin-Ciocalteu (Sigma Co.), 0.5 mL 10% Na_2CO_3, and 2 mL distilled water. The solution was allowed to stand for 25 min at room temperature and then centrifuged at 5,000 rpm for 10 min. The supernatant liquid was collected and absorbance reading at 725 nm was recorded. Gallic acid (100~500 $\mu g\ mL^{-1}$) was used for the standard curve.

Fatty acid analysis

Fatty acid analysis was done following the procedure of Ruibal-Mendieta *et al*. (2004). Brown rice sample was methylated by reacting with methanolic potassium hydroxide and analyzed for fatty acid using GC-MS (GC-MS: HP 6890 series, Hewlett Packard, Japan) equipped with DB-225 column (30 m × 0.25 mm × 0.25 μm). The injector temperature was 250℃ while the column temperature was 140℃ for 2 min and gradually increased to 200℃ at 5℃ min^{-1} and finally increased to 220℃ at 10℃ min^{-1}. The injection volume was 1 μL injected at a flow rate of 1 mL min^{-1}.

Squalene and phytosterols content

Brown rice extract was prepared by dissolving brown rice in 1 mL 50% KOH and boiled for 10 min in water bath (80℃). After rapid cooling, the precipitate was mixed thoroughly in ethyl ether solution, added with Na_2SO_4 to dry, and then the concentrate was recovered. The concentrate was added with 1 mL chloroform and filtered using 0.2 μm syringe filter. The filtrate was analyzed for squalene and phytosterols content using GC-MS (HP 6890, Hewlett-Packard Co., Japan) equipped with HP-5 column (30 m × 0.25 mm × 0.25 μm). The injector temperature had an initial temperature of 250℃ for 1 min and gradually increased at 2℃ min^{-1} until a temperature of 300℃ was reached. The carrier gas used was Helium and the injection volume was 1 μL at a flow rate of 1 mL min^{-1}.

RESULTS

Effect on antioxidant contents

The effect of rice bran on the antioxidant property of rice grain was analyzed by determining its electron donating ability, total phenolic compounds and squalene contents. In the electron donating capacity, results showed that fertilization at 1 DBT obtained high values (~65%) in the RF+RB and HRF treatments but low values (55.4~55.8%) in the HRF+RB treatments. The trend was reversed at 10 DBT wherein the values in HRF+RB and 100% RB treatments obtained higher electron donating capacity (53.9~55.3%) than those of RF+RB treatments (50.6~52.8%). At 5 DBT, only RF+50% RB and 100% RB obtained high electron donating capacity (59~65%). In case of the total phenolic compounds, values decreased from 1 to 10 DBT which were 92.7~128.3, 92.6~114.4 and 55.4~95.1%, respectively. In 1 and 10 DBT treatment days, RF+RB treatments generally obtained lower values than those of HRF+RB. For squalene content, results at 10 DBT showed higher values in 100 and 200% RB treatments but lower in 50% RB while the highest value was at HRF+50% RB (~32 $\mu g\ g^{-1}$). At 5 DBT, RF and HRF combined with RB treatments obtained high values (27.1~30.2 $\mu g\ g^{-1}$) while only 200% RB obtained high value (31.8%) at 1 DBT.

Effects on fatty acid contents

Effect of rice bran treatment on major fatty acid contents of rice grain was determined. The fatty acids analyzed were myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), palmitoeic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. The results showed that the treatments had no apparent significant effects on the saturated fatty acids (data not shown) except for palmitic acid. At 1 and 5 DBT, palmitic acid content was lower at rice bran and HRF treatments. However, higher palmitic acid content was obtained in rice bran treatments at 10 DBT. Results also showed that the treatments affected unsaturated fatty acids contents (Table 2). Palmitoeic acid content was not affected at 5 and 10 DBT. At 1 DBT, highest value was obtained at HRF+200% RB (0.08%) while RF plus RB treatments had significantly higher (0.06~0.08%) palmitoeic acid content than RB treatments (0.03~0.05%) alone. Oleic acid was significantly higher at HRF, RF and RF+50% RB (43.4~43.6%) than the other treatments at 10 DBT. At 5 DBT, results showed higher oleic acid content at 50 and 100% RB (42.7~43.7%) and HRF plus 50 and 100% RB (42.5~43.1%) treatments. At 1 DBT, high values were obtained at 50 and 100% RB (45.2~45.7%). Linoleic acid content at 10 DBT was high at 200% RB (37.0%), RF plus 100 and 200% RB (36.8~37.3%), and HRF plus 100 and 200% RB (36.7~37.1%). It was also high at 5 DBT treatments of HRF plus 100 and

Table 1. Effect of rice bran and chemical fertilizer on the antioxidant properties of rice grains.

Treatment†	Electron donating capacity (%)			Phenolic compounds (mg GAE 100 g D.W^{-1})			Squalene ($\mu g\ g^{-1}$)		
	10‡	5	1	10	5	1	10	5	1
200% RB	51.1§ef¶	51.0g	56.6de	95.1a	101.3bcd	117.2bc	31.2ab	23.7c	31.8a
100% RB	54.7ab	58.6b	56.9de	80.7e	107.2b	114.0c	28.7bc	20.5d	29.5b
50% RB	52.8cd	56.6c	53.5f	84.5c	114.4a	127.4a	29.4ab	21.6d	25.4c
RF+200% RB	52.0de	55.0d	67.3a	78.2g	92.6e	92.75f	30.3ab	30.2a	23.9cd
RF+100% RB	50.6f	52.5ef	65.9ab	78.2g	103.3bc	92.7f	29.8ab	27.1b	23.9cd
RF+50% RB	52.8cd	65.1a	65.5ab	80.0f	94.9de	97.4e	26.5cd	28.8ab	23.5cd
HRF+200% RB	55.3a	51.9fg	58.1cd	55.4i	92.6e	106.6d	31.7a	29.3a	19.5e
HRF+100% RB	54.4ab	49.4h	55.8e	83.4d	101.3bcd	120.0b	32.0a	28.3ab	24.9c
HRF+50% RB	53.9bc	53.6e	55.4e	90.7b	96.8cde	128.3a	25.9d	29.3a	25.4c
RF	44.3h	52.8ef	59.1c	66.2h	46.0g	79.4g	26.1cd	19.8d	22.1d
HRF	45.4g	52.5ef	65.1b	79.9f	61.0f	90.8f	26.1cd	21.0d	24.5c

†Treatments: 200% RB (500 kg 10a^{-1}), 100% RB (250 kg 10a^{-1}), 50% RB (150 kg 10a^{-1}), RF (Recommended fertilizer dose: N-P_2O_5-K_2O, 11-5.5-4.8 kg 10a^{-1}), HRF (Half-recommended fertilizer dose: N-P_2O_5-K_2O, 5.5-2.75-2.4 kg 10a^{-1}).
‡Application day of fertilizer days before rice transplanting (DBT): 1, 5, and 10 DBT.
§All values are mean of 15 samples and 3 replications.
¶Within DBT, same letters are not significantly different $P < 0.05$ level by DMRT.

200% RB (37.8~38.1%) and 1 DBT HRF+200% RB (37.9%) treatments. In case of linolenic acid, there was no significant difference in the percent content between treatments at different DBT treatments.

Effect on phytosterol contents

The effect of rice bran treatment on the phytosterol contents of rice grain was determined. The major phytosterols in rice were campesterol, stigmasterol, sitosterol and cycloartenol. Campesterol content was high at 1 DBT treatments of 100% RB (39.3 μg g^{-1}) and HRF (40.6 μg g^{-1}). The opposite was observed at 5 DBT wherein only HRF+50% RB obtained low value (28.2 μg g^{-1}). At 10 DBT, low campesterol content was obtained in the low fertilized treatment: 50% RB (30.9 μg g^{-1}), HRF+50% RB (27.6 μg g^{-1}), HRF (29.9 μg g^{-1}) and RF (30.1 μg g^{-1}), but was high at 100 and 200% RB (33.0 μg g^{-1}), RF plus RB treatments (32.7~33.0 μg g^{-1}) and HRF plus 100 and 200% RB (33.1~33.6 μg g^{-1}). In the case of stigmasterol, high value was obtained at 1 DBT treatments of 100% RB (29.0 μg g^{-1}) and 50% RB (29.6 μg g^{-1}). At 5 DBT, the RB treatments generally obtained high stigmasterol contents with the highest obtained in only 50% RB (24.4 μg g^{-1}). At 10 DBT, 100 and 200% RB obtained values (22.1 and 22.3 μg g^{-1}, respectively) comparable with those obtained in RF plus 100 and 200% RB (23.2 and 22.3 μg g^{-1}, respectively), HRF plus 100 and 200% RB (21.6 and 22.4 μg g^{-1}, respectively), HRF (22.0 μg g^{-1}), and RF (22.9 μg g^{-1}). In sitosterol, high values were obtained 1 DBT treatments of 100 and 200% RB and HRF (90.0~92.4 μg g^{-1}). At 5 DBT, HRF+50% RB obtained the highest value (87.6 μg g^{-1}), but RF plus 100 and 200% RB, and HRF+200% RB also obtained high values (84.0, 84.0 and 80.4 μg g^{-1}, respectively). At 10 DBT, RF+200% RB obtained the highest value (90.9 μg g^{-1}), but HRF+200% RB, HRF+50% RB, RF and HRF obtained high values (88.8, 88.4, 89.7 and 89.8 μg g^{-1}, respectively) as well. In the case of cycloartenol, 1 DBT 100 and 200% RB treatments obtained high values (34.9 and 34.7 μg g^{-1}, respectively). At 5 DBT, RF+50% RB (32.1 μg g^{-1}) and HRF+50% RB (32.5 μg g^{-1}) obtained high values. At 10 DBT, RF+50% RB (37.9 μg g^{-1}) obtained high value compared to other treatments.

DISCUSSION

Results in the analysis of antioxidant property showed that HRF plus 100 or 200% RB treatments obtained comparable if not better antioxidant property than RF and RF plus RB treatments. Values obtained were also higher at HRF plus

Table 2. Percent (%) fatty acid content in rice grains at different rice bran and chemical fertilizer treatments.†

Treatment‡	Palmitic (16:0)			Palmitoeic (16:1)			Oleic (18:1)			Linoleic (18:2)			Linolenic (18:3)		
	10§	5	1	10	5	1	10	5	1	10	5	1	10	5	1
200% RB	18.6¶ab#	17.6c	16.5bc	0.10a	0.08a	0.05de	41.2b	42.0c	44.2bcd	37.0ab	37.2b	36.2cd	1.23a	1.23ab	1.20a
100% RB	18.7ab	17.7bc	16.1c	0.09a	0.08a	0.04ef	41.9b	42.7bc	45.7a	36.3cd	36.5c	35.1f	1.18a	1.20ab	1.15ab
50% RB	19.0a	18.2abc	16.6bc	0.08a	0.08a	0.03f	42.1b	43.7a	45.2ab	35.8d	34.7e	35.5ef	1.17a	1.16b	1.13ab
RF+200% RB	18.5ab	18.7a	17.8ab	0.10a	0.08a	0.07abc	41.0b	40.9d	42.0fg	37.3a	37.3b	37.2b	1.25a	1.23ab	1.16ab
RF+100% RB	18.0abc	18.0abc	17.6ab	0.09a	0.09a	0.07abc	41.9b	42.5bc	42.2ef	36.8abc	36.1c	37.4ab	1.21a	1.23ab	1.20a
RF+50% RB	18.0abc	18.2abc	17.5ab	0.09a	0.09a	0.07ab	43.4a	43.1ab	42.7def	35.9d	35.4d	36.4c	0.88b	1.21ab	1.15ab
HRF+200% RB	18.1abc	17.9abc	18.2a	0.09a	0.09a	0.08a	41.7b	40.9d	40.8g	37.1ab	38.1a	37.9a	1.18a	1.24a	1.20a
HRF+100% RB	18.0abc	18.1abc	17.1abc	0.08a	0.09a	0.06bcd	42.2b	41.0d	43.4cdef	36.7abc	37.8ab	36.5c	1.22a	1.24a	1.14ab
HRF+50% RB	18.3ab	17.5c	16.5bc	0.08a	0.08a	0.06cd	42.1b	42.1c	44.9abc	36.4bcd	37.3b	35.5ef	1.20a	1.26a	1.14ab
RF	17.1c	18.3abc	17.5ab	0.09a	0.09a	0.07bcd	43.6a	41.1d	43.7cde	36.1cd	37.4b	35.8ef	1.23a	1.27a	1.09b
HRF	17.7bc	18.5ab	17.7ab	0.08a	0.08a	0.07ab	43.4a	42.4bc	43.0def	35.8d	35.9cd	36.3cd	1.16a	1.20ab	1.13ab

†Total fatty acid contents equals 100% including those not reported in the table.
‡Treatments: 200% RB (500 kg $10a^{-1}$), 100% RB (250 kg $10a^{-1}$), 50% RB (150 kg $10a^{-1}$), RF (Recommended fertilizer dose: N-P_2O_5-K_2O, 11-5.5-4.8 kg $10a^{-1}$), HRF (Half-recommended fertilizer dose: N-P_2O_5-K_2O, 5.5-2.75-2.4 kg $10a^{-1}$).
§Application day of fertilizer days before rice transplanting (DBT): 1, 5, and 10 DBT.
¶All values are mean of 15 samples and 3 replications.
#Within DBT, same letters are not significantly different $P < 0.05$ level by DMRT.

Table 3. Phytosterols content (μg g^{-1}) in rice grains at different rice bran and chemical fertilizer treatments.

Treatment†	Campesterol			Stigmasterol			Sitosterol			Cycloartenol		
	10‡	5	1	10	5	1	10	5	1	10	5	1
200% RB	33.0§ab¶	31.9a	28.8d	22.3ab	23.6ab	20.1e	85.5cd	75.9de	92.4a	29.9cd	23.7e	34.7a
100% RB	33.0ab	31.7a	39.3a	22.1abc	23.3bc	29.0a	85.5cd	75.8de	90.0a	29.8cd	24.8de	34.9a
50% RB	30.9bc	31.3a	31.6cd	21.2bc	24.4a	23.6bcd	87.2bcd	73.6e	78.9cde	31.3bc	25.0de	29.9c
RF+200% RB	32.7ab	32.8a	36.0b	22.3ab	22.0def	25.4b	90.9a	84.0ab	80.7cd	27.8d	28.8bc	24.4f
RF+100% RB	33.0ab	30.7ab	32.9c	23.2a	21.2fg	23.6bcd	87.2bcd	84.0ab	78.0de	28.9d	28.9b	26.8e
RF+50% RB	32.8ab	31.2a	31.7cd	20.4c	21.4efg	22.8cd	82.2e	78.5cd	80.7cd	37.9a	32.1a	27.8de
HRF+200% RB	33.6a	31.9a	25.7e	22.4ab	22.2de	21.6de	88.8ab	80.4bc	66.8f	31.4bc	28.5bc	23.3f
HRF+100% RB	33.1ab	30.4ab	28.7d	21.6abc	21.1fg	24.2bc	84.9de	75.8de	77.3e	33.0b	27.3c	28.8cde
HRF+50% RB	27.6d	28.2b	29.5d	18.4d	20.6g	23.9bcd	88.4abc	87.6a	81.4c	31.6bc	32.5a	32.9b
RF	30.1c	31.1a	38.3ab	22.9ab	22.5cd	25.6b	89.7ab	77.2cde	85.3b	33.5b	25.7d	28.6cde
HRF	29.9c	30.3ab	40.6a	22.0abc	22.6cd	29.6a	89.8ab	74.1e	90.0a	32.2bc	25.8d	29.4cd

†Treatments: 200% RB (500 kg 10a^{-1}), 100% RB (250 kg 10a^{-1}), 50% RB (150 kg 10a^{-1}), RF (Recommended fertilizer dose: N-P_2O_5-K_2O, 11-5.5-4.8 kg 10a^{-1}), HRF (Half-recommended fertilizer dose: N-P_2O_5-K_2O, 5.5-2.75-2.4 kg 10a^{-1}).
‡Application day of fertilizer days before rice transplanting (DBT): 1, 5, and 10 DBT.
§All values are mean of 15 samples and 3 replications.
¶Within DBT, same letters are not significantly different $P < 0.05$ level by DMRT.

RB treatments at 10 DBT, but RB or RF treatments obtained higher values at 1 and 5 DBT. Only total phenolic compounds obtained observable decreasing trend in values from 10 to 1 DBT treatment. Squalene content (20~30 μg g^{-1}) was quite high compared to that (14.3 μg g^{-1}) reported by Ha *et al*. (2006), which maybe attributed to rice variety or the effect of treatment. For fatty acid, no general trend was observed on the effect of treatments on fatty acids. Palmitic acid content was decreasing from rice bran to pure chemical fertilizer treatments. Palmitoeic acid was not affected at 5 and 10 DBT treatments while linolenic was not affected at all at different application times. At 1 DBT, palmitoeic acid obtained high values at RF plus RB treatments while oleic acid obtained high values at 50 and 100% RB. Only linoleic acid obtained high values at 10 DBT HRF plus RB treatments (36.7~37.1%) that were comparable with those obtained in RF plus 100 and 200% RB (36.8~37.3%). HRF plus 100 and 200% RB treatments also obtained high linoleic acid at 1 and 5 DBT. This may be a good measure of grain quality since linoleic acid is an important essential fatty acid in rice. The observed percent composition of palmitic, oleic and linoleic acids were in agreement with those reported by Son *et al*. (1996) (20.3, 35.0, and 41.1%, respectively) and Cho *et al*. (2006) (24.0, 35.4, and 36.5%, respectively).

In the case of phytosterol contents, similar observations with that of antioxidant property were recorded. At 1 DBT, higher values were obtained at 100 and 200% RB. At 5 DBT, higher values were obtained at RF plus RB treatments, and at 10 DBT, higher values were obtained at HRF plus RB treatments. Only cycloartenol, did not obtain high value at 10 DBT HRF plus RB treatments. The values obtained also agreed with those reported by Ha *et al*. (2006) except for cycloartenol which was higher by almost 10 μg g^{-1}.

In general, the results showed better antioxidant property at HRF plus RB treatments at 10 DBT. Other studies on application of organic fertilizer combined with reduced dose of chemical fertilizer also showed better antioxidant property (Na *et al*., 2007; Rico *et al*., 2007) compared to the recommended dose of chemical fertilizer alone. Similar observation was also reported in oats wherein phenolic compounds were not negatively affected in organic farming system (Dimberg *et al*., 2005). There were varying trends in the fatty acid contents at different application times, but in general it could be said that it was not negatively affected. Lee *et al*. (2004) reported also a not significant decrease in fatty acid content of rice at reduced dose of chemical fertilizer. Similar study on fatty acid content in red raspberry seed oil did not show significant effects of application times and application rate of nitrogen fertilizer and organic manure (Gercekcioglu *et al*., 2007). In the case of phytosterols, there is no available study yet on the effect of organic fertilizer on the phytosterol contents of rice. There is one study though showing the effect of spraying stigmasterol on the hormones production in sesame seeds (El-Greedly *et al*., 2005).

In terms of the best application time, there seemed to be better grain nutritional quality at 10 DBT. Although total phenolic compounds and palmitoeic acid obtained high values at HRF plus RB treatments 1 DBT, HRF plus RB treatments 10 DBT seemed favorable in most parameters studied. Mintah *et al*. (2007) reported a better yield and grain quality at 10 DBT application time of reduced dose of chemical fertilizer and biofertilizer. This observation was attributed to enough time for the fertilizer to get assimilated with the soil.

However, what is unique in this study was that 100 and 200% RB treatments at 1 DBT gave similar, and sometimes even higher, nutritional values compared with RF treatment. This was opposite with what Mintah *et al*. (2007) reported wherein RF treatments obtained higher values compared with biofertilizer at short time gap before transplanting. Xu (2000) also reported a low nutrient availability at the early growth stages of crops in organically fertilized fields. The good performance of 100 and 200% RB treatments could not be easily explained by high and readily available nutrient contents of rice bran and should be explored for further studies.

In case of vegetable and fruit crops, increased nutritional quality was obtained due to increased production of secondary metabolites for defense mechanism against pest infestation (Brandt & Molgaard, 2001; Benbrook, 2001). In case of combined application of organic fertilizer and reduced dose of chemical fertilizer in rice farming, review of literature (Wang *et al*., 2007; Xu *et al*., 2008) showed it could maintain soil nutrients balance, amend soil physical and chemical properties, increase the soil organic matter and nutrient availability, decrease fertilizer loss rate, and then enhance soil fertility and ecosystem productivity.

Use of rice bran in organic farming has shown beneficial effect on microbial growth in spinach production (Khan *et al*., 2007a), weed control in organic farming system (Khan *et al*., 2007b), weed control and fertilizer on transplanted rice (Kuk *et al*., 2001), and mulching in tomato (Rodriguez, 2007). Rice bran is also a major component of Japanese bio-organic fertilizer ‘Bokashi’ that is widely used in Japan for organic farming (Xu *et al*., 2003; Khan *et al*., 2007a; 2007b).

This study has shown that combined application of rice bran and reduced dose of chemical fertilizer has similar effect on the nutritional and antioxidant quality of rice grain produced in recommended dose of chemical fertilizer. However, more studies are needed to validate its long-term efficacy.

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