

Evaluation of the Physio-Chemical and Microbial Properties of Green Tea Waste-Rice Bran Compost and the Effect of the Compost on Spinach Production

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Abstract : To study the physio-chemical and microbial properties of green tea waste-rice bran compost (GRC), and feasibility of GRC as the alternative of agrochemicals for quality spinach production, five types of compost were prepared by mixing green tea waste and rice bran. The compost temperature was increased by adding rice bran to the green tea waste. The compost GC made from green tea waste alone contained a relatively large amount of nitrogen (7.55%). On the other hand, the compost RC made from rice bran alone contained a relatively large amount of minerals, such as phosphorus (0.49%), potassium (4.96%) and magnesium (2.28%). Addition of rice bran increased the total number of bacteria, viable bacteria and organic matter decomposing bacteria as well as actinomycetes population in the mature compost. The frequency of organic matter decomposing bacteria in GRC was in the following order: cellulolytic bacteria < pectolytic bacteria < lipolytic bacteria < amilolytic bacteria except RC. The growth of spinach was significantly increased over the untreated control (only soil) when GRC was applied in the field and growth pattern was depended on the nature of the composting materials. The highest fresh weight of 27.5 g plant⁻¹ and 45.4 g plant⁻¹ were obtained by applying the compost with 30% green tea waste + 70% rice bran (GRC-3) under field condition in autumn of 2004 and 2005, respectively. The highest growth enhance effect was also obtained from GRC-3 when three spinach varieties were grown with GRC under greenhouse condition. The amount of nitrate and oxalate in the spinach grown with GRC were ranged from 114.0 to 146.0 mg 100 g⁻¹ and 612.0 to 748.0 mg 100 g⁻¹ fresh weight, respectively. These values were below from the safety standard of health level of nitrate and oxalate for spinach. The amount of ascorbic acid, glutamic acid and iron in the spinach were increased by applying the compost. Among the five combinations of GRC, GRC-3 provided the best results in spinach production.

Key words : Composting, Green tea waste, Microbes, Physio-chemical properties, Rice bran, Spinach.

The characteristics of modern agriculture are mechanization and application of large amount of chemical fertilizers, pesticides and herbicides. Although these agrochemicals increase land productivity and labour potentiality, they destroy the diversity of biota and sacrifice human health (Balogh and Anderson, 1992; Nelson et al., 2004). Because of these problems, much attention is being focused on alternative methods of crop production by using compost which is safe for environment and public health.

Approximately, worldwide 2.5 million metric tons of dried tea manufactured per year and 20% of it is green tea (Graham, 1992). In Japan, consumption of green tea beverages has continued to increase remarkably in recent years (Kondo et al., 2004). After the extraction of green tea, the remainder is discarded as waste. Generally, the waste has been disposed by dumping or burning. Dumping obviously creates environmental problems, whereas burning of the waste

is expensive. In Japan, it costs 10 yen to dispose kg⁻¹ of tea waste. But, green tea waste, for their high content of organic matter (>90%) and nitrogen (5–7%), could be used as composting materials to restore the soil fertility. Especially in Saga Prefecture, Japan, it is easy to collect the green tea waste from the tea processing factories without any type of costs. On the other hand, rice is the staple food of over half the world's population. Rice bran accounts for 5–8% of the weight of the whole-grain rice (FAO, 1964). It contains oil, protein, vitamins and some essential minerals as well as enzymes, microorganisms, natural toxicant constituent, harmful contaminants and adulterants (Barber and De Barber, 1980). Parts of the Japanese organic farmers are now using rice bran (200 g m⁻²) for weed control and fertilizer on transplanted rice (Kuk et al., 2001). Green tea waste contains high amount of water (> 80%) which enhance the anaerobic decomposition, whereas drying of the waste is expensive. There is a possibility to get the good quality compost, if it

Table 1. Determination of the culturable bacteria in mature compost by substrate-specific plate counting techniques.

Target microbes	Media	Principle of detection	Incubation temperature (°C)	Incubation period (days)	References
Cellulolytic bacteria	Carboxymethyl cellulose (CMC)	Decomposition of CMC	30°C	5	Hankin et al., 1974 and Suyama et al., 1993.
Pectolytic bacteria	Pectin	Decomposition of pectin	30°C	3	Hankin et al., 1974.
Lipolytic bacteria	Tween20	Decomposition of Tween20	30°C	5	Hankin et al., 1974.
Amilolytic bacteria	Starch	Decomposition of starch	30°C	5	Hankin et al., 1974.
Actinomycetes	Albumin	Mycelial growth	30°C	30	De Azeredo et al., 2001.

is mixed with rice bran (water content 15–20%). Moreover, rice bran helps to increase the microbial activity as well as compost pile temperature during composting (Kato et al., 2005). Considering the above facts, this research was designed to establish a compost from green tea waste (rich in organic matter and nitrogen) and rice bran (rich in microbial biomass and minerals) which have a possibility to serve as soil conditioner and fertilizer that can help to grow organic vegetables and reduce the use of hazardous synthetic agrochemicals.

To study the effect of the compost on vegetables production we used spinach (*Spinacia oleracea* L.), because spinach is a highly valuable leafy vegetable with good cooking adaptability (Nishihara et al., 2001). Numerous investigations have been reported that chemical fertilizer promotes the accumulation of nitrate in spinach. However, slow release of N in compost can modify the nitrate content in spinach. The specific objectives of this research were to evaluate the physio-chemical and microbial properties of green tea waste-rice bran compost (GRC), and the feasibility of GRC as an alternative of agrochemicals for quality spinach production.

Materials and Methods

1. Composting procedure

The GRC was prepared by mixing green tea waste and rice bran at five ratios in a 45 L plastic bucket (height: 49.5 cm and diameter: 42.7 cm, Belc, Risu Corporation, Japan) with three replications (maintaining 3 buckets separately) and allowed to decompose for 4 mo (composting started from March, 2005) in the glasshouse of Field Science Center, Saga University. Green tea waste and rice bran were mixed at the following five ratios (v v⁻¹): green tea waste alone (GC), 70% green tea waste+30% rice bran (GRC-1), 50% green tea waste+50% rice bran (GRC-2), 30% green tea waste+70% rice bran (GRC-3) and rice bran alone (RC). The moisture content was adjusted to 55

–60% at the start of composting. The composting buckets were turned upside down once a wk. The temperature was measured hourly at a depth of 10–15 cm from the surface of compost by Thermo Recorder (RT-12, ESPEC MIC Crop, Japan). Sample of compost was taken by mixing sub-samples from ten places of the bucket, covering a wide span of the profile (from top to bottom of the bucket). The microbial properties of the compost were measured immediately after samples collection. The samples were air-dried and ground to 0.5 mm size and then oven dried (70°C for 72 h), and kept frozen (–20°C) until for the use of physio-chemical analyses.

2. Physio-chemical analyses of compost

The mature compost was analyzed for moisture content which was measured after drying at 70°C for 72 hr, electrical conductivity (EC) (1 : 5 w v⁻¹ compost: water extract) (Rhoades, 1996), pH and oxidation reduction potential (ORP) (1 : 5 w v⁻¹ compost:water extract) (HM-21P, TOADKK, Japan), organic carbon (Tyurin, 1931), total nitrogen (Bremner, 1996), exchangeable K (Kundsen et al., 1982), P, Ca, Mg, Fe and Na (ICP-MS, OPTIMA 3100RL Spectrometer, Perkin Elmer, Norwalk, USA) (Thompson and Wood, 1982).

3. Estimation of the number of bacteria by direct counting techniques

The Nuclepore filter technique (Hobbie et al., 1977; Roser, 1980; Someya, 1995) was used for staining the bacteria of mature compost (4 mo old compost) suspension with nucleic acid intercalating agent ethidium bromide (EB, Sigma), and a nonspecific esterase substrate, carboxy fluorescein diacetate (CFDA; mixed isomers, Molecular Probes Inc.) to determine the total (EB method) and viable (CFDA method) bacteria in compost suspension. An initial suspension was made by adding 10 g (wet weight) of sample to 95 ml of sterile particle-free distilled

water and homogenized in a blender with stainless steel blades (Ace homogenizer, Nihonseiki Ltd.) at 15000 rpm for 15 min. The bacterial cells were counted under an epifluorescent microscope (Nikon Optiphot-2, Tokyo, Japan) fitted with a Neofluar objective (100x, NA=1.30). At least 10 randomly selected microscopic fields were counted in each filter preparation using an eyepiece micrometer disk (5 × 5 grids). Three replicated filters were prepared for each sample. The means and standard deviations were calculated.

4. Estimation of number of bacteria by substrate-specific plate counting techniques

Culturable bacteria, such as cellulolytic, pectolytic, lypolytic, amilolytic, and actinomycetes were enumerated by the substrate-specific plate-dilution frequency method. Table 1 shows the counting procedures. An initial suspension was made following the procedure of direct counting techniques. The suspension was further diluted 10-fold with sterile particle-free distilled water. Ten-fold serial dilutions were then made in saline water (NaCl 8 g L⁻¹) and used as inoculum. Aliquots (100 µl) of each dilution were spread onto agar in petri plates, in triplicate. Quantitative determinations were made on the basis of colony forming units (CFU). All results were expressed on CFU g⁻¹ of dry weight (after drying aliquots of the samples at 105°C for over night).

5. Productivity and qualitative analyses of spinach

Experiments were conducted consecutively two years, autumn of 2004 and 2005 under field conditions to test the effect of GRC on the production of spinach variety Jiromaru (Takii Seed, Japan) with the compare of non-treated control (only soil). The field experiment was conducted at the organic farm of Field Science Center, Saga University, Japan where use of the synthetic agrochemicals is strictly prohibited. So, the effect of GRC on spinach production was compared with the non-treated control. The field was divided into small plots with an area of 2 × 1 m² with three replications in a completely randomized block design. Composts were applied during the final land preparation (7 day before sowing of seeds) at the rate of 2 kg m⁻². Three spinach seeds hill⁻¹ was sown maintaining line to line distance 25 cm and plant to plant 15 cm at the first wk of October both in 2004 and 2005. After germination, one healthy plant was kept hill⁻¹. Using twenty seven plants from each treatment, the plant height (cm), leaf area (cm² plant⁻¹) and fresh weight (g plant⁻¹) were measured at harvesting (45 days after sowing of seeds). Additionally, a preliminary experiment was conducted at the conventional farm of Field Science Center with mixed chemical fertilizer (N : P : K = 14 : 18 : 14; application rate 157.1 g m⁻²) using the soil of organic farm. The other practices of this

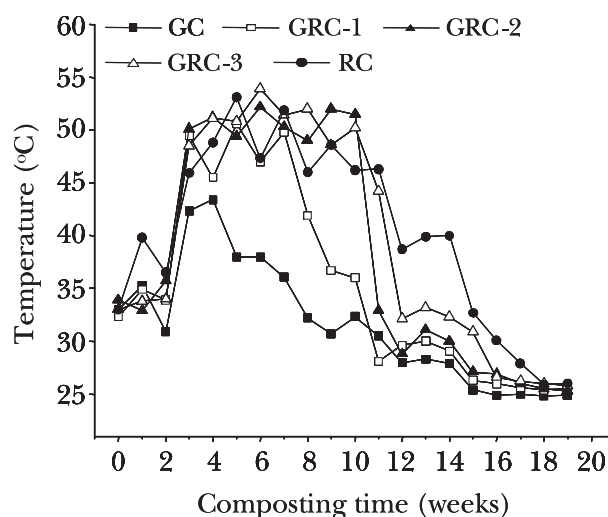


Fig. 1. Temperature fluctuations during composting of the mixture of green tea waste and rice bran. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 2.

experiment were same to the main experiment.

To test the effects of GRC on different varieties of spinach, Jiromaru, Hoyo and Shin-nihon (Takii Seed, Japan) were grown in plastic pots (pot height: 11.5 cm and surface area: 153.9 cm²) using GRC under greenhouse condition in the autumn of 2005 with the compare of non-treated control (only soil). Finally, three healthy plants were grown pot⁻¹ and replicated 3 times in a completely randomized block design. Fresh weight of the plant was measured 45 days after sowing of seeds. Twenty percent GRC (v v⁻¹ of soil) were mixed in pot soil except the control (only soil). Soils of the organic farm were used in these experiments.

Spinach leaves including stems were analyzed for nitrate, oxalate, iron, ascorbic acid and glutamic acid concentration. The nutrient analyses were done only in 2005 and conducted spectroscopically (Tagliavini et al., 2000) using the Merck RQflex (E. Merck D 64271 Darmstadt, Germany) with specific tests (nitrate test n. 1.16971; iron test n. 1.16982; ascorbic acid test n. 1.16981 and oxalic acid test n. 01873-96).

6. Statistical analyses

All the experiments were conducted with three replications and average results were used in table and figure. Statistical analyses were performed using SAS package version 6.12 (SAS Institute, 1996). Differences among the treatments were determined by Tukey's test ($P < 0.05$).

Results and Discussion

1. Temperature changes during the compost processing

Fig. 1 shows the change in temperature of five GRCs during decomposition. The initial temperature was 32.3–32.9°C, and the weekly average temperature

Table 2. Physio-chemical characteristics of green tea waste-rice bran compost (GRC).

Characteristics of compost	Combination of GRC				
	GC	GRC-1	GRC-2	GRC-3	RC
pH	6.8	6.5	6.4	6.4	6.1
EC (dS m ⁻¹)	2.4 d	5.4 c	6.5 c	7.9 b	10.8 a
ORP (mV)	147.3 a	97.0 b	84.7 b	82.7 b	0.7 c
Carbon (%)	43.24 a	42.22 a	37.56 b	38.54 b	34.36 c
Total N (%)	7.55 a	6.53 b	6.08 c	5.33 d	4.35 e
C/N ratio	5.73 c	6.47 b	6.18 c	7.23 a	7.90 a
P (%)	0.04 d	0.20 c	0.24 bc	0.32 b	0.49 a
K (%)	1.07 d	2.19 c	2.50 bc	2.86 b	4.96 a
Ca (%)	0.83 a	0.69 b	0.48 c	0.34 d	0.12 e
Mg (%)	0.33 c	1.17 b	1.27 b	1.67 b	2.28 a
Fe (%)	0.08	0.05	0.04	0.03	0.08
Na (%)	0.02	0.02	0.03	0.01	0.01
Moisture (%)	54.2 b	58.2 a	51.8 b	48.0 c	44.2 d

Values in the row with the same letter are not significantly different at $P < 0.05$. GC=green tea waste alone, GRC-1=70% green tea waste+30% rice bran, GRC-2=50% green tea waste+50% rice bran, GRC-3=30% green tea waste+70% rice bran and RC=rice bran alone.

was highest at the 6th wk of composting, which was 53.9°C in GRC-3. The temperature of the GC was lower than that of RC and temperature was increased by mixing rice bran with green tea waste. The high temperature was maintained from the 3rd wk to 10th wk of composting time. The temperature consistently increased immediately after turning over, but after 12th wk, the temperature fluctuation after turning over was very low and the temperature reached the ambient temperature (24.9–26.0°C) at 18th wk. Kato et al. (2005) reported that as the compost approached maturity stage, the temperature started to drop. Composting of GRC exhibited a classical temperature pattern, commencing with a brief latency phase followed by a rapid increase in temperature lasting for about 2 mo and gradual cooling for another mo. Similar patterns have been reported in a variety of waste products, e.g., hardwood bark (Hoitink et al., 1977), sewage sludge (Nakasaki et al., 1985), grape pulp (Faure and Deschamps, 1990) and poultry manure, rice husk and rice bran (Kato et al., 2005).

2. Physio-chemical properties of compost

Table 2 shows the physio-chemical properties of GRC. All composts were slightly acidic (pH ranging from 6.1 to 6.8) and pH decreased slightly with the increase of rice bran percentages in GRC. ORP was also decreased from 147.3 mV (GC) to 0.7 mV (RC) with the increase of rice bran percentages in GRC. Esaki et al. (1993) stated that the evolution of organic acids that reduce the ORP. The lowest ORP in RC indicates the evolution of high amount of organic acids which hampers the plant growth. EC was increased

with the increase of rice bran percentages in GRC. The highest EC was found in RC (10.8 dS m⁻¹) and lowest in GC (2.4 dS m⁻¹). Rice bran is rich with mineral salts which would likely be responsible for the increase of EC. Normally, the high EC of compost optimizes after the application in soil. Abad et al. (2001) reported that the EC value of an ideal growth medium is ≤ 0.5 dS m⁻¹. The largest amount of nitrogen was obtained in GC (7.55%) and smallest in RC (4.35%). The C/N ratio of the compost was ranged from 5.73 (GC) to 7.90 (RC). Whereas, at the beginning of composting, C/N ratio of green tea waste and rice bran was 9.3 and 19.5, respectively. Due to the utilization of carbon by the microorganisms, C/N ratio was reduced finally. Bernal et al. (1998) proposed that $C/N < 12$ is the maturity index of compost. Phosphorus, potassium and magnesium contents of GRC were increased with the increase of rice bran percentages in GRC. The moisture content of the mature compost ranged from 44.2% to 58.2%. Above 50% moisture in mature compost is congenial for the regrowth of microbes. Among the five combinations of GRC, GC was rich in nitrogen and RC was rich in minerals. The amount of nitrogen and minerals in the other combinations of GRC were depended on the proportion of green tea waste and rice bran presence in the mixture.

3. Microbial properties of compost

Traditionally, studies on compost bacteria have been concerned with taxonomic identification and populations. Such studies do not inform us which degradative enzymes are produced by the bacteria present in the given compost. Composting is the

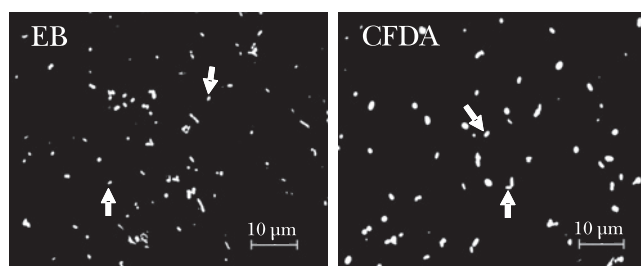


Fig. 2. Micro image of GRC-3 bacteria (40x) by ethidium bromide (EB) and carboxy fluorescein diacetate (CFDA) staining method. Arrows indicate the bacterial cell. For GRC-3 see Table 2.

process of degradation of complex and heterogeneous organic material by a mixture of microorganisms. Among the microorganisms, bacteria play an important role in organic matter decomposition. Various hydrolytic enzymes are believed to control the rate at which various substrates are degraded (Tiquia, 2002). So, the population of cellulolytic, pectolytic, lipolytic and amylolytic bacteria which are responsible for hydrolysis of cellulose, pectin, fat and starch, respectively were studied in this experiment to understand the degradation of various organic wastes. We also studied the actinomycetes population in mature compost. Actinomycetes are well known as exoenzyme producers (Peczynska-Czoch and Mordarski, 1988). Moreover, mature composts produced soil smell due to the presence of actinomycetes (Hayashida et al., 1988). We did not show the data on compost smell in this paper but it is an important factor of compost quality.

Three techniques were used to investigate the microbial properties of GRC; EB and CFDA methods for total and viable bacteria respectively (Fig. 2), and substrate-specific plate count techniques for the bacteria that produce the specific degradative enzymes. In mature GRC, the population of total bacteria ranged from 2.2×10^{11} in GC to 6.5×10^{11} cells g^{-1} dry sample in RC and viable bacteria ranged from 5.0×10^{10} in GC to 1.9×10^{11} cells g^{-1} dry sample

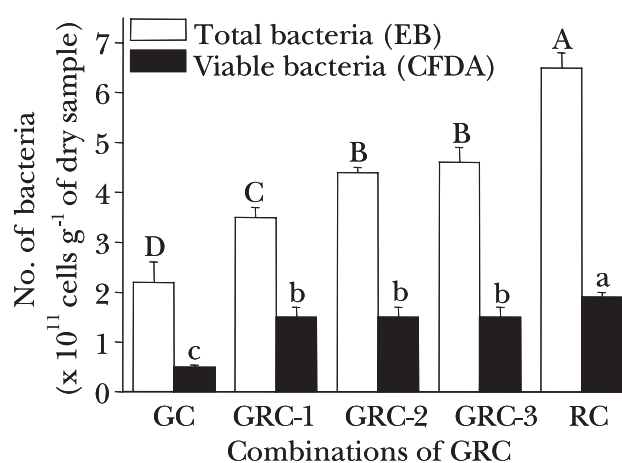


Fig. 3. The number of total bacteria (EB method) and viable bacteria (CFDA method) in green tea waste-rice bran compost (GRC). Error bars are the standard deviation of the means of three replications. Values on the column with the same letter are not significantly different at $P < 0.05$. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 2.

in RC (Fig. 3). Golueke (1977) reported that heat generated during composting due to the metabolic activity of microorganisms helps to enhance the composting process and destruction of pathogen and weed seeds. In our experiments, we found that rice bran increased the composting temperature when it was mixed with green tea waste (Fig. 1). Lukito et al. (1998) reported that the available P in soil considered as necessary to increase the microbial activity. RC contained comparatively higher amount of P than GC (Table 2). Thus, there is a possibility of the increase of microbial population with the increase of rice bran percentages in GRC (Fig. 3). No sooner had vitamins been discovered to be essential in the growth and reproduction of certain of the higher animals than similar claims were put forth in regard to their importance in the physiology of microorganisms. Substances of the nature of vitamin B have been claimed to be essential or markedly stimulating to the growth and reproduction of various microorganisms

Table 3. Number of organic matter-decomposing bacteria in green tea waste-rice bran compost (GRC).

Combination of GRC	No. of bacterial colonies ($\times 10^9$ CFU g^{-1} of dry sample)				
	Cellulolytic bacteria	Pectolytic bacteria	Lipolytic bacteria	Amilolytic bacteria	Actinomycetes
GC	2.6 c	2.9 c	5.6 d	6.6 d	3.9 d
GRC-1	2.2 c	2.9 c	12.0 c	25.0 c	11.0 c
GRC-2	2.5 c	2.9 c	13.0 b	21.0 d	11.0 c
GRC-3	6.0 b	21.0 b	36.0 a	52.0 b	41.0 b
RC	8.4 a	56.5 a	36.0 a	54.6 a	61.0 a

Values in the column with the same letter are not significantly different at $P < 0.05$. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 2.

(Werkman, 1927). Barber and De Barber (1980) reported that rice bran contains oil, protein, vitamins and some essential minerals as well as enzymes. Thus, rice bran increased the total and viable bacterial population and the composting temperature (Figs. 1 and 3).

Table 3 shows the number of the organic matter decomposing bacteria, i.e. the populations of the bacteria able to produce the degradative enzymes. The frequency of organic matter decomposing bacteria in mature compost was in the order of cellulolytic bacteria < pectolytic bacteria < lipolytic bacteria < amylolytic bacteria except RC. In RC, the frequency was in the order of cellulolytic bacteria < lipolytic bacteria < amylolytic bacteria < pectolytic bacteria. In this study, the Actinomycetes population was increased by the increase of rice bran percentages in GRC. The highest population (61.0×10^9 CFU g⁻¹ of dry sample) was obtained from RC and lowest (3.9×10^9 CFU g⁻¹ of dry sample) from GC (Table 3). The type and numbers of bacteria present in the compost, and their ability to produce specific enzymes, would be strongly influenced by the composting materials. Among the five combinations of GRC, the proportion of pectolytic bacteria was in the order RC > GRC-3 > GRC-2 = GRC-1 = GC. The presence of higher amount of rice bran in GRC-3 (70%) might be increased the population of pectolytic bacteria. Hankin et al. (1974) reported that the bacterial population in soil generally in the following order: cellulolytic bacteria < pectolytic bacteria < amylolytic bacteria < lipolytic bacteria < proteolytic bacteria. Our results were partially in accordance with these findings. Cellulose and pectin are more complex than starch and fat. It degrades step by step from complex form of carbohydrate to simple form (glucose) by the microorganisms. At the end of the composting (when temperature goes down), complex carbohydrate turns to comparatively simple form of carbohydrate. Thus, the population of amylolytic and lipolytic bacteria was higher than cellulolytic and pectolytic bacteria in mature compost.

4. Growth and quality of spinach

The growth pattern of spinach (var. Jiromaru) with GRC was evaluated at the organic farm of Saga University, Japan in the autumn of 2004 and 2005 (Fig. 4). The highest results in 2004: plant height = 28.2 ± 0.5 cm, leaf area = 544.9 ± 31.3 cm² plant⁻¹ and fresh weight = 27.5 ± 1.1 g plant⁻¹ were found from GRC-3. Significantly lowest growth was found from control treatments. Among the five types of GRC, RC provided the lowest plant height (19.8 ± 1.1 cm), leaf area (384.7 ± 5.3 cm² plant⁻¹) and fresh weight (18.0 ± 1.6 g plant⁻¹). In 2005, results showed that plant height was nearly same in GC, GRC-1, GRC-2, GRC-3 and RC

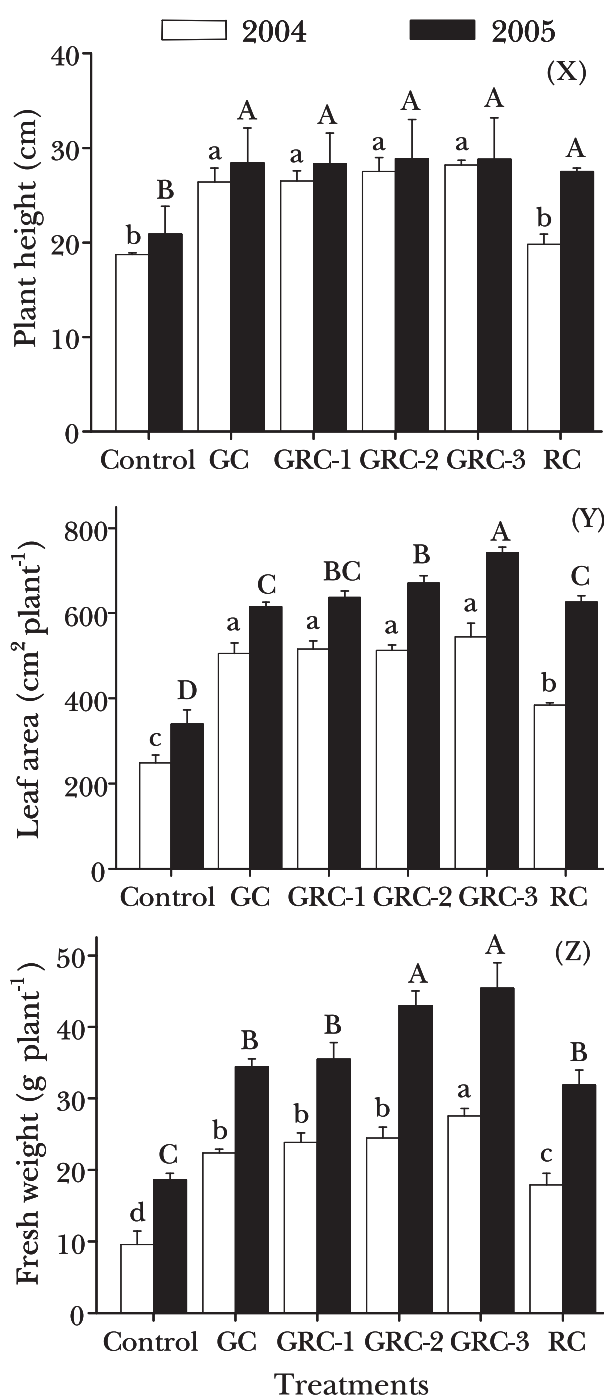


Fig. 4. Effects of the green tea waste-rice bran compost (GRC) on plant height (X), leaf area (Y) and fresh weight (Z) of spinach in 2004 and 2005. Error bars are the standard deviation of the means of three replications. Values on the column with the same letter are not significantly different at $P < 0.05$. In control used only soil. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 2.

plots ranging from 27.5 ± 0.4 cm to 28.9 ± 4.1 cm, but was significantly shorter (20.9 ± 2.9 cm) in the control plot (only soil). The largest leaf area was obtained from GRC-3 plot (741.2 ± 13.1 cm² plant⁻¹) and smallest in the control plot (339.2 ± 34.1 cm² plant⁻¹). There

was no significant difference ($p < 0.05$) among the GC, GRC-1 and RC treatment. GRC-1 and GRC-2 treatments were also provided significantly same results. The fresh weight was highest for the GRC-3 ($45.4 \pm 3.6 \text{ g plant}^{-1}$) and significantly similar results were obtained from GRC-2 ($43.0 \pm 2.1 \text{ g plant}^{-1}$). GC and GRC-1 provided significantly similar and second highest results $34.4 \pm 1.1 \text{ g plant}^{-1}$ and $35.5 \pm 2.4 \text{ g plant}^{-1}$, respectively. RC was provided $31.9 \pm 2.1 \text{ g plant}^{-1}$ fresh weight. Significantly lowest result was obtained from control plots ($18.6 \pm 0.9 \text{ g plant}^{-1}$). Presence of the lower amount of nitrogen, ORP and higher EC, growth

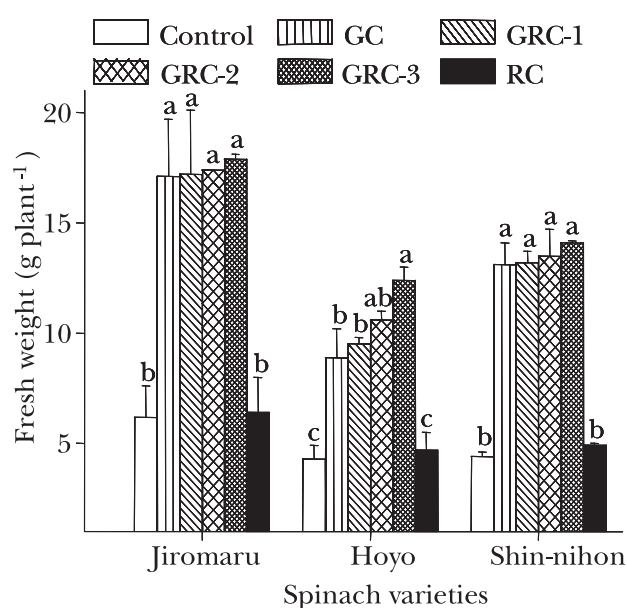


Fig. 5. Effects of green tea waste-rice bran compost (GRC) on the fresh weight of three spinach varieties under greenhouse conditions in the autumn of 2004. Error bars are the standard deviation of the means of three replications. Values on the column with the same letter are not significantly different at $P < 0.05$ and data was analyzed with individual variety basis. In control used only soil. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 2.

inhibitors (data not shown) in RC might be provided significantly lower growth of spinach (Table 2 and Fig. 4). The results of the preliminary experiment using soils of organic farm with chemical fertilizer in 2004 and 2005 provided plant height = $26.4 \pm 2.9 \text{ cm}$ and $26.6 \pm 3.6 \text{ cm}$; leaf area = $458.5 \pm 24.3 \text{ cm}^2 \text{ plant}^{-1}$ and $538.9 \pm 16.2 \text{ cm}^2 \text{ plant}^{-1}$; fresh weight = $21.8 \pm 1.1 \text{ g plant}^{-1}$ and $36.5 \pm 1.1 \text{ g plant}^{-1}$, respectively. The results of the spinach grown with chemical fertilizers were more or less similar to the results of GC. The growth differences between 2004 and 2005 were due to the weather and soil condition variation of the experimental site. In 2004, due to high rainfall during cultivation time and conducted experiment in the continuously cultivated area were the main reason for lower growth. In 2005, experiment was conducted in the area where no crops were grown within the year. Additionally, naturally grown weeds were mixed with soil several times within the year. Overall, the results of two years experiment showed that GRC-3 provided the significantly highest leaf area and fresh weight and there was no significantly different effect among the GC, GRC-1, GRC-2 and GRC-3 on plant height.

Three spinach varieties such as Jiromaru, Hoyo and Shin-nihon were used to test the effect of GRC on plant growth under green house conditions with the compare of non-treated control (only soil). Changes in fresh weight (g plant^{-1}) in each treatment are presented in Fig. 5. Overall, results showed that GC, GRC-1, GRC-2 and GRC-3 have the statistically same effect on the fresh weight of Jiromaru and Shin-nihon. In case of Hoyo, the largest fresh weight was obtained from GRC-3, and then followed by GRC-2, GRC-1, GC, RC and control. In an average, GRC-3 provided the highest result for all the three varieties used in this experiment and there was no significant difference between RC and control treatment.

Numerous investigations on the accumulation of nitrate and oxalate in plants have been reported that the plant species, cultivars, level and type of fertilization, light conditions, temperatures, humidity

Table 4. Concentration of nutrients in spinach (var. Jiromaru) grown on soil applied green tea waste-rice bran compost (GRC) at the rate of 2 kg m^{-2} .

Characteristics	Spinach leaf nutrient ($\text{mg } 100 \text{ g}^{-1}$ fresh weight) grown with GRC					
	Control	GC	GRC-1	GRC-2	GRC-3	RC
Nitrate ¹	88.0 c	146.0 a	135.3 a	130.7 ab	117.7 b	114.0 b
Oxalate ²	962.0 a	748.0 b	686.0 b	614.0 c	617.0 c	612.0 c
Iron	1.4 b	1.4 b	1.4 b	1.4 b	1.6 ab	1.7 a
Ascorbic acid	68.3	77.3	75.0	72.7	79.3	72.0
Glutamic acid	31.0 b	42.0 ab	45.3 a	44.0 a	45.7 a	43.3 a

¹Safety standard of health level of nitrate is $250 \text{ mg } 100 \text{ g}^{-1}$ fresh matter and ²safety standard of health level of oxalate is $894 \text{ mg } 100 \text{ g}^{-1}$ fresh matter. Values in the row with the same letter are not significantly different at $P < 0.05$. In control used only soil. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 2.

and structure of the soil, and even the application of plant protection agents affect the accumulation of nitrate and oxalate in plant (Takebe et al., 1995; Kabaskalis et al., 1995; Jaworska, 2005). The nitrate contents of the spinach grown on the compost ranged from 114.0 to 146.0 mg 100 g⁻¹ fresh weight (Table 4). In Poland, the regulation issued by the Ministry of Health permits the content of 250 mg nitrate 100 g⁻¹ fresh spinach (Jaworska, 2005). The amount of nitrate in spinach grown with GRC was below from the safety standard of health level. GRC released N in a slower rate than chemical fertilizers which leads to accumulate lower nitrate in spinach. Vogtmann et al. (1984) conducted pot and field trials with spinach, Swiss chard, head lettuce and corn salad that fertilized with composted farmyard manure—a slow release N-source that led to significantly lower nitrate concentrations in these vegetables compared to an N-equivalent NPK-application. They also reported that there is a very low risk of high nitrate concentrations in vegetables even with an overdose of composted farmyard manure (300 kg N ha⁻¹). Although, there are many reports on the accumulation of nitrate in spinach, thus compost could be used to decrease the spinach nitrate content. The oxalate contents of the spinach grown on the compost ranged from 612.0 to 748.0 mg 100 g⁻¹ fresh weight (Table 4). The limit values of total oxalate in the New Zealand spinach is 894 mg 100 g⁻¹ fresh matter (Yamanaka et al., 1983). Elia et al. (1998) reported that the amount of oxalate in spinach petioles grown with the unfertilized control treatment was higher than the N fertilized treatments and blades oxalates were not influenced by N treatments. We also found the highest amount of oxalate (962.0 mg 100 g⁻¹ fresh weight) from the unfertilized control treatment (Table 4). Further intensive study is necessary to find out the causes of the accumulation of oxalate in spinach. The largest amount of ascorbic acid (79.3 mg 100 g⁻¹) and glutamic acid (45.7 mg 100 g⁻¹) was obtained in the plants grown on GRC-3-treated plots, and the highest Fe content spinach (1.7 mg 100 g⁻¹) was obtained in the plants grown on RC-treated plot (Table 4). Though the taste of the vegetables depends on various factors, glutamic acid is one of the main factors. The average quantity of free glutamic acid in spinach is 39 mg 100 g⁻¹ (Bellisle, 1999). In our experiments, the amount of glutamic acid in spinach grown on GRC-treated plots ranged from 42.0 to 45.7 mg 100 g⁻¹ fresh matter.

Compost made from green tea waste and rice bran had an ability to serve as an alternative of chemical fertilizers for spinach production. Rice bran increased temperature, microbial biomass and minerals of compost when it was mixed with green tea waste. Rice bran also played an important role for optimizing the water content in green tea waste and created an aerobic environment during composting. But the high EC and low ORP in RC might suppress the spinach

growth when it was applied to the field. Moreover, lower amount of N in RC was not sufficient for spinach growth. GC was rich in N but did not provide the best growth performance. It may be due to the high weed incidence in GC treated plot (data not shown). Considering the physio-chemical properties of GRC, and the growth and quality of spinach, a mixture of the green tea waste and rice bran is better than either applied alone. Among the five combinations of GRC, GRC-3 provided the best results for quality spinach production. Further studies should be undertaken to generalize the effect of GRC for the other popular vegetables.

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