



## Evaluation of the Upland Weed Control Potentiality of Green Tea Waste – Rice Bran Compost and Its Effect on Spinach Growth

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**Abstract:** This study evaluated the upland weed control potentiality, germination inhibition ability and growth suppression efficiency of the five combinations of green tea waste – rice bran compost (GRC). GRC was prepared by mixing green tea waste and rice bran at five ratios, and allowed to decompose for 5 mo. Application of GRC suppressed weed growth up to 93.4% in number and 95.4% in dry weight in 2004, and 80.7% in number and 73.4% in dry weight of weeds in 2005, as compared with the untreated control (only soil) under the greenhouse condition. Among the five combinations of GRC, rice bran alone (RC) showed the significantly highest and green tea waste alone (GC) showed the lowest weed suppressing activity in both years. The weed control potentiality of GRC was increased by the increase of rice bran percentages in the mixture. The exudates of GRC inhibited the hypocotyl and radicle elongation of lettuce seedlings when examined by the sandwich method. The water extracts of GRC also inhibited the germination and radicle elongation of the test species in the seed germination tests. The growth inhibitory activity of RC was greater than that of GC, and radicle elongation was more sensitive than seed germination and hypocotyl elongation in all bioassays. The inhibitory activity of GRC water extract varied with the test species and was higher for the dicotyledonous species than monocotyledonous species. The inhibitory effect on seed germination and seedling growth increased as the extract concentration increased. The concentration dependent responses of test species to the water extract of GRC indicated that it might contain phytotoxic substances that were responsible for growth inhibition. Moreover, GRC promoted spinach growth significantly compared with the untreated control. These results suggest that the use of 30% green tea waste + 70% rice bran mixture compost (GRC-3) might be useful to control the upland weeds and enhance spinach growth among the five combinations of GRC, and it may reduce the use of hazardous synthetic agrochemicals. Optimization of the combination of composting materials is necessary for the multipurpose use compost.

**Key words:** Compost, sandwich method, seed germination test, selective inhibition, weeds control.

### INTRODUCTION

A significant portion of the agricultural land in developing countries in the tropics is heavily infested with weeds, and weed control is a big challenge for Asian farmers. Weeds in upland vegetables are usually controlled by cultural methods and herbicides. Although herbicides contribute greatly in alleviating weeding labour and stabilizing production, the increase use of herbicides raise serious environment and public health concern<sup>[1]</sup>. Because of these problems, the present trend in weed control is to reduce the dependency on herbicides by using natural products, which possess allelopathic potential<sup>[2]</sup>. In the past two decades, much work has been done on plant derived compounds as environmentally safe alternatives to

herbicides for weed control<sup>[3]</sup>. Weed growth suppression is one of the most important effects of mulches, and the use of plant derived mulches is an important method of weed control prior to the development of herbicides<sup>[4]</sup>.

Rice bran is one of the major by-products which accounts for 5-8% of the weight of rough rice and has weed control potential. Moreover, it is rich in minerals and microbes and helps to enhance composting process by increasing the compost pile temperature. Parts of the Japanese organic farmers are now using rice bran (200 g m<sup>-2</sup>) for weed control and fertilizer on transplanted rice<sup>[5]</sup>. On the other hand, green tea waste contains more than 90% organic matter and 5-7% nitrogen, which might be used as a good source of composting materials. Therefore, tea processors are now searching

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for the most cost-effective option for utilizing the tea waste, because dumping creates environmental problems and burning is expensive<sup>[6]</sup>.

Although many studies have been made separately on the effect of individual compost on weed control and crop production but to our knowledge, there are few reports comparing the effects of various combinations of composting materials on weed control and vegetable production in the same study. Additionally, there are some reports on seed germination tests for detecting the growth inhibiting potential of compost. However, to our knowledge, there are no reports on the use of sandwich method for detecting the growth inhibiting potential of the compost. Considering the above facts, this research was designed to evaluate the upland weed control potentiality of the five combinations of GRC and their effect on spinach growth under greenhouse condition, and also to evaluate the growth inhibiting potential of GRC by sandwich method and seed germination test.

## MATERIALS AND METHODS

The experiments were conducted in the Field Science Center, Saga University, Japan (33°18'20"N and 130° 20'12"E) from 2004 to 2005.

**Preparation of the five combinations of Green tea waste – rice bran compost (GRC):** Five combinations of GRC were prepared by mixing green tea waste and rice bran 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 5 months (composting started from March) in a glasshouse. Green tea waste and rice bran were mixed at the following five ratios (v/v): 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 to 60% at the start of composting. The composting buckets were turned upside down once a week. The mature compost was kept frozen (-20 °C) until use for the bioassay.

**Greenhouse experiment:** The upland weed control potentiality of GRC compared with the non-treated control (only soil) was tested in autumn of 2004 and 2005, under greenhouse conditions. The soil in this experiment was collected from the Organic Farm of Saga University, Japan where no agrochemicals were used in the last 10 years and contaminated with a large number of weed seeds naturally. The physio-chemical properties of the soil were: pH = 6.39, EC = 0.18 dS

cm<sup>-1</sup>, C = 3.67%, T-N = 0.39%, C/N ratio = 9.45, P = 0.01%, K = 0.02%, Ca = 0.1%, Mg = 0.01%, Fe = 0.0002% and Na = 0.0006%. The soil was air dried and sieved with 3 mm plastic sieve for the equal distribution of weed seeds. Composts were applied during the final bed preparation (7 day before sowing of seeds) at the rate of 15% (v/v) of soil. The bed size was 0.24 m<sup>2</sup>. Three spinach seeds (Jiromaru, Takii Seeds, Japan) per hill were sown with 10 cm row spacing and 8 cm hill spacing at the first week of October both in 2004 and 2005. After germination, they were thinned to one healthy plant per hill. Weed data such as the name, number and dry weight of weeds were collected 30 day after sowing of spinach seed. In order to find the effects of GRC on spinach growth, the plant height (cm), leaf area (cm<sup>2</sup> plant<sup>-1</sup>) and fresh weight (g plant<sup>-1</sup>) of fifteen plants from each treatment were measured at harvesting (45 days after sowing of seeds). No agrochemicals were used during experimentation. Only water was supplied when necessary. Experiment was conducted in a completely randomized block design with three replications.

**Test of the growth inhibiting potential of GRC by sandwich method:** Growth inhibiting potential of the five combinations of GRC was evaluated by the sandwich method<sup>[7]</sup>. Forty five mg of each dried samples (dried at 60 °C, over night with forced air) were added in between the two layers of 0.75% sterilized aqueous agar medium (Agar powder, gelling temperature 30 to 31 °C, Nacalai Tesque Inc., Kyoto, Japan) in each well (10 cm<sup>2</sup>) of the sandwich multidish plate. Control well contained only two layers of agar medium. Volume of each layer of agar medium was 5 ml. After gelatinizing the agar, five lettuce seeds (*Lactuca sativa* L. Great Lakes 366, Takii Seed Co. Ltd., Japan) were seeded on the surface of agar in the well of the multidish plate. The multidish plates were covered with plastic lid and sealed with Parafilm (Parafilm `M` Laboratory Film American National Can TM). Radicle and hypocotyl length of lettuce seedlings were calculated after incubation for 72 hr at 25 °C in complete darkness in comparison with the control. Each treatment was replicated five times in a completely randomized design.

**Test of the growth inhibiting potential of GRC by seed germination test:** Growth inhibiting potential of the five combinations of GRC was also evaluated by the seed germination test<sup>[8]</sup>. The specificity and activity of the phytotoxic compounds in GRC was evaluated through oat (*Avena strigosa*) var. Hei ootsu, Snow Brand Seed, Japan; radish (*Raphanus sativus* L.) var.

Tokinashi-daikon, Yae Seed, Japan; tomato (*Lycopersicon esculentum*) var. Oogata-fukuju, Takii Seed, Japan; lettuce (*Lactuca sativa*) var. Cisco, Takii Seed, Japan.

Hundred grams of dried compost (dried over night with forced air at 60 °C) and 1 L of distilled water were mixed and shaken for 12 hr at high speed (250 rpm) at <5 °C temperature after which the mixture was filtered with four layers of cheesecloth and two layers of filter paper (Advantec No. 5A). The filtrates were then used for the seed bioassay. Eleven seeds of each test species were imbibed and incubated for 72 hr at 25 °C temperature under completely dark conditions on a double sheet of filter paper (Advantec No. 2) wetted with 5 ml of either GRC water extract or distilled water (control) in a covered 9 cm petridish. The petridishes were sealed with Parafilm (Parafilm 'M' Laboratory Film American National Can TM). The percentage of seed germination and radicle elongation were calculated by reference to the control treated with distilled water. The experimental design was a completely randomized design with treatment replicated five times.

All combinations of GRC at the concentration of 1, 3, 10, 30 and 100 g L<sup>-1</sup> were also prepared by using distilled water to determine the effect of the concentration on the seed germination and radicle elongation of lettuce.

**Statistical analysis:** Data of this study were analyzed using SAS 6.12 version using ANOVA with Tukey's test (P < 0.05).

## RESULTS AND DISCUSSION

**Weed control potentiality of green tea waste – rice bran compost (GRC):** This study was conducted to determine whether GRC would have any effect on the suppression of weeds of upland vegetable. In 2004, application of GRC reduced weed number up to 93.4% and dry weight 95.4% as compared with the untreated control (only soil) under greenhouse condition (Table 1). Among the five combinations of GRC, only the green tea waste alone (GC) increased the weed dry weight (3.9% of control), though it reduced the weed number (35.4% of control). Rice bran alone (RC) showed the highest inhibition activity both in weed number and dry weight. In 2005, GRC reduced the weed number by 52.3% - 80.7% and dry weight of weeds by 25.2% - 73.4% as compared with the untreated control (Table 1). The results of these experiments clearly showed that GC has the lowest and RC has the highest weed control potentiality among the five combinations of GRC. In both years, the weed

control efficiency of GRC was increased by the increase of rice bran percentages in GRC. On the average, the weed controlling effect of GRC was in the order of RC > GRC-3 > GRC-2 > GRC-1 > GC. Seven weed species such as, *Lamium amplexicaule* L., *Capsella bursa-pastoris* Medius, *Galium spurium* L.,

*Spergula arvensis* L., *Stellaria media* Villars, *Amarenthus* sp. and *Poa annua* L. were observed both in 2004 and 2005. Among these species, *Lamium amplexicaule* L. was more sensitive to GRC than the other species. The population of *Lamium amplexicaule* L. was observed highest in 2004 and that of *Amarenthus* sp. was highest in 2005. The mature and stable composts applied to vegetable crops reduced the weed growth because of the high concentration of phytotoxic substances including organic acids, ammonia, ethylene oxide, and phenolic compounds<sup>[9]</sup>. But, the phytotoxicity of organic wastes depends on various factors; high electrical conductivity is one of them<sup>[8]</sup>. In our previous study, we found that the electrical conductivity (EC) of RC was higher (10.8 dS m<sup>-1</sup>) than that of GC (2.4 dS m<sup>-1</sup>) and it was increased by the increase of rice bran percentages in GRC<sup>[6]</sup>. The oxidation-reduction potential (ORP) was found very low in RC (0.7 mV) and high in GC (147.3 mV), and it was decreased by the increase of rice bran percentages in GRC<sup>[6]</sup>. So, possible toxic concentrations of salts (high value of EC) and lower ORP in RC might also be responsible for reducing the weed biomass.

**Evaluation of the growth inhibiting potential of GRC by sandwich method:** Figure 1 shows the inhibitory effect of GRC on hypocotyl and radicle elongation of lettuce seedling in the sandwich method. RC was more effective in suppressing the growth of lettuce than GC and hypocotyl elongation was less affected by GRC than radicle elongation. The minimum hypocotyl and radicle inhibition were obtained by applying GC, and maximum inhibition by RC. Increased the rice bran percentages in GRC increased the inhibition potentiality. The inhibitory effect of GRC on the hypocotyl and radicle elongation in the sandwich method, was in the order of RC > GRC-3 ≥ GRC-2 ≥ GRC-1 ≥ GC and RC ≥ GRC-3 ≥ GRC-2 > GRC-1 > GC, respectively (Fig. 1). The extract of GRC was more effect for the root growth than for the shoot growth in the same species. Such an outcome might be expected, because roots are the first to absorb the allelochemicals or toxic compounds from the environment<sup>[10]</sup>.

Table 1: Effects of green tea waste – rice bran compost (GRC) application on weed biomass under greenhouse condition in the autumn of 2004 and 2005

Treatments	Weed number (plant m <sup>-2</sup> )		Dry weight (g m <sup>-2</sup> )	
	2004	2005	2004	2005
Control	1392.3 a	1824.0 a	39.0 a	57.5 a
GC	899.7 b (-35.4)	869.3 b (-52.3)	40.5 a (+3.9)	43.0 b (-25.2)
GRC-1	772.9 bc (-44.5)	679.0 c (-62.8)	18.2 ab (-53.3)	32.3 c (-43.8)
GRC-2	775.8 bc (-44.3)	588.0 cd (-67.8)	17.1 ab (-56.2)	27.3 d (-52.5)
GRC-3	448.4 c (-67.8)	523.1 d (-71.3)	8.7 b (-77.8)	25.0 d (-56.5)
RC	91.4 d (-93.4)	351.5 e (-80.7)	1.8 b (-95.4)	15.3 e (-73.4)

Compost was applied at the rate of 15% (v v<sup>-1</sup>) of soil. Values in the column with the same letter are not significantly different at P < 0.05. Data in parentheses indicate percent increase (+) or decrease (-) over control. In control used only soil. 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

Table 2: Effects of green tea waste – rice bran compost (GRC) on growth of spinach (var. Jiromaru) under greenhouse condition in the autumn of 2004 and 2005

Treatments	Plant height (cm)		Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )		Fresh weight (g plant <sup>-1</sup> )	
	2004	2005	2004	2005	2004	2005
Control	9.1 c	12.5 b	42.2 c	45.2 b	1.8 d	2.1 b
GC	17.0 a	20.9 a	283.4 a	276.9 a	10.2 b	11.7 a
GRC-1	19.2 a	22.3 a	285.7 a	285.7 a	12.8 ab	13.1 a
GRC-2	18.4 a	21.9 a	293.4 a	293.4 a	13.7 ab	14.0 a
GRC-3	18.5 a	22.3 a	309.6 a	344.6 a	16.3 a	16.5 a
RC	13.4 b	16.5 b	138.3 b	153.3 b	5.6 c	6.2 b

Compost was applied at the rate of 15% (v v<sup>-1</sup>) of soil. Values in the column with the same letter are not significantly different at P < 0.05. For control, GC, GRC-1, GRC-2, GRC-3 and RC see Table 1.

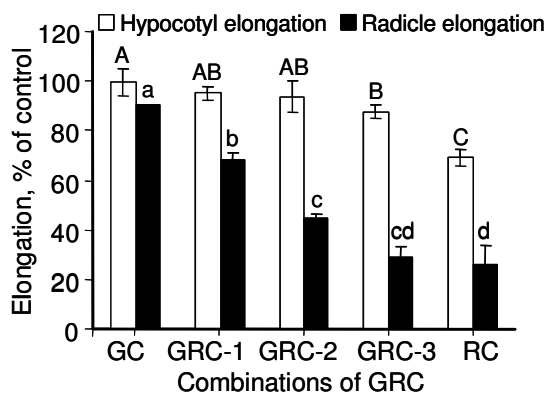


Fig. 1: Effects of green tea waste – rice bran compost (GRC) on lettuce hypocotyl and radicle elongation in sandwich method. Error bars represent  $\pm$  SD of the mean of five replications. Values on the column with the same letter are not significantly different at P < 0.05. Hypocotyl and radicle length of control was  $21.9 \pm 1.2$  mm and  $32.4 \pm 2.3$  mm, respectively. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 1

There are many reports on seed germination test for detecting the compost toxicity<sup>[8,11]</sup>. To our knowledge, however, there are no reports on the sandwich method

for detecting the growth inhibiting potential of the composted materials. Use of the sandwich method for detecting the allelochemicals in the exudates from GRC, is a new aspect of seed bioassay. The merit of using agar in the sandwich method is to transfer the water-soluble chemicals from the sample to medium by osmotic potential though in the seed germination test, water-soluble chemicals are directly in contact with the seeds and/or seedlings of the test species. The results of the sandwich method suggested that GRC might contain water-soluble phytotoxic compounds, which may be originated and/or, activated by the decomposition of plant residues.

**Evaluation of the level of inhibitory effect of GRC by the seed germination test:**

The level of inhibitory effect of GRC water extract on seed germination and radicle elongation of lettuce was tested. Several recent studies suggested that phytotoxicity tests using seed germination rate and root elongation are a valuable for ecotoxicology in higher terrestrial plants<sup>[12]</sup>. The water extract of GRC inhibited the seed germination and radicle elongation of lettuce seedlings. Among the extracts of five combinations of GRC, the extract of RC was most effective and that of GC at least (Fig. 2 and Fig. 3). To obtain the dose-response curves of the GC, GRC-1, GRC-2, GRC-3 and RC, we plotted the seed germination and radicle elongation (% of control)



against the logarithm of their concentrations. The concentrations required for 25% germination inhibition in the assay (defined as  $I_{25}$ ) were 48.0, 32.0, 19.0, 7.0 and 4.0  $g L^{-1}$  for the GC, GRC-1, GRC-2, GRC-3 and RC, respectively as interpolated from the dose-response curves (Fig. 2). Comparing  $I_{25}$  values, the germination inhibiting activity of the water extract of RC was 12.0 fold greater than that of GC. In the same way, the concentration required for 50% radicle elongation inhibition (defined as  $I_{50}$ ) in the assay were 57.0, 16.0, 11.0, 5.0 and 2.0  $g L^{-1}$  for the GC, GRC-1, GRC-2, GRC-3 and RC, respectively (Fig. 3). Comparing  $I_{50}$  values, the radicle elongation inhibitory activity of the water extract of RC was 28.5 fold greater than that of GC. The seed germination and radicle elongation were significantly reduced as the extract concentration increased. Such concentration dependent responses of the test plant to the water extract of GRC suggest that

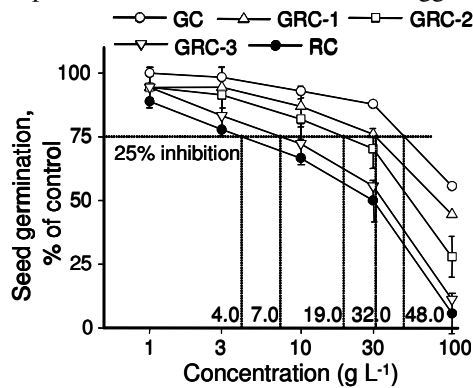


Fig. 2: Effects of the water extract of green tea waste – rice bran compost (GRC) on seed germination of lettuce. Error bars represent  $\pm$  SD of the mean of five replications are not shown when smaller than symbols. Germination rate of control was  $100 \pm 0.0$  %. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 1

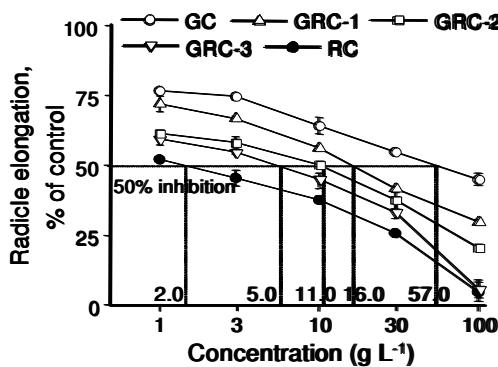


Fig. 3: Effects of the water extract of green tea waste – rice bran compost (GRC) on radicle elongation of lettuce. Error bars represent  $\pm$  SD of the mean of five replications are not shown when smaller than symbols. Radicle length of control was  $25.2 \pm 0.7$  mm. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 1

GRC might contain phytotoxic substances. The present observations partially agreed with that of Kato-Noguchi<sup>[10]</sup>, who found that increasing the concentration of n-hexane, acetone and water soluble extract of *Evolvulus alsinoides* increased the inhibitory activity for both seed germination and radicle elongation of lettuce. The aqueous extracts of allelopathic rice plant shoots decreased the root growth of weed species and the inhibition potentiality was increased with the increase in extract concentration<sup>[13]</sup>. Water extracts of GRC might contain phytotoxic substances which was responsible for the inhibition of seed germination and radicle elongation. Additionally, the growth inhibiting potential was depended on the nature of the composting materials and extract concentration.

#### Evaluation of the selective growth inhibitory activity of GRC:

To evaluate the species specificity of the growth inhibitory effect of GRC, we applied 100  $g L^{-1}$  water extract to oat, radish, tomato and lettuce seeds (Fig. 4 and Fig. 5). The results showed that there was

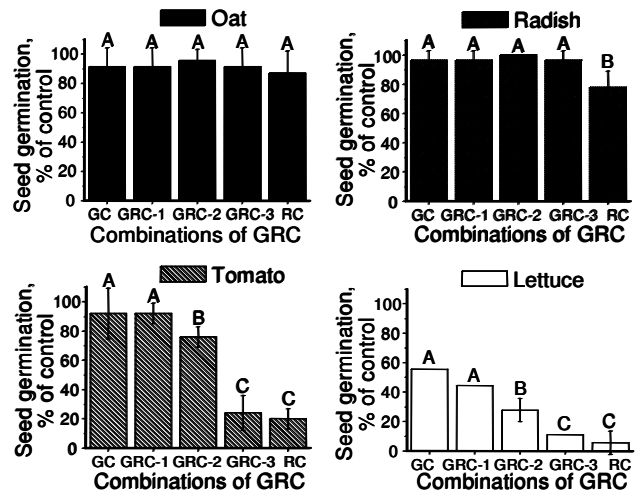


Fig. 4: Effects of the water extract of green tea waste – rice bran compost (GRC) on seed germination of oat , radish, tomato and lettuce. Error bars represent  $\pm$  SD of the mean of five replications. Values on the column with the same letter are not significantly different at  $P < 0.05$ . Seed germination of oat, radish, tomato and lettuce in control was  $85.2 \pm 6.4$  %,  $100 \pm 0$  %,  $92.6 \pm 6.4$  % and  $98.8 \pm 2.1$  %, respectively. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 1

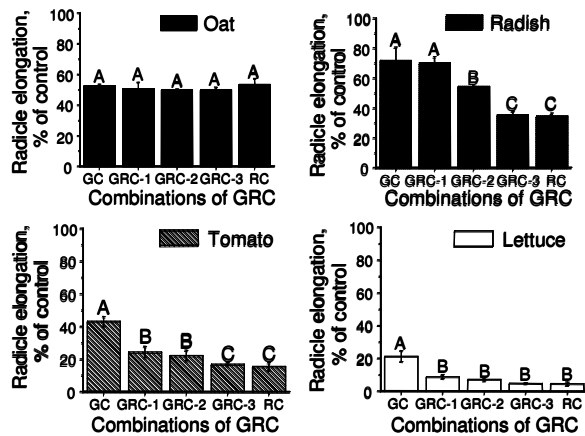


Fig. 5: Effects of the water extract of green tea waste – rice bran compost (GRC) a on radicle elongation of oat, radish, tomato and lettuce. Error bars represent  $\pm$  SD of the mean of five replications. Values on the column with the same letter are not significantly different at  $P < 0.05$ . Radicle elongation of oat, radish, tomato and lettuce in control was  $31.6 \pm 0.9$  mm,  $25.6 \pm 1.6$  mm,  $19.6 \pm 1.9$  mm and  $22.6 \pm 1.6$  mm, respectively. For GC, GRC-1, GRC-2, GRC-3 and RC see Table 1

no significant difference among the effects of the five combinations of GRC on the seed germination in oat but RC significantly inhibited the seed germination of radish, tomato and lettuce. Tomato and lettuce were more sensitive than radish (Fig. 4). There was no significant difference among the inhibitory effects of five combinations of GRC on the radicle elongation in oat, but the radicle elongation of radish, tomato and lettuce was significantly and differently inhibited by the water extract of five combinations of GRC. Lettuce was more sensitive than tomato and radish (Fig. 5).

Overall results of the present experiments indicate that the inhibition potentiality of GRC is species specific and were increased by the increase of rice bran percentages in GRC. It was shown that rice bran was effective for the dicotyledonous species (radish, tomato and lettuce) than monocotyledonous species (oat). There are some reports on the species specific growth inhibitory effect of compost. They reported that phytotoxicity of the compost depended both on the age of the composting materials and the plant species<sup>[8,10,11]</sup>.

**Spinach growth response to GRC:** As a whole, results showed that GC, GRC-1, GRC-2 and GRC-3 had the statistically same effect on the growth of spinach var. Jiromaru both in 2004 and 2005 under greenhouse conditions (Table 2). The highest values of growth were observed by applying GRC-3: plant height = 18.5 cm,

leaf area =  $309.6 \text{ cm}^2 \text{ plant}^{-1}$  and fresh weight =  $16.3 \text{ g plant}^{-1}$  in 2004, and plant height = 22.3 cm, leaf area =  $344.6 \text{ cm}^2 \text{ plant}^{-1}$  and fresh weight =  $16.5 \text{ g plant}^{-1}$  in 2005. Significantly lower growth values were observed in RC and control treatments. Previously, we found that a lower nitrogen content (4.35%), higher EC ( $10.8 \text{ dS m}^{-1}$ ) and lower ORP (0.7 mV) in RC were responsible for the inferior growth of spinach<sup>[6]</sup>. Moreover, a larger amount of growth inhibitors in RC (Fig. 1, Fig. 2 and Fig. 3) might provided the significantly lower growth of spinach. On the other hand, GC contained high nitrogen (7.55%) and low growth inhibitors (Fig. 1, Fig. 2 and Fig. 3) but did not provide the best growth performance of spinach. It may be due to the high weed incidence in GC treated bed (Table 1 and Table 2). Considering both the physio-chemical properties of the five combinations 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<sup>[6]</sup>. We also found that among the five combinations of GRC, GRC-3 provided the best results for quality spinach production<sup>[6]</sup>.

These studies suggest that optimization of the combination of composting materials is important for the multipurpose use of compost. Additionally, selection of the composting materials is also important for compost quality as well as plant growth. Compost made from the plant materials or their by-products like GRC, could successfully control the upland vegetable weeds and also enhance spinach growth. Among the five combinations of GRC, GC had the lowest weed control potential. RC was the most effective for controlling the upland weeds under greenhouse condition but it inhibited the growth of spinach when applied at a rate of 15% GRC of soil. Thus, considering both the growth of spinach and weed control potentiality, GRC-3 provided the best results among the five combinations of GRC in this experiment. Further research under large scale vegetables production is necessary to validate this technology.

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