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Evaluation and Comparison of Composting Rabbit Manure Mixed with Mushroom Residue and Rice Straw

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ABSTRACT

The purpose of this study was to look for carbon materials for composting with rabbit manure. Rabbit manure was composted with rice straw and mushroom residues. During the composting process, different related parameters were evaluated. Results showed that the rabbit manure could be normally co-composted with rice straw or with mushroom residues. The *GI* value showed that both composts could help seed growth. By comparing the effects of the two composts, the rabbit manure co-composted with rice straw had a much greater effect on improving seed growth. The results showed that co-composting rabbit manure with rice straw and mushroom residues was a much more efficient method to utilize the material. Rabbit manure mixed with rice straw has also been shown to compost much more efficiently than with mushroom residues.

Keywords: Composting, Livestock, Microbial Diversity, Mixture, Recycle.

INTRODUCTION

The livestock industry represents a large segment of the agricultural economics in China. The country produces an abundance of manure as a result, which must be managed. In China, the domestic rabbit breeding has developed so fast that more than 400 million domestic rabbits must be slaughtered each year. One rabbit can produce around 28.8 kg of manure in its entire lifespan. A total of more than 1.2 million tons of rabbit manure must therefore be managed each year (XU *et al*., 2005). The rabbit livestock production scale has become larger and larger in recent years and, as a result, production system develops towards intensification and much bigger production output. More and more rabbit manure will, therefore, need to be treated in the future. Rabbit manure contains a much larger amount of nutrients than many other animals' manure. One ton of rabbit manure contains 108.5 kg ammonium sulfate, 100.9 kg superphosphate (SSP), and 17.85 kg sulfuric acid potassium (Anon, 1998). Since the nutrient content and water content in rabbit manure are very high, if the manure were used directly, it would be unsustainable in the long-term and lead to pollution of soil (Hao and Chang, 2003), contamination of water (Chang and Entz, 1996) and air quality (Chang *et al*., 1998) as well as public perception issues. On the contrary, the rabbit manure could be converted to a source of valuable fertilizer and soil amendment if handled properly. Utilization of livestock wastes in any environmentally friendly way is very important.

Composting is a bio-oxidative process characterized by a succession of different microbial populations with a large variety of

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mesosphericthermo-tolerant and thermophilic aerobic microorganisms (Haruta *et al*., 2005; Mondini and Insam, 2003). Composting will benefit the environment, because manure nutrients are converted to more stable forms and will be less likely to reach groundwater or move into surface runoff (Larney and Hao, 2007). Compost can be stored until land application conditions are suitable or can be more economically transported to watersheds with low nutrient loadings (Abouelenien *et al*., 2009). However, there is limited research about rabbit manure compost. Most of the smaller farms in China do not have adequate capital to invest in composting equipment. Convenient, practical, and cost saving technology for composting should be therefore developed to manage rabbit manure.

Since rabbit manure contains larger amounts of nitrogen, it should be composted with other carbon sources to adjust the initial C/N ratio for normal composting processing (Hao *et al*., 2004). In rural China, there are plenty of wasted carbon sources like rice straw and mushroom residues. How to manage these carbon source wastes has become a very obvious and serious problem after the harvest of summer crops. The reason behind this is because the farmers usually burn these carbon source wastes after the crops are harvested. That will produce harmful effects on the environment as a side effect. The researches reviewed by Hubbe *et al*. (2010) demonstrated that plant-derived cellulose materials, especially cellulosic and lignin components in plant, play a critical role when organic wastes are composted to produce a beneficial amendment for topsoil.

The mushroom residues and rice straw have many differences in physical and chemical properties. For example, the mushroom residues are very exquisite, while the rice straws are empty and tough, and the moisture content and C/N ration between them are also different. The rice straw and mushroom residues could, therefore, represent two different types of carbon sources: plant straw and microbial digestion residues, respectively.

The objective of this research was to compare the composting of rabbit manure with mushroom residues and rice straw and to find a practical as well as feasible method to utilize the rabbit manure and other wasteful carbon sources in China's rural areas. Besides, during the experiments, the physicochemical properties, as well as the microbial diversity, were to be detected during the composting process. This research aimed at collecting fundamental data consisting of composting rabbit manure as well as reducing the damaging influence of rabbit manure on the environment.

MATERIALS AND METHODS

Composting Facility and Sampling

Rabbit manure was taken from Sichuan Grassland Research Institute, Rabbit Breeding Farm. Mushroom residues and rice straws were provided by farms near the research institute. The main physical and chemical properties of the starting materials are summarized in Table 1. Before the composting was conducted, the materials were smashed by a grinder and then sieved (35 mm mesh) to remove large bark pieces (Figure 1-A). To facilitate the objectives of this study, two groups of composting piles were set up. One group consisted of rabbit manure composted together with mushroom residues (RMM), while in the other group, rabbit manure was composted together with rice straw (RMS). There were three piles for each group. In each group, different amounts of carbon sources were mixed with rabbit manure to make the initial physicochemical properties of C/N ratio, moisture, and pH value roughly equal. The ratio of the different initial materials used is shown in Table 2. The external shapes and parameters of composting piles are shown in Figure 1-B. The mixed rabbit manure and its co-composting materials were located on nylon gauze (0.2 m above the ground) with 0.5

Table 1. Physical and chemical properties of composting materials.

Parameters [a]	Composting materials		
	Rabbit manure	Straw	Mushroom residues
Moisture (%)	64.86	11.43	29.3
pH value	8.95	8.29	8.32
Total carbon (C) (%)	44.83	48.86	41.52
Total nitrogen (N) (%)	1.79	0.70	0.87
Total phosphorus (P) (%)	0.59	0.02	0.05
Total potassium (K) (%)	0.67	2.04	0.39
C/N ratio	25.10	69.74	47.82
Organic matter content (%)	77.29	84.24	71.60

[a] Note: The percentage of total carbon (C), total nitrogen (N), total phosphorus (P), and total potassium (K) were calculated by their relative contents in 100 g dry samples.

Table 2. Different materials and rabbit manure treatments[a].

Items	RM: Straw (RMS)	RM: Mushroom residues (RMM)
Mixing ratio	7.4:1	2.6:1
C/N	31.45	30.59
Moisture (%)	62.26	57.33
pH	8.3	8.2

[a] Note: RM: Rabbit manure; RMS: Rabbit manure-Straw, RMM: Rabbit manure-Mushroom residues.

mm aperture to hold composting mixture and distribute air equally. The external shapes of the piles looked like a vertebral with 1m bottom diameter and 1 m high (Figure 1-B). The whole composting process was conducted indoors, and the mixtures were composted for 35 days.

Approximately 100 g of homogeneous compost was collected every 3 days from each pile. Each compost sample was a representative mixture from 3 different locations (about 0.3, 0.55 and 0.8 m from the bottom) at the center of the piles with three replicates in each pile. They were gently mixed for physicochemical properties and biological analysis. All samples were

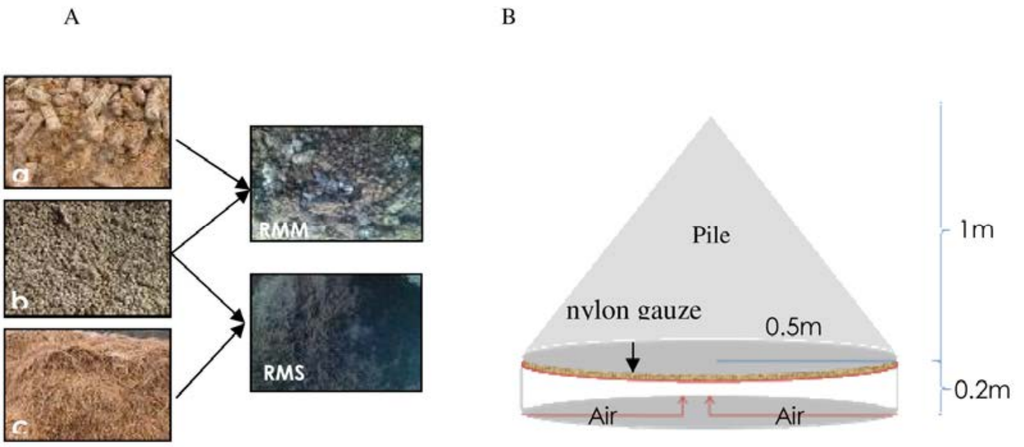

Figure 1. Materials used for composting. (A) The materials used for composting: Images (a): Represents mushroom residues; (b): Is rabbit manure and (c): Is rice straw. RMM represents the mixtures of rabbit manure and mushroom residues, RMS represent the mixtures of rabbit manure and rice straw. (B) Schematic of a composting pile: height and width of the pile are labeled on the image.

sieved (< 10 mm mesh) before storage. Each sample was divided into three parts. The first part of the sample was kept at 4°C for pH value and total bacterial number analysis; the second part was dried out and then stored at 4°C for physicochemical properties analysis. The last part was placed in 75% alcohol and then kept at -20°C for DNA isolation.

Physical and Chemical Properties Analysis

Temperature was recorded as the average temperature at different elevations (about 0.3, 0.55 and 0.8 m above the bottom) at the center of the piles. Moisture content was determined from weight loss upon oven drying at 105°C for 24 hours. The fresh samples were used to determine pH. The suspensions were shaken for 1 hour, centrifuged at 12,000 rpm for 20 minutes, and filtered through 0.45 mm membrane filters. The pH was determined using a PHS-25 meter. Compost samples were digested for total Kjeldahl nitrogen (TKN). Total phosphorus (TP) was measured using vanadium-molybdenum-yellow photometric method. Total potassium (TK) was measured using the flame photometer method. Total carbon (TC) concentrations were determined by oxidation to CO_2 with potassium dichromate according to the Walkley–Black method (Methods of Soil Analysis, 1996). Total organic content was calculated by multiplying the TC values by 1.76 (Nelson and Sommers, 1982).

Microbiological Analyses

Total Bacteria Count

The total number of bacteria was determined by plate count agar (HB0101, Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) according to the manufacturer's instructions.

DNA Isolation and PCR

Total bacteria genomic DNA was isolated by manure DNA extracted Kit (TIANDZ Co., Ltd, China), conducted according to the manufacturer's instructions. The DNA was confirmed by electrophoresis on a 1% gel and then diluted to the same concentration in preparation for PCR. The nested PCR procedure was used to obtain a highly specific PCR product. To amplify the bacteria 16S ribosomal V3-V5 fragment RNA genes, the universal primers 341F and 907R were used as described previously (Stach *et al*., 2001). The primers are as follows: 341F*:5'-CCT ACG GGA GGC AGC AG-3'*(17bp), 907R: *5'-CCG TCA ATT CCT TTR AGT TT-3'*(20bp). A GC clamp (5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G-3'(40bp)) was attached to the 5' end of the forward primer (341F) to improve the separation of the PCR fragments. The expected product size of the PCR was 626bp (contained the GC-clamp)(Muyzer *et al*., 1993; Stach *et al.,* 2001). The PCR reaction volume was 50 μL and was prepared with 1.5 μL of the template DNA (20 ug ml^{-1}), 1.5μL of each primer (10 pmol μL^{-1}), 25 μL PerfectShot ™Taq PCR MasterMix, 1.0 μL BSA V (10▯ mL^{-1}) and 19.5 μL ddH_2O. The reaction system was conducted with the following procedure: initial denaturation at 94°C for 4 minutes, 10 cycles of denaturation at 94°C for 30 seconds, annealing at 68°C for 30 seconds, and extension at 72°C for 45 seconds, 20 cycles denaturation at 94°C for 30 seconds, annealing at 68°C (decrease 1°C every 2 cycles) for 30 seconds, and extension at 72°C for 45 seconds, and 15 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 45 seconds, and final extension at 72°C for 15 minutes; and cooling at 4°C. The PCR products were confirmed by electrophoresis analysis on a 1% gel to show if the size of the PCR was equivalent to the expected product size.

DGGE (Denaturing Gradient Gel Electrophoresis)

DGGE analysis of the amplified bacterial 16S ribosomal RNA gene was performed on the D-Code universal mutation detection system (Bio-Rad), according to the manufacturer's instructions. An 8% polyacrylamide gel with a denaturing gradient of 30–75%. Electrophoresis was performed at 200 V for 3.5 hours at 60°C in 1×TAE buffer (40 mMTris, 40 mM acetic acid, 10 mM EDTA-2Na·2H2O). Samples with a volume of 100 µL including 50 uL 16S ribosomal RNA gene PCR products and 50 uL 2×Gel Loading Dye were added to each well in the gel. After staining the gels with silver nitrate staining methods established by van Orsouw *et al.* (1997), an image was taken with a digital camera (Bio-Rad, USA).

Microbial Diversity

Microbial diversity index was analyzed by Shannon-Weaver index using the following formula (Patil *et al*., 2010):

$$Dsh = -\sum P_i \ln P_i = -\sum \left({N_i}/{N}\right) \ln \left({N_i}/{N}\right)$$

Where, *Dsh* represents Shannon-Weaver index, *Pi*, is the frequency of the number *i* DGGE band, *Ni*, is the amplified products of the number *i* DGGE band and *N,* represents the total ampilfied PCR products of all microbal DNA(N= ∑Ni).

Evaluation of the Effects of Compost on Germination Index (GI)

Seed germination and root length test were carried out on water extracts by mechanically shaking the fresh samples for 2 hours at a solid: double distilled water ratio of 1:10 (w/v, dry weight basis). About 3.0 mL of each extract was transferred into a sterilized glass petri dishes lined with 3mm Whatman filter paper. Chinese cabbage seeds (*Lepidium sativum L.*) were evenly placed on the filter paper and incubated at 25°C in the dark for 48 hours. Samples were analyzed in triplicate from each composting mixture. Deionized water was used as a control for all experiments. Treatments were evaluated by counting the number of germinated seeds and measuring the length of the roots. The responses were calculated by *GI*, which was determined according to the following formula (Zucconi *et al*., 1981):

GI= (Seeds germination% of treatment×Root length of treatment)/(Seeds germination% of control×Root length of control)

Statistical Analyses

Data was subjected to analysis of variance (ANOVA) using the SAS software (USA). Statistical differences were observed at $P<0.05$ and written as mean±stand error.

RESULTS AND DISCUSSION

Physical and Chemical Properties Changing during Composting Processing

Rabbit manure contains excellent nutrients for garden soil. It consists of 2.5% nitrogen, 1.4% phosphoric acid, and 0.6% potassium (Table 1). Composting biowaste and the application of compost play an important role in sustainable agriculture. Composting allows organic waste to be recycled and returned to the soil as well as providing a solution for managing much of the waste materials. As the nitrogen content in rabbit manure is very high, it should be supplied with some carbon sources for normal composting. This experiment was designed in order to determine if the rice straw and mushroom residues could be used as a carbon source and composted together with rabbit manure. The main factors in controlling composting processes include environmental (temperature, moisture, pH, and aeration) and material parameters (C/N ratio,

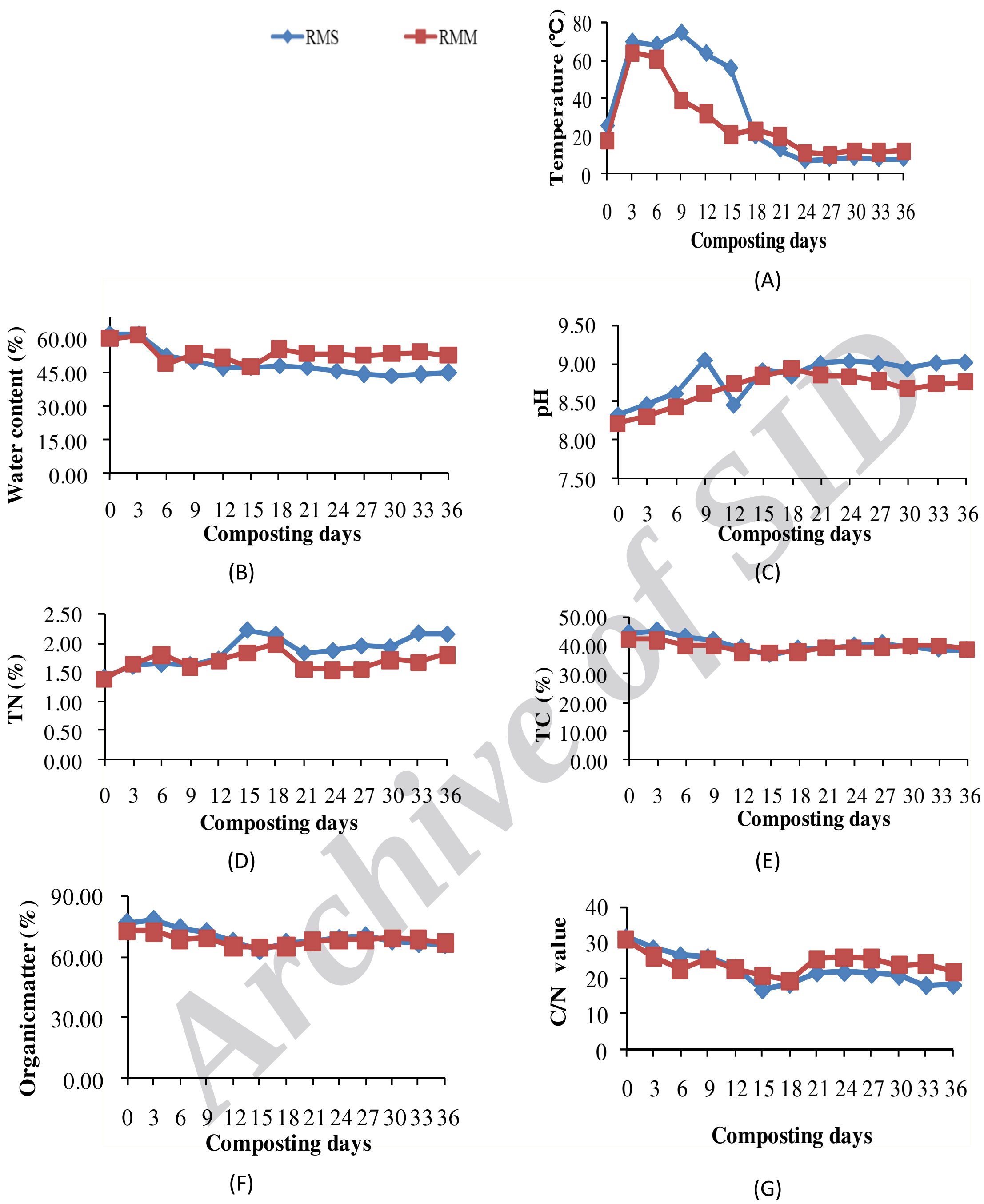

Figure 2. Physical and chemical properties change during composting process. RMS represents the rabbit manure co-composted with the straw group; RMM represents rabbit manure co-composted with the mushroom residues group. For each item and time point, *n*= 8. (A) Temperature changes during composting process in the two composting piles; (B) Water content changes; (C) pH changes; (D) Total nitrogen (TN) changes; (E) Total carbon (TC) changes; (F) Organic matter content changes; (G) Ratio of carbon and nitrogen (C/N); (H): Phosphorus content changes, (I) Potassium content changes.

particle size, nutrient content, and free air space)(Diaz *et al*., 2001). Figure 2-A shows that the mean temperature in the piles changed during the composting process. During the initial days of composting, the temperature in both of the two groups were sustained at a higher level and then decreased to below 20°C. Temperatures above 60°C were observed from day 3 to 15 in the RMS group and from day 3 to 6 in the RMM group. The rise and fall in temperature have been shown to be strongly correlated with the microbial activity (de Guardia *et al*., 2010; Tiquiaa *et al*., 1996). The optimum temperature range for composting was observed between 40-65°C, while 52-60°C is the most favorable temperature for decomposition (Liang *et al*., 2003). For most of the rabbit manure composting stages, the temperature was moderate for normal composting. During the composting process, a large quantity of water evaporated and, as a result, the moisture in the compost decreased gradually (Figure 2-B). The moisture content in the two mixtures sustained at a regular level during the composting process was not significantly different between the RMS and RMM groups. Result of this work is supported by Bernal *et al*. (2009), who found that the optimum water content for composting varied with the waste to be composted. The mixture should have 50-60% moisture content. The pH value is another important parameter that could influence compost's effects. A pH value of 6.7-9.0 supports good microbial activity during composting. Figure 2-C shows that the pH value in the RMS group changed from 8 to 9 during composting and varied from 7.5 to 8 in the RMM group. During the initial days of composting, the pH was sufficient for microbial growth, while in the lateral stages, the pH became harmful for microbial activities. Industry productions must therefore consider keeping the pH at a level sufficient for microbial growth.

Figures 2-D and -E show, that the content of TN and TC in the two composts changed dramatically during the early composting stages and then sustained regular levels at later stages. The changing trends of the TN and TC were similar in both the RMS and the RMM group. The TN content reached its maximal value after the temperature rapidly increased around 15 days. The utilization of TC had a similar trend between the two groups. Both had begun to decrease during the first few days of composting and then sustained a stable level for the remaining days of the entire 12 day time period. The changes of organic matter had a similar trend with TC, which indicated that the carbon sources were utilized at all times (Figure 2-F). It has been reported that the nutritional balance is mainly defined by the C/N ratio and the ideal range for the C/N value is 25-35(Bernal *et al.,* 2009; Hao *et al.,* 2004). In this study, the C/N ratio changed and experienced a decreasing trend during the first few days of the composting and then sustained a regular level for the remaining days in the entire 15 day time period. For most of the rabbit manure composting stages, the C/N value was sustained in the range 20-30, which also indicated good composting effects. A similar trend was also observed in the content of total phosphorus and potassium in the two studied groups. Phosphorus and potassium in the RMM group were lower than in the RMS group during the composting process, however (Figures 2-H and -I). The whole composting process is usually divided into three phases: Initial, High Temperature, and Final. The initial phase is characterized by the activity and growth of mesophilic organisms leading to a rapid increase in temperature. Thermophilic organisms then take over the degradation process, which inhibits the growth and activity of non-thermo-tolerant organisms. The final phase is a cooling and maturing period, which is characterized by the development of a new mesophilic community (Albrecht *et al*., 2009). The first three days of composting could be easily distinguished as the initial phase because the temperature had increased and the quantity of bacteria had decreased. The composting days from 3 to 15 were distinguished as the high temperature stage, because the temperature was sustained at high levels, indicating that the thermophilic organisms had taken over the degradation process. The final stage for rabbit manure composting began around 15 days when the temperature decreased and eventually remained at a stable level. Analysis of this data showed that rabbit manure could compost, as it normally would, with rice straw and mushroom residues.

The Microbial Diversity during Composting Process

Composting is mainly dependent on the activities of microorganisms. Compost processing could be greatly influenced by changes in the physical and chemical parameters as well as direct effects by the microbe. The microbial activity is, therefore, highly correlated with the composting process. In this research, the microbial quantity was estimated by calculating the number of colonies in each gel (Figure 3-A). A decreasing bacterial number rate was observed. From day 1 to 7 of composting, the bacteria number decreased quickly, while from days 7 to 14 they decreased slowly. After this time point, the bacteria number increased and reached their peak value on day 21 in RMS group and day 28 in RMM group. This suggested that there was a greater change in the total number of bacteria in the RMS group than in the RMM group (Figure 3-B). During the initial stages of the composting, the microbial quantity decreased rapidly, reflecting that some microbes could not adapt to the environmental changes in the composting piles and died.

Composting is a bio-oxidative process characterized by a succession of different microbial populations with a large variety of mesophilic, thermo-tolerant, and thermophilic aerobic microorganisms involved in the process (Haruta *et al.,* 2005; Mondini and Insam, 2003; Tang *et al*., 2004). In this study, the microorganisms' diversity was then analyzed by PCR-DGGE based on the differences of 16S ribosomal RNA genes at the molecular level. The PCR products were conducted utilizing electrophoresis on an SDS-PAGE gel. Results showed that the bands at different stages were different, suggesting that the diversity of the microorganisms had changed during the composting process. Similar results were observed by Tang *et al*. (2007), who found that the diversity of microbial communities increased with cattle manure co-composted with rice straw. The electrophoresis results were then generated to inclusion (Figures 3-C and -D). The diversity indexes (Dsh) and Similarity coefficient (*Cs*) value were calculated to show the microbial diversity during composting (Figure 3-E). The *Dsh* value shows the diversity index of the microorganisms in the two groups. The results showed that the diversity of the microbes increased during the initial days of composting until day 9 in the RMM group and day 12 in the RMS group. After these time points, the diversity of micro-organisms in RMS group decreased until day 21 and was then sustained at a stable level. For the two composting mixtures, there were some differences regarding the micro-organisms' diversity. Results of the *Cs* value showed that the diversity between the RMS and RMM group were very different during composting days 9 to 15, which supported the results analyzed by *Dsh*. It was interesting to note that the total number of bacteria decreased during composting, while the microbial diversity increased gradually, indicating more and more microbial adaptation to the new environment and their rapid reproduction to become the most populated species of micro-organisms.

Evaluation of the Manure Composts

Agricultural use of organic residues offers an attractive method for their safe disposal and a valuable source of organic amendments and nutrients. In this study, the rice straw and mushroom residues were used as carbon source. Both the rice straw and mushroom residues contain a large amount of organic matter (Table 1), which is beneficial for composting. Brunetti *et al.*

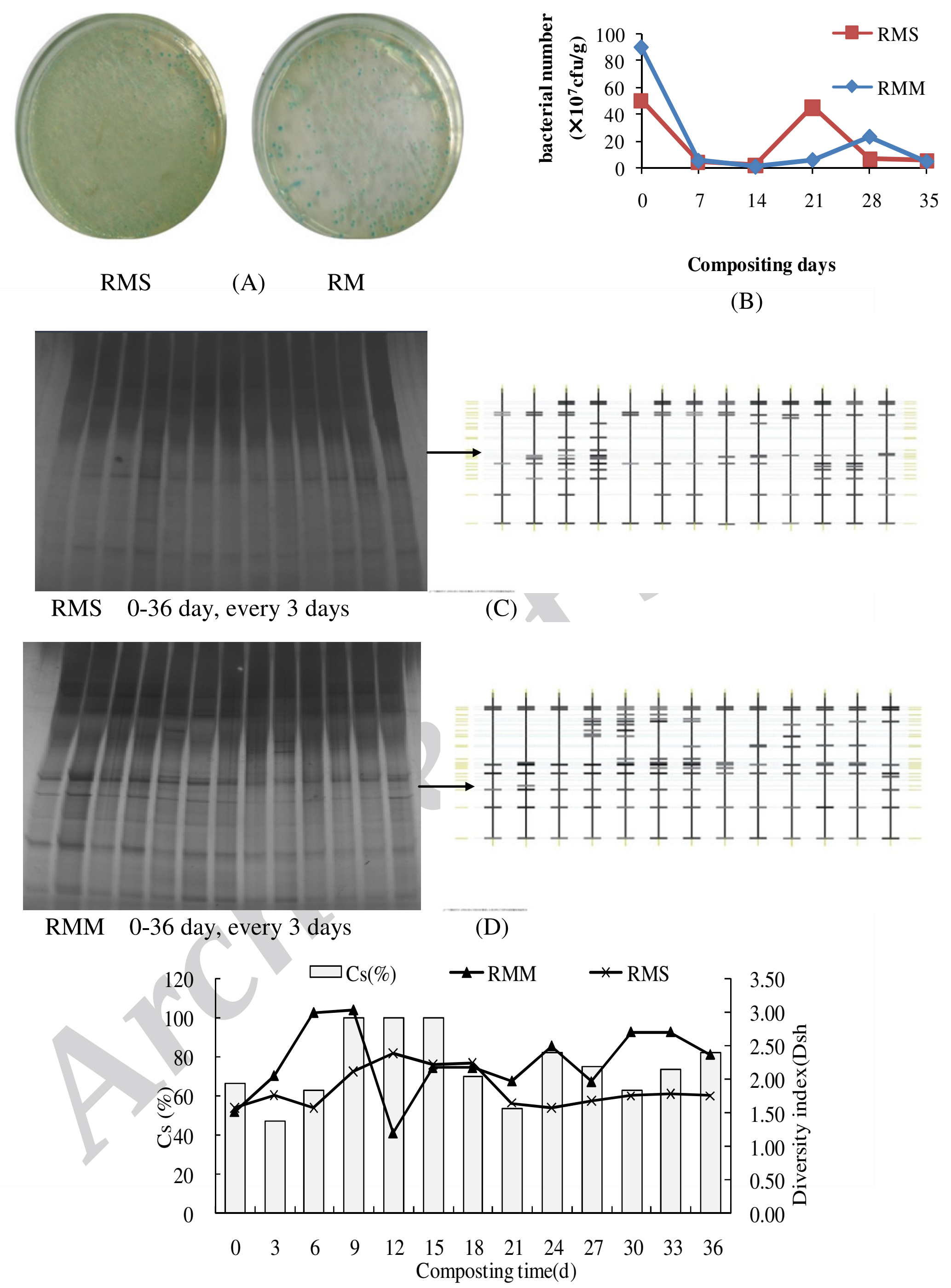

Figure 3. Microorganism changes during composting process: (A) Morphological differences observed with the microorganism in composting mixtures RMS and RMM; (B) Changes of the bacterial number during composting processing; (C) The DGGE electrophoresis results of RMS group; (D) The DGGE electrophoresis results of RMM group, (E) Diversity index (Dsh) and percentage of *Cs* changes in the two different composting mixtures during composting process.

(2009) studied the mushroom residues as compost materials and demonstrated that mushroom residues could serve as an effective compost material. Rice straw is also a potential food source for microorganisms like bacteria, fungi, and actinobacteria. It could be converted into a valuable end product in a short period of time through a microbial composting process (Hatamoto *et al*., 2008). Kausar *et al.* (2011) studied biodegradation of the rice straw, which has complex structures, and suggested that composting should be an effective way to utilize it. In the current study, the physicochemical properties of organic materials in the two groups were determined before and after composting (Table 3). It was found that the TN, TC and C/N value, as well as the total phosphorus and total potassium, had changed immensely through composting. This suggested that the composts obtained in this study could serve as nutrients source in organic farming. Therefore, the composts were further supplemented into media containing Chinese cabbage seeds. The *GI* value showed that the composts could help the seeds and roots growth, indicating that the content of the original materials had been changed through composting.

The rice straws and mushroom residues used for composting represent two different types of carbon sources. Table 3 shows that the parameters including TN, TC, and C/N value improved much more in RMS group than in RMM group. At the initial, mid, and final composting stages, the *GI* value of RMS group was larger than that of RMM group. It also shows that the compost effects developed into a more suitable environment for the growth of seeds during the composting process (Figure 4). Together, these results show that the rabbit manure could successfully co-compost with rice straw or mushroom residues. Rabbit manure

Table 3. Physical and chemical changes before and after the composting[a].

Items	RMM			RMS		
	Before composting	After composting	Changes (%)	Before composting	After composting	Changes (%)
Water (%)	60.33±0.14	52.83±0.13	-12.43	62.26 ±0.95	44.91±0.64	-27.87
TN (%)	1.38±0.02	1.80±0.06	+23.33	1.42±0.01	2.15±0.05	+51.41
TC (%)	42.29±0.34	38.99±0.51	-24.36	44.62±0.48	38.56±0.84	-13.58
C/N (%)	30.59	21.69	-29.09	31.45	17.94	-42.96
P (%)	0.33±0.00	0.41±0.01	+19.51	0.43±0.01	0.61±0.01	+41.86
K (%)	0.55±0.04	0.76±0.01	+38.18	1.13±0.01	1.65±0.01	+46.02

[a] Note: RMM: Represents the rabbit manure and mushroom residues group, RMS: Represents the rabbit manure and rice straw group.

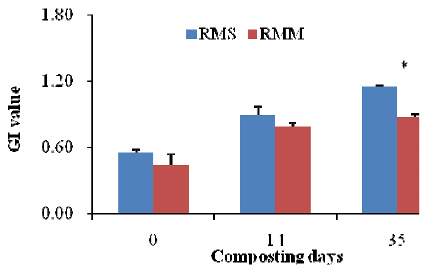

Figure 4. (GI) values in the RMS and RMM composting groups, *n*> 100 for each treatment. The label '*' on the bar means there is a significant difference between the RMM and RMS group (P< 0.05).

co-composting with rice straw produces better results than co-composting with the mushroom residues.

CONCLUSIONS

Composting not only allows organic waste of agricultural origin to be recycled and returned to the soil, but also provides a solution for managing it. In this study, the rabbit manure co-composting with rice straw and mushroom residues potentially were used to solve this problem. Throughout this study, the rabbit manure co-composting with either rice straw or the mushroom residues could process normally and increased the rate of seed growth. The composts from rabbit manure mixed with rice straw have been shown to be more efficient in helping seed growth than co-composting with mushroom residues.

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ارزیابی و مقایسه کمپوست کود خرگوشی مخلوط با باقیمانده های قارچ خوراکی و کاه برنج

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چکیده

هدف از این پژوهش یافتن مواد کربنی مناسب برای اختلاط با کود خرگوشی وتهیه کمپوست بود. به این منظور، کود خرگوشی با باقیمانده های قارچ خوراکی و با کاه برنج مخلوط و کمپوست سازی شد. در طی فرایند کمپوست سازی پارامتر های مختلف ارزیابی شدند. نتایج نشان داد که کود خرگوشی در اختلاط با باقیمانده های قارچ خوراکی یا کاه برنج قابل کمپوست شدن است. نیز، با توجه به نتایج آزمون درصد جوانه زنی و طول ریشه، هر دو نوع کمپوست مزبور در رشد بذر موثر بودند. با مقایسه نتایج این دو کمپوست می توان گفت که کود خرگوشی کمپوست شده با کاه برنج تاثیر بسیار بیشتری روی بهبود رشد بذر داشت. همچنین، بر پایه نتایج به دست آمده، اختلاط وکمپوست کردن کود خرگوشی با باقیمانده قارچ خوراکی و کاه برنج روش موثری در استفاده از این مواد است. این آزمایش نشان داد که کمپوست شدن کود خرگوشی مخلوط با کاه برنج کارآیی بسیار بیشتری از مخلوط با باقیمانده های قارچ خوراکی داشت.