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Production, Characterization and Phytotoxicity Evaluation of Compost Produced from Rice Straw and Goat Manure Slurry

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Abstract: Approximately 1.2 million ton of rice straw waste is generated annually in Malaysia. The rice straw is usually managed through open burning which leads to haze and environmental pollution. This study aims to recycle this waste through composting of rice straw with goat manure slurry and both the biomass and compost were characterized for selected physical, chemical and biological characteristics and phytotoxicity test. The compost was produced by mixing shredded rice straw with goat manure slurry, chicken feed and molasses. Compost temperature were recorded twice daily. The compost mixture before and after composting were analysed for physico-chemical properties. The surface morphological changes of compost mixture sample before composting and after composting were investigated using a Scanning Electron Mcroscope (SEM) and Energy Dispersion X-ray spectroscopy (EDX). Spread plate count method was carried out to quantify the viable bacterial count on the first day of composting and final composting day. A phytotoxicity test based on germination bioassay was carried out to evaluate whether the finished compost is safe to be applied for agricultural use. The final rice straw compost had no foul odour and low in heavy metals. The seed germination indices of phytotoxicity test were above 90% for the finished compost. The SEM image of the finished rice straw compost indicated uneven compost surface structure with small pores while EDX indicated that the compost had more minerals such as carbon, oxygen, potassium and magnesium. Composting rice straw with goat manure slurry can convert the agricultural wastes into value added organic fertilizer.

Key words: Composting, rice straw, goat manure slurry, phytotoxicity test, SEM-EDX, agricultural

INTRODUCTION

Rice is a monocotyledon plant, botanically known as Oryza (Lim et al., 2012). It is an important food staple for approximately half of the world population (Slayton and Timmer, 2008) thus, massive quantity of rice is being planted year-round. Most places in the South and South-East Asia Regions have large rice cultivation areas whereby the rice straw is generally being left on the field as farm residue after each planting cycle (Gadde et al., 2009). In Malaysia, the average national paddy (Oryza sativa) production is approximately 3.66 metric tonnes per hectare and approximately 1.2 million ton of rice straw is generated annually. A study by Lim et al. (2012) defined rice straw as left over rice stalk on the field upon harvesting of the rice grain. Only little farmers used it for other activities such as animal feed, mulching material and as medium to grow mushroom. Nevertheless, it is usually being burnt in the rice field to get rid this waste (Gadde et al., 2009). However, this unsustainable practice leads to severe environment problems particularly haze and air pollution.

Numerous studies had been done in order to search for alternative ways to manage these residues wisely. According to, Silalertruksa and Geewala utilization of rice straw increased the job opportunities, improved rural economy and at the same time, reduced air pollution due to burning of rice straw. One of the sustainable ways to handle these agricultural wastes is recycling agricultural wastes through composting. According to, Das et al. (2011) composting is defined as mineralization and humification of organic matter to produce a stable end product that is phytotoxicity free and pathogens free. According to, Raj and Antil (2012) direct application of organic wastes without composting exert a high risk to the environment and human health due to presence of pathogens and weeds. Composting of several agricultural residues might able to reduce excessive chemical fertilizers application by farmers. Furthermore, composts basically could improve soil fertility by improving the physico-chemical and biological properties. Palanivell et al. (2013) indicated that compost can serve as slow-released fertilizers during mineralization compared to mineral fertilizers like urea, muriate of potash and triple

superphosphate which are known for being highly soluble upon soil application. Recycling of these agricultural wastes through composting can serve as an alternative to conventional fertilizer to increase the crop production.

A study by Bhattacharyya et al. (2012) concluded that combination of rice straw with manure or organic materials will produce a good fertilizer. Composting of rice straw with goat manure slurry would benefit countries such as Malaysia in terms of agricultural wastes management where both dairy manure and rice straw are produced in large quantity annually. Goat manure slurry has lower C-N ratio (C/N) whereas rice straw has the opposite property. Therefore, by mixing these 2 materials it can provide a balanced nutrient for microorganisms to carry out the composting process (Xiujin et al., 2008). Therefore, the aim of this study was to produce an organic fertilizer through composting of rice straw with goat manure slurry and both the biomass and finished compost were characterized for selected physical, chemical and biological characteristics and phytotoxicity test.

MATERIALS AND METHODS

Biomass collection, processing and characterization:

The rice straw was obtained from a paddy field at Pasir Puteh, Kelantan, Malaysia. The samples were bulked, air dried and shredded. Sampling of goat manure was done in a dairy farm located in Kemahang, Kelantan, Malaysia. Prior to composting, selected proximate and ultimate analysis of the shredded rice straw, goat manure, chicken feed and molasses were carried out. According to, Peech (1965) the rice straw was analyzed for pH and EC in a 1:5 solution of compost and water. Combustion method described by Chefetz et al. (1996) was used to determine the total organic matter and total C. Micro-Kjeldahl method was used to determine the total N (Bremner and Lees, 1949) whilst total P was extracted using the method described by Tan (2005) and the P concentration was determined using the blue method (Murphy and Riley, 1962). Afterwards, C/N and C/P ratios were calculated. Total K, Ca, Mg, Zn, Cu, Fe and Mn were also determined. The leachate collected from the leaching process was used to determine the Cation Exchange Capacity (CEC) of the rice straw (Schollenberger and Simon, 1945). The method described by Keeney and Nelson (1982) was used to determine the ash content, ammonium (NH₄-N) and Nitrate (NO₃-N) contents in rice straw. Goat manure, chicken feed and molasses were analyzed for pH, total OM, total C, total N, total P, C/N and C/P ratio, total cations (K, Ca, Mg, Zn, Cu, Fe and Mn) and ash content, using the aforementioned methods. All analyses were done in triplicate.

The composting of the rice straw with goat manure was carried out at the open space of research area of Universiti Malaysia Kelantan Jeli Campus, Malaysia. Three composting bins with 425 (diameter)×435 mm (height) were used for composting. Twelve holes with the holes size of 0.5 cm diameter each were made on the sides of the bins to enable good aeration during composting. The compost was produced by mixing 80% of shredded rice straw +5% of chicken feed +10% of goat manure slurry +5% of molasses in composting bin. This formulation ratio was based on the previous study by Ch'ng et al. (2013) with the justification that rice straw has high C/N ratio and the process to decompose by itself is very slow. Therefore, by composting rice straw with goat manure slurry which have a lower C/N ratio and also, serves as source of microorganisms, rapid decomposition of the rice straw can be achieved. In this study, 400 g of goat manure were dissolved in 3.0 L of water and filtered to produce goat manure slurry. The rice straw used in this study served as a substrate mean while the goat manure slurry was used as a source of nutrients, moisture and microbes for the composting process. The chicken feed was added during composting to provide energy for the microbes. Besides, molasses was also added as a source of carbohydrate for the microbes. During mixing of rice straw and goat manure slurry the chicken feed and molasses were added slowly in order to obtain a uniform mixture. The composting material was turned when necessary for aeration and water was sprinkled when required to maintain the moisture content of 60% (Das et al., 2011). The composting process was carried out in 3 replications to verify repeatability in minimizing error and they were completed within 60 days. The ambient and compost temperatures were recorded daily at 8 am and 5 pm using a digital thermometer with accuracy of ± 0.5 .

The compost mixture before composting and after composting were characterized for pH and Electrical Conductivity (EC) total organic matter, total P, total cations (K, Ca, Mg, Na, Zn, Cu and Fe) ash content, total C and N contents, CEC and C/N and C/P ratio. Humic acid of the compost mixture before composting and after composting was isolated using the standard procedures described by Stevenson (1994) and Ahmed et al. (2004). Changes in the texture, colour, odour and size of the compost were recorded via. physical observation. The surface morphological changes of compost mixture sample before composting and after composting were investigated using a Scanning Electron Microscope (SEM) (FEI Quanta FEG 650; ThemoScientific, USA) equipped with an Energy Dispersion X-ray spectroscopy (EDX). All analyses were done in triplicate.

Spread plate count method was performed in order to quantify the viable bacterial count during the first and final day of composting day (Brock and Madigan, 1991). First, one gf compost was weighed and was placed into 9 mL sterile distilled water tube. Next, serial dilution was carried out. The solution was shaken for 15 min. After that a sterile cheese cloth was used to filter the solution. Then, 1 mL of the solution was pipetted into another tube containing 9 mL of sterile distilled water to produce a 1:100 dilution factor. Similar procedure was repeated to produce 10⁻³-10⁻⁷ dilution factors. Next, 0.1 mL of solution from each tube was pipetted into Nutrient Agar and spread by hockey stick. Samples were then incubated at 28°C for 48 h. Bacterial colony was counted by using colony counter under optical microscope (×40). The value of CFU was calculated using the equation as follows:

$$\begin{array}{l} \text{CFU} \\ \text{/mL} \end{array} = \left(\begin{array}{l} \text{Number of colony counted/amount} \\ \text{of solution spread on plate} \end{array} \right) / \text{ Dilution factor}$$

Compost phytotoxicity test: A germination bioassay method was carried out to assess the compost phytotoxicity (Zucconi et al., 1981). A 10 g of compost was weighed and mixed with 100 mL of distilled water. The sample was shaken for 24 h. Then, the samples were centrifuged at 10,000 G for 20 min and the supernatants were filtered through Whatman No. 42 filter study. The extract was diluted 5 times and another one with distilled water only served as control. Ten mung beans seeds (Vigna radiata L.) were placed in 9 cm diameter petri dishes filled with a filter study then 5 mL of extract was pipetted on each petri dish. Petri dishes with 5 mL distilled water only served as a control. Parafilm was used to seal each petri dish. This was to prevent water loss while allowing air penetration. The petri dishes were placed in a dark area for seeds germination. Each replicate was made up of ten seeds. Results were reported as means of the 10 seeds. Seed germination and measurement of the length of root and shoot were done after 72 h for all the extracts and the control. The Germination Index (GI) was obtained by multiplying Germination (G) and Relative Root Growth (RRG) both expressed as percentage (%) of the control values. The equation is as:

Germination index = $(G\% \times RRG\%) \times 100$

Where:

G% = Number of seeds germinated in a sample/number of seeds germinated in the control

×100; RRG% = Mean root length in a sample/mean root length in the control×100

Meanwhile, for Vigour Index, the formula is as follows:

$$Vigour\ index = Ger\,min\,ation\%\times\left(\begin{matrix} mean\,root\,lenght+\\ mean\,shoot\,length\end{matrix}\right)$$

Statistical analysis: All the experiments were carried out in triplicate and the results were expressed in average values. The data for phytotoxicity test was analysed using SPSS (SPSS Inc., Version 24.0). One-way Analysis of Variance (ANOVA) was used to detect the significant difference while Tukey's HSD test was used to compare the means of seed germination indices for the composts at $p \le 0.05$.

RESULTS AND DISCUSSION

Selected proximate and ultimate analysis of the raw materials used in composting: The selected proximate and ultimate analysis outcomes of raw materials used in composting were presented in Table 1. The pH of rice straw was 7.9 while the pH of goat manure was 6.5. The values were within the range reported by Jusoh et al. (2013). This indicates the pH of rice straw and goat manure were near neutral. In Table 1, the K, Ca and Mg in the rice straw were high in order Mg>Ca>K with values of 2,480, 1,797 and 1,727.6 mg/kg, respectively. The rice straw had low concentrations of Zn (34.1 mg/kg) Cu (2.7 mg/kg) and Fe (220.1 mg/kg) compared to finished compost (Table 2). These values were in the standard range reported by wood end research Laboratory (2005). The goat manure was high in K, Ca and Mg with values of 7,646.33, 85,103.3 and 10,505 mg/kg, respectively (Table 1). Lower C/N ratio was observed in chicken feed (5.8) compared to shredded rice straw (28.2). However, chicken feed had the same N concentration with shredded rice straw which was 1.1%. Besides, chicken feed also contained high nutrient composition such as total P, K, Ca and Mg. The molasses had higher N concentration (1.2%).

Composting and temperature profile: Temperature is one of the parameters that affect the composting process due to its essential role in selective various microorganism that degrade organic compound and promote compost maturation (Xiao et al., 2009). The compost temperature profile of this study was in Fig. 1. The ambient temperature throughout this study was between 22-35°C throughout the composting period. The 3 typical composting phases were observed during the composting process of rice straw compost. The initial temperature of the rice straw compost was at mesophilic stage which was

Table 1: Selected proximate and ultimate analysis of raw materials used in composting

·	Chicken feed Goat manure Mean (± SE)			
Properties			Molasses	Rice straw
Proximate analysis				
pH	7.1	6.5	7.5	7.9
Ash content (%)	nd	15.4	nd	3.8
Moisture Content (%)	nd	nd	nd	14.1
Total organic matter (%)	1.2	44.6	10.6	55.4
Ultimate (elemental) analysis				
Total C (%)	6.5	25.88	6.1	32.1
Total N (%)	1.1	2.9	1.2	1.1
Total P (mg/kg)	24.8	246.3	16.8	312
C/N ratio	5.9	7.9	4.6	28.2
C/P ratio	2,620.9	1,050.7	3,630.9	1,028.8
CEC (cmol _c /kg)	nd	nd	nd	9.8
Total K (mg/kg)	14.1	7,646.3	12.1	1,727.6
Total Ca (mg/kg)	215.6	85,103.3	177.6	1797
Total Mg (mg/kg)	2,570	10,505	148	2,480
Total Zn (mg/kg)	Trace	927.5	Trace	34.1
Total Cu (mg/kg)	Trace	22.4	Trace	2.7
Total Fe (mg/kg)	Trace	682.5	Trace	220.1
Total Mn (mg/kg)	Trace	201.1	Trace	28.3
NH ₄ N (mg/kg)	Trace	Trace	Trace	48.6
NO ₃ N (mg/kg)	Trace	Trace	Trace	23.2

nd = not determined; C/N = Carbon to Nitrogen ratio; CEC = Cation Exchange Capacity. Values in bracket represent standard error of the mean

Table 2: Selected chemical properties of the rice straw compost before and after composting

Property	Before composting	After composting	
pH	7.34	7.55	
Electrical conductivity (dS/m)	1.47	1.53	
Total organic matter (%)	81.08	73.53	
Total C (%)	47.03	42.63	
Total N (%)	1.67	2.14	
Total P (%)	0.21	0.34	
C/N ratio	28.16	19.92	
C/P ratio	223.95	125.38	
Total K (%)	5.96	8.71	
Total Na (%)	0.44	0.55	
Total Ca (%)	0.26	0.34	
Total Mg (%)	13.80	10.6	
Total Zn (μg/g)	54.00	54.2	
Total Cu (μg/g)	12.60	8.00	
Total Fe (μg/g)	623.2	1,362.4	
Humic acid (%)	5.7	6.50	
Ash content (%)	11.8	12.81	
NH ₄ -N (mg/kg)	49.5	37.5	
NO ₃ -N (mg/kg)	21.4	24.5	
Bacterial count (CFU/mL)	1.86×10^{8}	1.62×10^{7}	

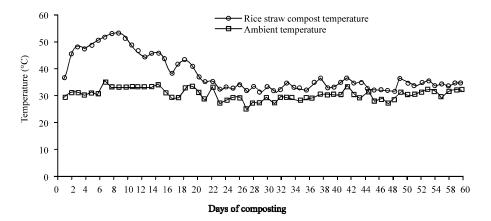


Fig. 1: Compost temperature profile

36°C on the first day of composting. During the initial stage, the compost was predominated by mesophilic bacteria at this stage where there were abundance of sugars, amino acid and protein. Mesophilic bacteria and fungi started to degrade the sugars, amino acid and protein during the mesophilic stage (Keener et al., 2000). The compost temperature remained at the mesophilic stage in the morning of the sec day of composting. The temperature was increased to thermophilic stage (45°C) in the evening (5.30 pm) on the second day. Once the temperature exceeds 40°C the high temperature condition did not favour mesophilic bacteria and the mesophilic bacteria became less competitive, hence, thermophilic bacteria took place. Alexander (1977) stated that the increased in temperature was due to high rate of organic matter decomposition. Keener et al. (2000) also indicated that thermophilic microorganisms degraded fats, cellulose, hemicellulose and some lignin along with the maximum degradation of the organic matter and destruction of pathogens during thermophilic phase. The thermophilic phase was maintained between 40.1-52.8°C from day 3 until day 16 of the composting process. The maximum temperature achieved was 52.8°C (day 7). Lu et al. (2015) concluded that temperature greater than 40°C was capable to kill pathogenic microorganism including pathogens and some bacteria in the compost. After that, the compost temperature dropped to 37.8°C on day 17 and raised again to thermophilic temperature on day 18 until day 20 (Fig. 1). Turning were done on day 17, 41 and 51 to obtain uniform composting process. The first turning was done on day 17. The temperature increased back to thermophilic phase (41.3°C) on day 18. Turning of the compost helped to improve the aeration for an aerobic microorganism. The compost thermophilic temperature in this study was compatible to the previous study by Ch'ng et al. (2014) which used sago bagasse as bulking agent.

The temperature decreased gradually to below 40°C after day 20 (Fig. 1). This indicates that the composting had entered curing phase. During this phase, the food sources available to thermophilic organisms started to deplete. The decrease in the compost's temperature indicated that the microbial activity was slowed down due to a decrease in oxygen, moisture and easily decomposable organic matter (Keener et al., 2000). A temperature range between 27.5-36.3°C was maintained from day 21 until day 60. During the completion of composting process, the average temperature of the finished compost product was 34.2°C. This was relatively near to the ambient temperature of 35°C. On day 31, fungus started to grow on the rice straw compost. A domination of fungus on the compost indicates the decline of the bacteria population and this was proven by the decreased in bacterial count after the composting

Table 3: Bacteria colony counts of the rice straw compost before and after composting

Sample	CFU/mL of sample
First day mixed compost (10 ⁻⁵ dilution)	1.86×10 ⁸
Final matured compost (10 ⁴ dilution)	1.62×10^7

CFU/mL = (Number of colony counted/Amount of spread on plate, mL)/Dilution factor

process (Table 3). The fungus observed in this study belongs to *Corprinus* sp. due to characteristics shaggy ink cap with white gills beneath the cap which later turned black and secret black liquid filled with spores which was similar to that discovered by Ch'ng *et al.* (2013).

Selected physiological and biochemical changes during composting: The finished compost in this study appeared to be dark in colour, soft, coarse with friable texture and it had an earthy smell while the rice straw was initially non-hollow yellowish-brown in colour, rough in texture. The humic acid and ash contents at day 60 of composting increased from the 5.7-6.5 and 11.8-12.8%, respectively. Nitrogen and P concentrations increased after the composting but C content decreased after composting (Table 2). The C/N ratio decreased from 28.16-19.92. The C/N ratio is a good indicator to determine the degree of compost maturity (Kausar et al., 2010; Harshitha et al., 2016). Goyal et al. (2005) stated that a compost having a C/N ratio below 20 indicates that the compost is matured. In this study, the final C/N ratio was 19.92 (Table 2) and this indicates that the rice straw compost in this study had reached maturity. The NH4-N content of the finished compost decreased from 49.5-37.5 mg/kg whereas NO₃-N content increased from 21.4-24.5 mg/kg. This was probably due to part of the NH₄ were mineralized to NO₃. This may have partly increased the compost pH via. ammonia evolution (Trautmann and Krasney, 1997).

The pH of the rice straw compost increased from 7.34 during the first day of composting to 7.54 at the end of composting (Table 2). The increased of pH at the end of composting was due to decomposition of protein to ammonium (Hu et al., 2009). The salinity level in the compost is indicated as EC which was used to reflect the risk of causing phytoxicity on the plant growth (Hou et al., 2017). The initial EC of rice straw compost was 1.47 dS/m and it increased to 1.53 dS/m at the end of composting. This was due to the release of several ions during the mineralization of organic matter (Hu et al., 2009). The organic matter content of final compost also, decreased from 81.08-73.53% due to degradation of organic matter into volatile compounds and the volatile compounds were lost from the solid compost during the composting process (Hu et al., 2009) (Table 2). The contents of N, P, K, Na, Ca and Mg in the finished compost were 2.14, 0.34, 8.71, 0.55, 0.34 and 10.6%,

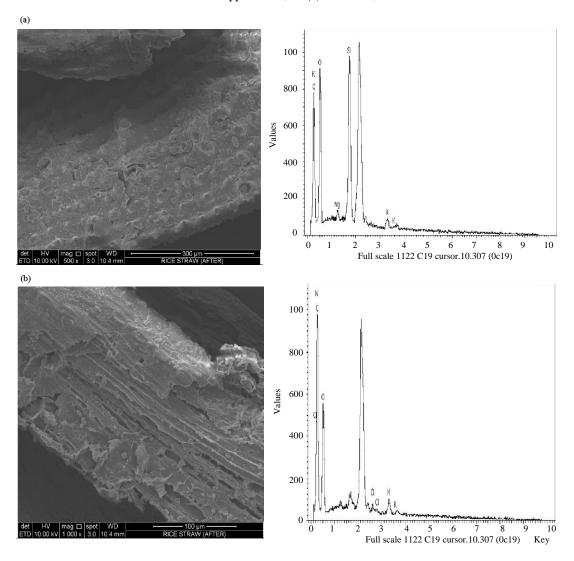


Fig. 2: a), b) Scanning Electron Micrographs (SEM) and EDX spectrograms of finished rice straw compost; spectrum 1

respectively. The micronutrients (Zn and Fe) increased except Cu was decreased in the matured compost (Table 2). The finished compost not only comprised of desired nutrients but it also contained low levels of heavy metals. Therefore, the finished compost in this study was safe to be used without causing any toxicity to plants. The initial total bacteria count was 1.86×10^8 CFU/mL wet substrate when the goat manure slurry was added and mixed with shredded rice straw, chicken feed and molasses. The bacteria count decreased to 1.62×10^7 CFU/mL at the end of the composting process (Table 3). The chicken manure slurry used in this study acted as microbial seeding, thus, the application of Effective Microbes (EM) can be omitted to reduce the cost of a compost production.

The SEM-EDX analysis of the finished rice straw compost sample is given in Fig. 2. The SEM images of the

finished compost that the compost had plane cleavage surface. This indicates that composting process would have made the volatile hydrocarbons to be stabilized and smoothened the surface of compost. The SEM image also uneven surface structure with small pores (Fig. 2a) and molded skeleton with broken edges (Fig. 2b) on the finished compost surface. This was due to the decomposition and fragmentation of organic materials in compost mixture, thus making the final compost more porous (Lim and Wu, 2016). This also, indicates that the lignin content was significantly reduced in finished compost. On the other hand, the pores on the finished compost surface was due to production of high volatile matter content (Lehmann et al., 2011). The Energy Dispersion X-ray spectroscopy (EDX) of the finished rice straw compost indicated that the compost had more minerals such as C, O, K, Mg, Si (Fig. 2a, b).

Table 4: Summary of phytotoxicity test (seed germination) of finished rice straw compost

	Mean root	Mean shoot	Mean seed	Relative seed	Relative root	Germination	Vigour
Compost	length (cm)	length (cm)	germination (%)	germination (%)	germination (%)	index (%)	index
Rice straw (original)	3.21ª	1.24^{nsd}	$100^{ m nsd}$	$100^{ m nsd}$	126.33a	$126.33^{\rm nsd}$	445.67ª
Rice straw (10×)	2.7^{ab}	$\mathrm{s}1.27^{\mathrm{nsd}}$	$100^{ m nsd}$	100^{nsd}	164.4ª	$104.4^{ m nsd}$	396.67ab
Rice straw (100×)	2.75^{ab}	$1.16^{ m nsd}$	$100^{ m nsd}$	100^{nsd}	107.43	$107.43^{\rm nsd}$	390.33ab
Rice straw (1,000×)	2.51ab	1.17^{nsd}	100^{nsd}	100^{nsd}	100.13 ^a	$100.13^{\rm nsd}$	368^{ab}
Rice straw (10,000×)	2.43 ^b	1.28^{nsd}	$100^{ m nsd}$	100^{nsd}	93.67ª	$93.67^{\rm nsd}$	371.67ab
Rice straw (100,000×)	2.37 ^b	$1.14^{ m nsd}$	$100^{ m nsd}$	100^{nsd}	93.84ª	$93.84^{\rm nsd}$	350.33 ^b
Control	2.68 ^{ab}	$1.44^{\rm nsd}$	$100^{ m nsd}$	100^{nsd}	100ª	100^{nsd}	411.67ab

Means within column with different letter (s) indicate significant difference by Tukey's test at $p \le 0.05$. nsd. No significant difference at $p \le 0.05$ by Tukey's test

Compost phytotoxicity test: The mung beans seeds germination indices in the finished compost were greater than 90% regardless of dilution factor (×10, ×100 and ×1,000) (Table 4). This indicates that the finished compost was free from phytotoxic and matured (Zucconi et al., 1981; Tiquia and Tam, 1998). According to Tiquia and Tam (1998) seed germination index is capable to detect different levels of toxicity that affects seed germination and root growth. Generally, stable compost leads to production of mature compost. However, some stable composts require longer period to achieve maturity due to different feedstock materials used in the composting process. As a result, both variables need to be assessed to ensure high-quality compost is produced. Wu and Ma (2001) that high levels of heavy metals can delay the maturity process of the compost.

CONCLUSION

The findings of this study suggests that composting of rice straw with goat manure slurry could be a practical and environmental friendly method in recycling both agricultural and dairy wastes. The final rice straw compost had no foul odour and low in heavy metals. The seed germination indices from the phytotoxicity indicated 90% for the finished compost. Shredded rice straw was high in carbon content and is suitable to be used as bulking agent. Thus, by composting rice straw with goat manure slurry it can convert the agricultural wastes into value added organic fertilizer. This can benefit agricultural sector especially the small-scale farmers. Nevertheless, several cycles of field trials are required to be carried out in order to assess the applicability and potential of the finished compost.

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