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TRICHO-COMPOSTING OF CROP RESIDUES WITH EASILY PRODUCED *T. HARZIANUM* LIQUID INOCULUMS

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ABSTRACT

This study aimed to develop a rapid composting method using *Trichoderma harzianum* culture of liquid state. Assessment of physico-chemical properties and nutritional qualities of composted crop residues generated by Tricho-composting is another concern. Different types of crop residues (Rice straw, Sugarcane bagasse, Black gram straw, and Mustard straw) were composted with *T. harzianum* IMI 392432 in a randomized block design with three replications. Composting was conducted in cement slabs, maintaining a pH range of 6.55 to 7.40. Temperature peaked at 10°-15°C during thermophilic activity before declining. Moisture content decreased gradually, with 15-20% retained in the finished compost. Weight loss ranged from 40.50% to 51.60%, and decomposition time varied from 67.33 to 96.00 days wherein mustard straw decomposed fast. Sugarcane bagasse decomposed slowly and black gram residues decomposed faster despite having less carbon. Mustard straw exhibited significant weight loss though their carbon content was abundant initially. Despite carbon losses in time, sugarcane bagasse and rice straw retained their properties longer than other residues. The highest phosphorus and potash content found with the decomposed residues of sugarcane in the treatment T_2. The treatment of T_4 comprise mustard straw showed the lowest phosphorus content and their potash content was 0.19 % which is non-significant with lowest potash containing rice straw generated compost. Easily produced *Trichoderma harzianum* liquid inoculums accelerated the composting process in accordance with their C:N ratio demonstrating its potential for efficient management of crop residues.

Keywords: *Trichoderma harzianum*, Composting, Tricho-composting, Crop residues.

1. INTRODUCTION

The widespread practice of burning crop residues in arid, semi-arid, and wet tropics exacerbates environmental degradation, contributing to air pollution, greenhouse gas emissions, and soil deterioration (Muni and Durge, 2024)). In India, where rice, wheat, and oilseed residues constitute a significant portion of total residue production (Verma *et al.*, 2024), their nutrient content, including nitrogen, phosphorus, and potassium, holds potential for enhancing soil fertility and crop productivity (Phiri *et al.*, 2023). Every year, agriculture produces a lot of crop residues, which are essentially unused resources. Bangladesh produces approximately 60 million tons of agricultural crops and approximately 36.48 million tons of recoverable crop residue each year (Halder *et al.*, 2014) Among these residues, rice leftovers make up a significant portion, making up about 70% of the total residues (Kamruzzaman *et al.*, 2024). However, the direct incorporation of crop residues into the soil presents challenges such as reduced grain yields, increased weed and pest populations, and soil acidification, necessitating sustainable alternatives for residue management (Meena *et al.*, 2024). Composting emerges as a viable solution, offering promise for rapid composting processes utilizing compost fungus activators. *Trichoderma harzianum* a ubiquitous soil fungus, renowned for its biocontrol properties (Rahman *et al*., 2023) and holds immense potential for accelerating the composting of organic materials, accelerates the decomposition of agricultural residues, thereby enhancing nutrient availability, suppressing soil-borne diseases, and improving crop vigor and yield (Muhammad *et al*., 2024). This approach, known as Tricho-composting, has become integral to organic agriculture, providing cost-effective and eco-friendly alternatives to chemical fertilizers (Sarangi *et al*., 2021). It improves plant nutrition by solubilization of mineral nutrients and benefits the crop to be more vigor in terms of disease resistance and crop yield. The present study aimed to develop a rapid composting method using *Trichoderma harzianum* as well as to assess the physico-chemical and nutritional qualities of composted crop residues.

2. MATERIALS AND METHODS

Location

The experiment was carried out at the laboratory of Agrotechnology, Department of Agronomy and Agricultural Extension, University of Rajshahi, Bangladesh, spanning from November 2022 to February 2023.

Experimental design and treatments

The experimental design utilized a Randomized Block Design (RBD) with three replications. The experiment consisted of four treatments viz. T_1= Rice straw, T_2 = Sugarcane bagasse, T_3= Black gram straw, T_4= Mustard straw.

Collection and preparation of different crop residues

Four types of crop residues, including rice straw, mustard straw, black gram, and sugarcane bagasse, were collected separately and dried thoroughly. The residues were gathered from various cropping seasons on the farm fields of the Agronomy and Agricultural Extension Department at the University of Rajshahi. They were then stored in poly bags for later use

Preparation of *T. harzianum* as liquid inoculants

T. harzianum IMI 392432; an effective strain used for composting of crop residues. Isolated *Trichoderma* strain cultured primarily in PDA plate and then grown in broth culture. Finally, *T. harzianum* IMI 392432 produced by bioreactor system as large number of liquid inoculums that was used as per treatment as ready inoculant for composting of different crop residues.

Process of composting in cement slab

Composting was done in cement slab (size: 69 cm diameter × 69 cm deep) upper the soil surface in a shade house. Rice, sugarcane, black gram, mustard trash used as crop residues for the treatment. Sun-dried residues of respective crop chopped at 10-15 cm and soaked with water by 2 kg straw. Moistened straw then poured in each digester with the treatment applied. 400ml liquid culture of *T. harzianum* (IMI392432) with surface spore was sprayed into crop residues and covered the slab. The surface of the all crop residues in each slab was sprayed with 500ml water/ residues to keep the residue moistened at 20-day intervals. After 20 days, the decomposing residues were overturned for mixing-well. Finally, last mixing of the contents in each slab was done at 60 day’s age of degradation. Composting residues was analyzed after certain duration for the parameter such as temperature, pH, moisture content, organic element and for *Trichoderma* viable cell count as colony forming unit (CFU) in finished compost.

Measurement of physical changes in crop residues during composting

Temperature

The temperature of compost pile was measured by inserting a thermometer into each compost content at about middle of residue substrate. The data taken from start point to end of the process at 15 day's interval and the temperature of ambience too recorded for evaluating the optimum condition of composting in each time.

pH

Buffer condition of composting is important for the growth of microorganism and for final product which will affect soil pH. Determination of pH is done at 15 day's interval in both cases. The fresh crop residues before composting and decomposed crop residues both were used for pH analysis. 10 g of grinded sample for each treatment were dispensed into 30 ml sterilized distilled water in 250ml conical flask and kept 6 hours at room temperature. The conical flask was set in a rotary shaker for the continuous shaking of solution at 100 rpm for 30 min. A pH meter (Shanghi jingke, China. model pHs 25) dipped into the supernatant to residue solution and reading was taken for each residue of treatment.

Moisture content (%)

Moisture content of decomposed crop residue is an important factor that makes and maintain the environment suitable for the growth of certain microorganism in the different stage of composting. Each sample were taken in measuring tin tray and measured the fresh weight. The sample were then kept in dry oven at 60°C temperature for 24 hours. The dried sample were weighted again to get dry weight. Calculation of moisture content was done following the formula provided by American Wood Preservers Associations (AWPA), given in 1986.

$$\text{Moisture content (MC)}\% = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

Weight loss (%)

Weight loss (%) of crop residue after degradation; the most important determinant that indicates the completion of process of compost. To determine the weight loss (%), weighted the dry crop residue (measuring with inoculants) before inoculation as initial weight(W). Dry compost weighted after degradation as final weight (W_1). Weight loss percentage was measured applying following formula on the basis of two weight.

$$\text{Weight loss }\% = \frac{W - W_1}{W} \times 100$$

Where, W=initial weight, W_1=final weight

Measurement of chemical changes of crop residues to compost

Organic carbon (%)

Dried crop residue and compost sample of known amount were taken in pre-weighted silica crucible and kept in muffle furnace at 600°c temperature for two hours. Cooled the substrate as soon as the crucible modified as desiccators and weighted (ash weight) immediately. The presence of organic carbon estimated by taking differences of dry weight and ash weight of sample. Organic carbon was calculated Finally by dividing percent organic matter using the factors 1.724 (Jackson, 1973).

$$\% \text{ of carbon} = \frac{\text{Sample of dry weight} - (\text{weight of ash})}{\text{Sample dry weight}} \times 100$$

Total nitrogen (%)

The sample residues were dried and then digested in concentrated sulfuric acid with a catalytic mixture containing potassium sulfate, copper sulfate, and selenium powder in a ratio of 50:10:1 using a micro Kjeldahl digestion unit. After digestion, the material was diluted with distilled water and made alkaline by adding sufficient sodium hydroxide (40%). Ammonia produced was trapped in a 2% boric acid solution along with a mixed indicator. The trapped ammonia was then titrated against 0.05N sulfuric acid, and the total nitrogen content was calculated from the volume of acid consumed using the formula provided by Jackson (1973).

$$\text{N}(\%) = \frac{\text{True value} \times \text{N of } \text{H}_2\text{SO}_4 \times 0.014 \times \text{dilution factor}}{\text{weight of the sample(g)}} \times 100$$

Carbon nitrogen ratio

The amount of total organic carbon and total nitrogen estimated by chemical analysis and calculated the C:N of each crop residue before degradation and after degradation. The devices used to measure this ratio are the CHN analyzer and the continuous-flow isotope ratio mass spectrometer (CF-IRMS). The C:N ratio was determined using the following formula (Erwan et al., 2012):

$$\text{C:N} = \frac{\text{Total Carbon}}{\text{Total Nitrogen}}$$

Phosphorus (%)

Finely ground samples (500mg each) were mixed with 3 mL of 37% HCl and 1 mL of 68% HNO_3 in a 50 mL Kjeldahl flask. The mixture was heated at 110°C until 1 mL of the solution remained. If the sample did not turn white, additional HCl and HNO_3 were added after cooling. Then, 10 mL of 1.2% HNO_3 was added, and digestion continued for 30 minutes at 80°C. Distilled water was added to maintain a 20 mL volume during heating, followed by cooling. Finally, distilled water was added to make up the volume to 20 mL. The resulting solution was used for phosphorus (P) and potassium (K) micronutrient analysis using Atomic Absorption Spectrophotometer according to Erwan et al. (2012).

Potassium (%)

An aliquot of the tri-acid digest from each residue was diluted to 50ml with distilled water. The concentration of potassium in the residue extract was determined using the flame photometric method by Jackson (1973). The flame photometer was calibrated using zero and 5 ppm standards. Flame photometer readings (FPR) were taken for other standards, and the diluted plant digest was analyzed, recording its FPR. A standard curve of potassium was plotted with FPR on the Y-axis and potassium concentration on the X-axis. The concentration of potassium in the sample was obtained by referring to this curve and calculated using the formula provided by Jackson (1973).

$$\text{K}(\%) = \frac{\text{Graph} \times \text{Dilution factor} \times \text{Vollum of the digest}}{10^6 \times \text{Weight of the sample}} \times 100$$

Determination of colony forming unit (CFU) of *T. harzianum* in Tricho-compost

CFU (colony forming unit) is a unit used to estimate the number of viable microorganisms in a sample. To determine the bacteria, fungi population in finished compost, the sample has gone through serial dilution technique for CFU count. Ten-gram air dried compost sample dissolve in 90 ml distilled water and sat for shake-well in a rotary shaker for 30 minutes to get stock sample.1 ml of stock sample diluted serially till 10^2 dilution factor for fungus cell count. 1ml of diluted solution spread over *Trichoderma* Selective Medium (TSM) [0.20 g of $MgSO_4.7H_2O$, 0.90 g of K_2HPO_4, 0.15 g of KCl, 1.0 g of NH_4NO_3, 3.0 g of glucose, 0.15 g of The Petri-plates were incubated at 25±2°C for three days. Finally, the counted fungus population was expressed using following formula as per unit dry weight of substrate.

$$\text{CFU} = \frac{\text{No of colonies on agar plate} \times \text{Dilution factor}}{\text{Amount of culture used to make a plate}}$$

Data record and analysis

Chemical characterization was performed at the Soil Resource Development Institution (SRDI), Regional office, Shampur, Rajshahi, Bangladesh. Data were statistically analyzed using the SPSS computer program (MSTAT-C) and means were compared using Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Determination of pH

Prior to the composting process, the sugarcane residues (T_2) exhibited the highest initial pH value of 7.96, surpassing all other residues (Table 1). In contrast, the black gram straw (T_3) displayed the lowest initial pH value of 6.12, indicating acidity. Following the composting process, treatment T_1 (Rice straw) displayed the highest final pH value of 7.40, surpassing all other decomposed materials. On the other hand, treatment T_3 (mustard straw) displayed the lowest final pH of 6.54. Various factors could have contributed to the observed fluctuations in pH values throughout the *Tricho*-composting process. Based on the initial pH values, it can be inferred that the residues possess an inherent chemical composition. The pH level experienced a rise during the process, potentially caused by the liberation of alkaline compounds as organic matter decomposed (Raymond *et al*., 2024). Conversely, the decrease in pH can be attributed to the build-up of acidic by-products (Gao *et al*., 2024) or the metabolic activities of specific microorganisms.

Determination of temperature

During the early stage of decomposition, treatment T_2 (Sugarcane bagasse) had the highest initial temperature of 29.26°C compared to other treatments (Table 1). In contrast, treatment T_4 (Mustard straw) had the lowest initial temperature of the experiment, measuring at 28.23°C. The temperature gradually increased from the 15th day of degradation. The highest temperature recorded was 26.86°C in treatment T_1 (Rice straw). Throughout the experiment, the compost in treatment T_3 (Black gram straw) reached a lowest temperature of 24.43°C. The temperature dynamics during composting indicate the activity of microorganisms and the level of decomposition (Su *et al*., 2024). As decomposition progresses, the temperature gradually rises, reaching its peak, indicating the beginning of thermophilic activity. Research has shown that certain microorganisms play a significant role in the decomposition of organic matter, leading to an increase in thermal activity (Acheampong *et al.*, 2024). Afterwards, the temperature dropped during the cooling phase of composting because of the reduced activity of microorganisms.

Determination of moisture content (%)

At first, it was found that mustard residues had the highest water absorption rate, measuring at 27.163%. (Table 1). On the other hand, the treatment T_3 (black gram straw) had the lowest moisture content, estimated at 23.44%. Following the decomposition process, there was a noticeable decrease in the moisture content. The treatment T_4 (mustard straw) had the highest moisture content of 20.07%, while treatment T_3 (Black gram straw) had the lowest moisture content of 12.97%. It is crucial to maintain optimal moisture levels during the phase of composting, as this has a direct impact on microbial activity and the rate of decomposition (Tang *et al.*, 2023). From the start, meticulous attention was given to providing adequate moisture for the treatments, promoting microbial growth and facilitating a seamless decomposition process. Over time, the moisture levels decreased gradually as a result of evaporation and microbial activity indicates the substantial influence of water during decomposition.

Table 1: Physical change of crop residues to Tricho-compost

Treatments	pH		Temperature		MC (%)	
	Initial	Final	Initial Ambient-25°C	Final Ambient-20°C	Initial	Final
T_1	7.137 c	7.400 c	29.267 a	26.867b	24.537a	15.563a
T_2	7.957d	7.153b	29.667 a	25.600ab	26.513a	15.427a
T_3	6.113 a	6.547 a	29.000 a	24.433a	23.447a	12.973a
T_4	6.543 b	7.083 b	28.233 a	25.033ab	27.163a	20.073b

Treatment denotes; T_1= Rice straw, T_2 = Sugarcane bagasse, T_3= Black gram straw, T_4= Mustard straw. In a column, data are the mean values with standard error having different letters within four different culture media differ significantly as per DMRT

Estimation of weight loss (%)

After the composting process, treatment T_4 appeared to be the most decomposed compared to the other treatments (Figure 1). T_4, which consisted of mustard straw, had the highest weight loss of 51.60%, suggesting a notable level of decomposition. On the other hand, the sugarcane residues had the lowest weight loss, estimated at 38.81%. As the composting process continued, the weight of the straw decreased as a result of the organic carbon being lost. The minimal weight loss suggests a lower level of decomposition compared to the rest.

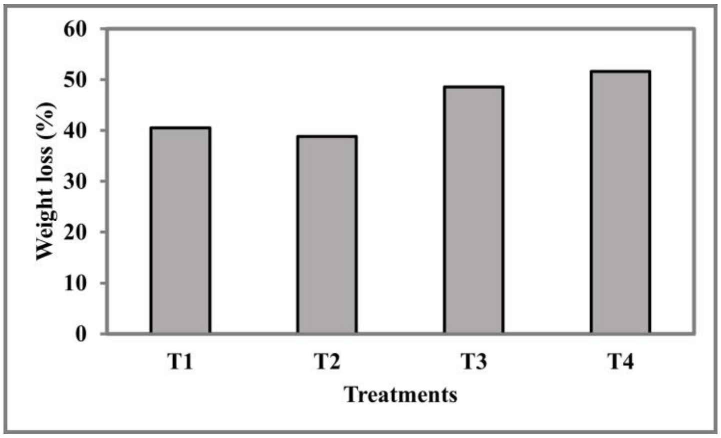

Figure 1: Weight loss of crop residues in *Tricho*-compost

Estimation of total time for decomposition

The degradation process of Treatment T_2 lasted for a total of 96.00 days, reaching complete decomposition (Figure 2). Contrarily, mustard residues decomposed in the shortest duration of time, in 67.33 days *Trichoderma* making it the fastest treatment. It is evident that mustard residues were the most efficient crop stubble for Tricho-composting among others. A study conducted by Wang *et al*., (2024) was found that the presence of *T. harzianum* significantly enhanced the decomposition process of organic materials.

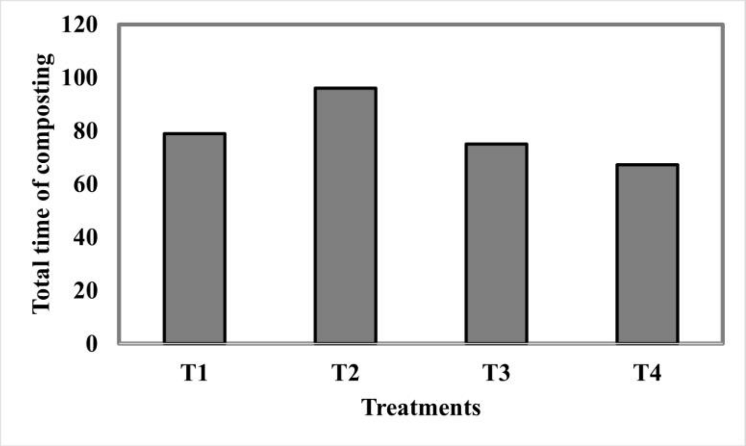

Figure 2: Total time required for decomposition

Chemical composition of crop residues and Tricho-compost

Organic Carbon (%)

All crop residue had higher carbon concentrations prior to composting. Initially, Sugarcane bagasse (Treatment T_2) had the maximum carbon content (50.04%), whereas Treatment T_3 (black gram straw) had the lowest carbon content (19.19%) among the crop wastes (Table 2). As decomposition began, carbon content declined over time, with significant differences among the four treatments. Finally, the treatment T_2 with sugarcane bagasse had the highest carbon content (18.63%). In comparison, Treatment T_4 had the lowest carbon level, at 5.32%. Carbon content drops during composting due to microbial respiration, in which microorganisms digest carbon molecules for energy and emit carbon dioxide (CO_2) into the atmosphere (Kong *et al*., 2024). As organic matter degrades, complex carbon molecules are transformed into simpler ones, lowering total carbon content (Koushika *et al*., 2024).

Nitrogen content (%)

The nitrogen concentration of crop residue varies according to the crop. Prior to composting, Treatment T1, which included rice straw, had the highest nitrogen concentration at 1.18%, while T4, which included mustard straw, had the lowest nitrogen level at 0.52% (Table 2). After composting, the nitrogen level of the final product differed. Treatment T_2, which consisted of sugarcane residues, had the greatest nitrogen concentration at 1.00%, surpassing all other composted wastes. Treatment T_4, which included mustard straw, had the lowest nitrogen content (0.45%). Nitrogen reduction occurs when denitrifying bacteria convert nitrate (NO_3^-) into nitrogen gas (N_2) in anaerobic conditions within a decomposing pile, resulting in nitrogen depletion (Liang *et al*., 2024). These findings were consistent with those of Pandey *et al*., 2024 and Luo *et al*., 2024, who found that nitrogen concentration was positively connected with organic matter loss and negatively correlated with the amount of carbon in hemicellulose and lignin. Sarma *et al*., (2013) investigated the NPK potential and manurial value of crop residue-generated compost and discovered that tapioca leaves had the most organic carbon (7.10%) and nitrogen (1.38%).

The carbon nitrogen ratio

The C:N ratio in composted materials was reduced in all treatments, to varying degrees (Table 2). Initially, treatment T_2 with sugarcane bagasse had the highest carbon nitrogen ratio of the experiment (59.32), while treatment T_3 with black gram straw had the lowest C:N ratio of 23.00. As time passed, the C:N ratio decreased. Following decomposition, Treatment T_2 (sugarcane bagasse) had the highest C:N ratio (18.64), whereas Treatment T_3 (black gram straw) had the lowest value (7.18). The C:N ratio declines during *Trichoderma* composting due to microbial use of carbon and nitrogen, nitrogen mineralization, microbial modification of organic nitrogen molecules (Li *et al*., 2024), and carbon loss by microbial respiration. Liang *et al*., (2024) found that combining livestock dung and plant residues reduced SOC sequestration effectiveness. Dwilaksono (2024) observed that mixing cattle dung and agricultural wastes would have helped to reduce the C:N ratio.

Phosphorus content (%)

Phosphorus concentrations in crop residues varied by the crop upon treatment (Table 2). Prior to the composting process, Treatment T_2, which consisted of sugarcane residues exhibited the highest concentration of phosphorus at 0.38%, the value surpassing remaining materials. In contrast, Treatment T_4 which incorporated mustard straw exhibited the least phosphorus content at 0.09%, a value is comparable to Treatment T_3. In a study by Sarma *et al.*, (2013) examining the manureal value and NPK potential of compost derived from crop residues, buckwheat compost contained the highest level of P_2O_5 (0.15%).

Potash content (%)

The initial potash content of rice straw was 1.35%, which was the highest among all residues (Table 2). On the other hand, the black gram straw (T_3) had the lowest potash content, with a percentage of 0.07% before degradation. Following the composting process, there was a significant decrease in the potash content. Treatment T_1 (rice straw) had the lowest potash content at 0.18%, while treatment T_2 had the highest potash content at 0.42%, which was found in the decomposed residues of sugarcane. In a study conducted by Sarma *et al*. (2013), the researchers examined the potential of NPK and the value of crop residues as manure. The results showed that compost made from wheat residue had the highest K_2O content, measuring at 0.29%.

Colony forming unit (CFU/g) of *Trichoderma* in compost

At the beginning, treatment T_1 (rice straw) had the lowest number of *Trichoderma* population (2.3×10^4 CFU/g) (Table 3), while mustard straw had the largest number (2.9×10^4 CFU/g) in compost. Following the application of the inoculum as a liquid culture, the population of *Trichoderma* increased. Treatment T_2 resulted final compost found with a very high *Trichoderma* population (6.2×10^4 CFU/g), In contrast treatment T_4, which included mustard straw, had the lowest *Trichoderma* population (5.0×10^4 CFU/g). Maximum *Trichoderma* colony may be found in compost degraded by sugarcane bagasse because to the bagasse's sugar residual content. The chemical characteristics of mustard, which release spicy-favoring myrosinase enzymes that might upset the *Trichoderma* population, result in the lowest amount of *Trichoderma* populations in mustard straw. According to Sebayang *et al*. (2024), the sixth week of composting produced the maximum population of bacteria and fungi, 300 x 10^7 CFU/g.

Table 3: Changes of *Trichoderma* population in cement slab compost ($\times10^2$ CFU/g)

Treatments	Initial (CFU/g)	Final (CFU/g)
T_1	2.3×10^4	6.0×10^4
T_2	2.5×10^4	6.2×10^4
T_3	2.6×10^4	5.9×10^4
T_4	2.9×10^4	5.0×10^4

T_1= Rice straw, T_2 = Sugarcane bagasse, T_3= Black gram straw, T_4= Mustard straw

Figure 3: Initial stage of composting of crop residues with *T. harzianum* inoculum in cement slab: T_1 = Rice straw, T_2 = Sugarcane bagasse, T_3 = Black gram straw, T_4 = Mustard straw

Figure 4: Degrading crop residues during 1 month of composting in cement slab: T_1 = Rice straw, T_2 = Sugarcane bagasse, T_3 = Black gram straw, T_4 = Mustard straw

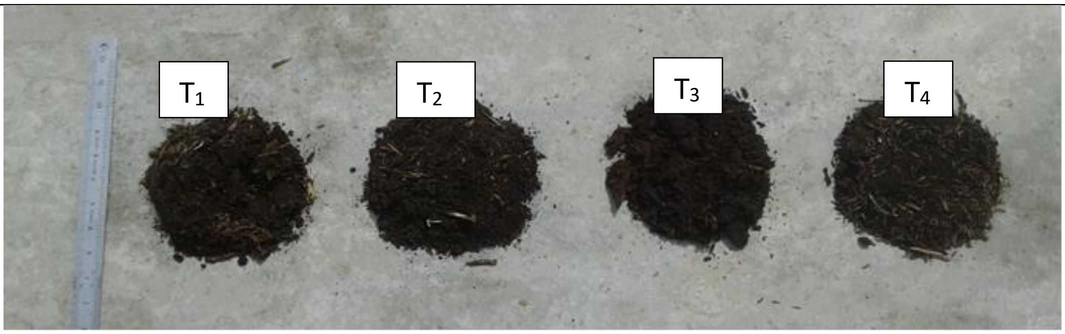

Figure 5: Final stage of Tricho-compost of crop residues in cement slab: T_1 = Rice straw, T_2 = Sugarcane bagasse, T_3 = Black gram straw, T_4 = Mustard straw

Conclusion

Applying *T. harzianum* in liquid form to organic waste like crop residues significantly expedited the process of biodegradation. Chemical analysis of compost from four crop straw revealed distinct differences. Sugarcane bagasse, rich in carbon, decomposed slowly, while black gram residues broke down faster despite containing less carbon. Mustard straw exhibited the highest weight loss though its abundant carbon content. Despite significant carbon loss, sugarcane bagasse and rice straw retained their physical and chemical properties longer than other residues.

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