



## Effect of rice straw and garbage enzyme addition on soil properties and plant growth of rice

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### ABSTRACT

The objective of the current study was to examine the impacts of rice straw and garbage enzyme generated from local vegetable and fruit waste on plant growth and carbohydrate or ammonium extraction from paddy soil after one month of growth in a pot experiment. Samples of topsoil were obtained from a depth of 0-15 cm, and the following treatments were applied: control (10 g soil), RS (adding 30 g soil + 0.6 g rice straw), GE (30 g soil + garbage enzyme), and combination (adding 30 g soil+ rice straw and garbage enzyme) maintained at room temperature. The study findings indicated that there were no observable impacts of rice straw and garbage enzyme application on biomass. However, RS addition seems to reduce root length but enhance shoot length. Soil carbohydrates that were extracted ranged from 61 to 207 mg kg<sup>-1</sup> soil, and treatments with rice straw addition exhibited significantly higher levels compared to those without it ( $p < 0.05$ ). The ammonium content was low. It could be concluded that at the initial seedling stage, rice straw has more effects on soil properties and plant growth than garbage enzyme. To fully assess the effects of rice straw and garbage enzyme on soil properties and plant growth, it is recommended that further research be conducted over longer periods.

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## 1. INTRODUCTION

Garbage enzymes (GE) which are generated by the fermentation of vegetables, fruit combined with water, and brown sugar, can be used for the purpose of speed up decomposition, transformation, and catalysis of the residues components (Arun & Sivashanmugam, 2015; Jiang et al., 2021; Sambaraju & Sree Lakshmi, 2020). GE has been widely used in wastewater treatment, soil fertilizer, and plant growth hormones. Leachate pollution was reported to be decreased by adding 20% GE after 4 weeks (Rani et al., 2020). In the case of dairy waste-activated sludge, garbage enzyme was found to reduce approximately 37% of total solids, 39% of suspended solids, and effectively eliminate most pathogens (Arun & Sivashanmugam, 2015). Using GE alone can also potentially improve honey locust fruit properties (Gu et al., 2021) or palm soil (Rasit & Chee Kuan, 2018).

However, to the best of our knowledge, no research evaluated the effect of GE on the soil properties especially rice paddy soils. In Vietnam, rice is one of the most important staple foods, making the country among the biggest rice exporters in the world. The cultivation of rice presents a range of environmental challenges, among which the management of rice straw residue is a particularly important concern, particularly in developing Asian countries (Allen et al., 2020; Van Hung et al., 2020; Wang et al., 2016). The management of rice straw can involve various methods, including removal or spreading within the field, or incorporation into the soil (Dobermann & Fairhurst, 2002; Toan et al., 2022). Rice straw is one crucial organic matter source for agriculture, improving soil quality properties (Cheng et al., 2016; Kautsar et al., 2020; Tang et al., 2016). It could be considered applying as a type of

organic fertilizer (Cheng et al., 2016; Dobermann & Fairhurst, 2002; Kautsar et al., 2020; Nguyen-Sy et al., 2020). Labile fractions were responsible for the slow decomposition of the total content of soil organic matter (Nguyen-Sy et al., 2020). Observations of increased soil organic matter stocks suggest that rice straw amendment plays a significant role in facilitating the rate of decomposition (Cheng et al., 2016; Kautsar et al., 2020; Tang et al., 2016). For instance, replacing plant residues on field may take 2-3 years to completely decompose. Adding chemical fertilizer could also speed up rice straw decomposition time. However, non-chemical addition methods will be sustainable method. Therefore, examining the impact of GE and rice straw separately as well as their interaction on soil paddy could be a potentially effective and eco-friendly approach for improving soil fertility.

With this scope, an experiment was carried out to assess the feasibility of utilizing rice straw and GE in combination may represent a promising approach towards the development of green fertilizers. In this study, GE was made of local waste material, making it more practical. The aim of this study was to investigate the impact of rice straw and GE by: 1) examining the effects of rice straw and GE on the growth of rice plants; and 2) investigate the change in important soil labile pools as extracted carbohydrate and inorganic nitrogen.

**2. MATERIALS AND METHODS**

**2.1. Site description and treatment design**

The paddy soils were collected from Hoa Vang District, Danang, Vietnam, which has been used for more than 100 years and is cultivated twice a year. The soil at the study site was alluvial soil type, with a composition of sand: silt: clay was 32%: 38%: 30%, respectively. Table 1 shows detailed information on soil properties at the study site. The mean annual temperature in the area was 26.2°C, and the average annual precipitation from 2013 to 2018 in local city was 2488 mm. In May 2021, following the rice harvest, topsoil samples were collected at a depth of 0-15 cm, which represents the plowed layer.

**2.2. GE preparation and experiment set up**

As described in Table 2, four treatments were set: control, RS, GE, and combination treatment. The application rate of rice straw was 2% (rice straw: soil weight) (Tang et al., 2016). GE could be diluted 1000 times to get the suitable pH (4.2), and 20 mL of the diluted GE was added into small volume bottles (height: 10 cm height, diameter: 5 cm, volume: 68 mL). The rice seeds were incubated for three days in a high-moisture, dark environment for germination. Afterward, germinated seeds were transplanted into bottles (15 seeds per bottle). Water and GE were added relatively, as shown in Table 1. The bottles were kept at room temperature for four weeks. After that, plant biomass and height were determined. Soil samples were extracted with distilled water and filtered to analyze their carbohydrate, ammonium, and nitrate contents.

**2.3. Plant growth measurement and soil extraction analysis**

In our study, the rice variety was the local variety Haphat 3, one of the most common varieties cultivated in Central Vietnam. This rice variety can tolerate drought stress for a short period (about 100 days). After one month of growth, we added 50 mL of distilled water into each pot bottle and shook it for 30 min to quickly remove the rice plant. Plant biomass (fresh and dry weight) and root and shoot lengths were measured after one month of growth. Fresh weight was calculated for each bottle (15 young plants), then dried at 70°C in 24 h to measure dry weight.

Water was added every three days and as necessary. The volume was recorded to adjust the total volume. To adjust the volume of water in each bottle for soil extraction, the Equation 1 was applied.

$$V \text{ (mL)} = W \text{ total} - W \text{ soil} - W \text{ plant} - W \text{ bottle} \dots\dots\dots[1]$$

where, W total: the total weight of pot bottle at the end of the experiment before taking out the plants; W soil: the weight of soil added in each pot bottle at the beginning of the experiment (30 g per bottle). We ignored the changes in soil weight absorbed by plants; W plant: fresh weight of plant in each bottle; W bottle: the net weight of each bottle.

The Dubois method, with minor modification was used to determine the total carbohydrate content (Albalasmeh et al., 2013). Extracted supernatant solution was reacted with 50 g/L phenol and 95% sulfuric acid. The final solution was quantified using absorbance measurements taken at a wavelength of 485 nm, and compared to a glucose standard to determine the concentration of the sample.

To measure the levels of inorganic nitrogen and mineralization nitrogen (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N), the samples were passed through a filter paper and subsequently analyzed using the nitroprusside (655 nm) and hydrazine reduction (540 nm) methods, respectively, with the help of a UVD-300 spectrophotometer.

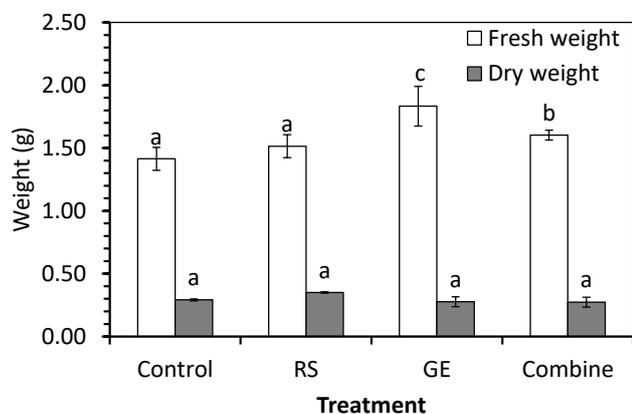
**Table 1.**The materials input description in the experiment\*

	Paddy soil	Rice straw	Garbage Enzyme
Organic Carbon (%)	1.49	25.12	-
Total Nitrogen (%)	0.16	0.37	-
NH <sub>4</sub> <sup>+</sup> (mg N kg <sup>-1</sup> soil)	13	-	undetected
NO <sub>3</sub> <sup>-</sup> (mg N kg <sup>-1</sup> soil)	3	-	undetected
Carbohydrate (mg C kg soil <sup>-1</sup> )	182	-	32
pH H <sub>2</sub> O	5.0	-	4.2
pH KCl	4.0	-	-

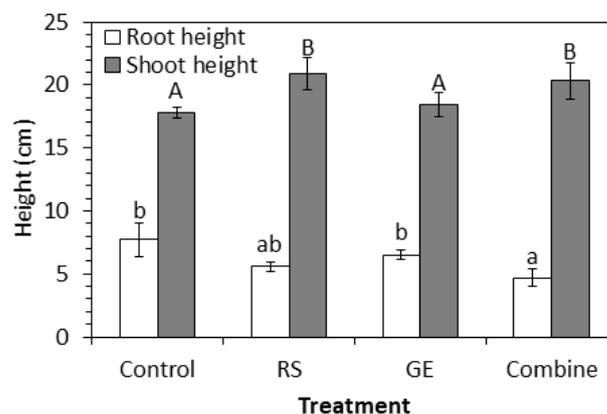
**Note:** \*soil and rice straw properties referred from Nguyen-Sy et al. (2021)

**Table 2.** Characterization of treatments in the current study

Treatment	Soil		Garbage Enzyme		Seedling
	g/bottle	Rice straw	ml/bottle	Seedlings/bottle	
Control	30	0	0	15	
RS	30	0.6	0	15	
GE	30	0	20	15	
Combine	30	0.6	20	15	



**Figure 1.** Plant biomass affected by 1-month rice straw and garbage enzyme application



**Figure 2.** Plant biomass affected by 1 month rice straw and garbage enzyme addition. The different letters indicate different significance at the 0.05 level

**2.4. Statistical analysis**

The present experiment was conducted for one month in February 2022. The treatment was designed following the randomized complete block design. The results shown are an average of three replicates, with the error bars or ± indicating the standard deviation.

**3. RESULTS**

**3.1. Changes in plant biomass**

Changes in plant biomass after one month of the experiment are shown in Figure 1. Fresh weight ranged from 1.41 to 1.83 g bottle<sup>-1</sup>. The lowest fresh weight was in the control and RS treatment (1.41 and 1.52 g bottle<sup>-1</sup>, respectively), while the highest fresh weight was in the GE treatment, followed by the combination treatment (1.60 and 1.83 g bottle<sup>-1</sup>, respectively; p < 0.05). Meanwhile, dry weight ranged from 0.27 to 0.35 g bottle<sup>-1</sup> and no significant difference among treatments was observed (p > 0.05). Adding GE likely enhanced the fresh biomass, but it is unexplainable why there was no effect on the dry biomass.

**3.2. Changes in plant height**

Root and shoot heights were investigated at the end of the experiment (Fig. 2). Root height ranged from 4.7 to 7.7 cm, while shoot height ranged from 17.8 to 20.3 cm. The longest root heights are shown in the combination and RS treatments (4.7 and 5.6 cm, respectively), while the longest was that of the control treatment (7.7 cm) (p < 0.05). Interestingly, a contrasting result was found in shoot height. The longest shoot height was in the RS treatment (20.9 cm), followed by the combination treatment, with the shortest being in control. RS and GE did not enhance but shortened the root length compared with that in the control, but they enhanced the shoot height.

**3.3. Changes in soil ECH**

As shown in Figure 3, the amount of ECHs in soil ranged from 61 to 207 mg kg<sup>-1</sup>. ECH is divided into two: the low ECH, which includes the control and GE treatment (61 mg kg<sup>-1</sup>), and the high ECH, which includes the RS and combination treatments (186 mg kg<sup>-1</sup> and 207 mg kg<sup>-1</sup>, respectively; p < 0.05). Notably, the initial soil ECH and GE were 182 and 32 mg kg<sup>-1</sup>, respectively, which are close to the ECH of the RS and combination treatments (Table 1). The result confirms that at least 66% of the initial soil ECH was absorbed by plants or emitted through CO<sub>2</sub> and CH<sub>4</sub> gases. Unexpectedly, GE did not promote the ECH amount after GE treatment; however, there was a slight enhancement in the combination treatment, but there was no significant effect observed.

**3.4. Changes in soil-extracted inorganic nitrogen**

Ammonium, together with nitrate are the two dominant forms that can be directly assimilated by plants. As NO<sub>3</sub><sup>-</sup> was not found, the quantity of NH<sub>4</sub><sup>+</sup>-N was considered for the total inorganic N (Fig. 4). The ammonium amount of all treatments ranged from 0.45 to 2.86 mg N kg<sup>-1</sup>. The amount of ammonium was also divided into two as in ECH: a high amount was observed in the GE treatment and control (2.86 and 1.97 mg kg<sup>-1</sup>, respectively), while a low amount was observed in the RS and combination treatments (0.48 and 0.45 mg kg<sup>-1</sup>, respectively; p < 0.05).

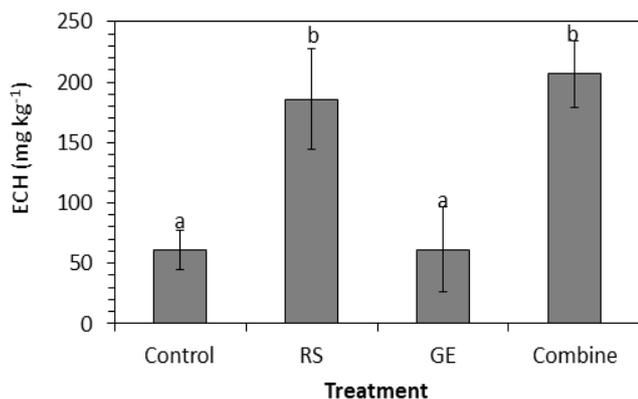
**3.5. Relationship between parameters**

As shown in Table 3, the fresh and dry weights have no significant correlation with other parameters (p > 0.05). The dry weight has a positive correlation with shoot height; however, there is no statistical significance (R = 0.46, p > 0.05). Meanwhile, ECH has a significant negative correlation

**Table 3.** The correlation among the rice growth and soil measurement parameters

	Fresh Weight	Dry Weight	ECH	NH <sub>4</sub> <sup>+</sup>	Root Height	Shoot Height
Fresh Weight	1.000					
Dry Weight	-0.123	1.000				
ECH	-0.280	0.201	1.000			
NH <sub>4</sub> <sup>+</sup>	0.451	-0.332	-0.892**	1.000		
Root Height	-0.158	-0.132	-0.664*	0.627*	1.000	
Shoot Height	-0.132	0.461	0.733**	-0.690*	-0.610*	1.000

**Note:** the correlation is significant at both the 0.01 level (\*\*) and the 0.05 level (\*)



**Figure 3.** Soil extracted as affected by 1-month rice straw and garbage enzyme application. The different letters indicate different significance at the 0.05 level.

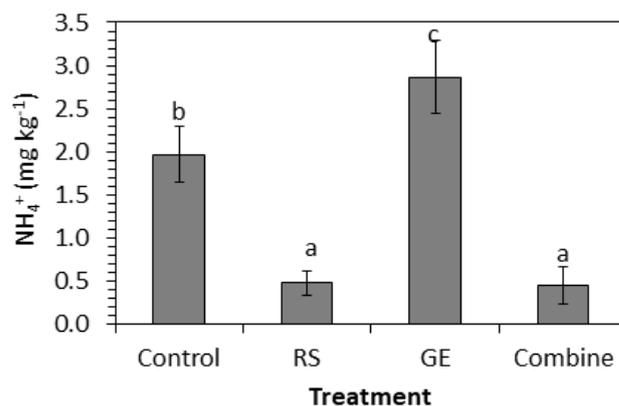
with ammonium and root height ( $p < 0.01$  and  $p < 0.05$ , respectively), but a positive correlation with shoot height ( $p < 0.01$ ). Ammonium has a negative correlation with ECH and shoot height ( $p < 0.01$  and  $p < 0.05$ , respectively) but a positive correlation with root height ( $p < 0.05$ ). Root height has a positive correlation with shoot height but a negative correlation with ECH ( $p < 0.05$ ).

**4. DISCUSSION**

Unexpectedly, the rice straw addition treatment showed the most extended shoot height as compared with the GE and combination treatments. It is supposed that the labile energy source from RS at the beginning (for example, ECHs) provided more nutrients for shoot height enhancement. There are a few research reports indicating that GE could be a promising enhancing plant growth factor in castor (Zhu et al., 2020) or honey fruit (Gu et al., 2021); Despite numerous investigations into the effects of GE, no research has yet explored its influence on the growth of paddy rice. In this study, we found that GE likely enhances the biomass rather than the length of the root.

Figure 1 illustrates that the RS and combination treatments yielded more carbohydrates than the control and GE treatments. This could be due to the RS using some of the carbohydrates for its growth and development. The study found that ECH contributed only 0.64% to 1.49% of SOC, which is lower than in earlier research (estimated range of 5% to 20% ) on crops such as vegetables and paddy rice (Nguyen-Sy et al., 2022; Thi et al., 2022), especially in the GE treatment and control (<1.0). Due to differences in the calculation method employed, the percentage of ECH to SOC in control

and RS treatment is slightly lower than in other studies.



**Figure 4.** Soil extracted ammonium extraction as affected by 1-month rice straw and garbage enzyme application. The different letters indicate different significance at the 0.05 level.

In our research, we cannot calculate the amount of ECH absorbed by rice plants, which is the main reason for the decline in the amount of ECH. As ECH is a labile component of SOC that is readily decomposed by soil microorganisms, thus making it available for plant uptake. The close association of ECH with soil microbial degradation highlights its importance as a potential source of nutrients for plants (Bongiorno et al., 2019; Ratnayake et al., 2013; Uzoho & Igbojonu, 2014).

Nitrogen is a crucial element in soil composition. Compared to the ECH model, the GE and control treatments had elevated ammonium levels, whereas the RS and combination treatments had lower levels. The amount of ammonium in the RS and combination treatments was about five times lower than those in the control and GE treatment (0.46 vs. 2.4 mg N kg soil<sup>-1</sup>). Elsewhere, there have been reports on the use of GE on rice plants, confirming it as a promising biofertilizer (Madhaiyan et al., 2010; Rekha et al., 2018). To the best of our knowledge, this is the first study to investigate the impact of GE treatment on fertilizer enhancement in paddy soil. The low ammonium amount in RS (RS and combination treatments) may result from immobilization. This result indicates that immobilization happens more than the mineralization process when RS is applied. We supposed that an amount of extracted soluble matter in RS (especially soluble organic carbon) enhanced the soil microorganism activity, leading to improved plant growth. The high amount of ECH content in RS treatments is shown in Figure 3. The findings were consistent with Pansu and Thuriès (2003), confirm that plant waste or green fertilizers can accelerate the process of immobilization. That

may explain why rice straw application in short term may be linked to the Min-N reduction.

Our previous research indicated that ECH has a high positive correlation with soil organic carbon (Toan et al., 2022; Toan et al., 2021); however, this research had no plant growth in soil. In this study, the amount of ECH is expected to correlate with soil ammonium negatively. This may be due to the different way plants absorb ECH compared with ammonium. Both the contents of ECH and ammonium declined after one month of growing rice; however, the reduced amount of ammonium was much higher than those in ECH. The main reason may relate to the ECH generated from rice straw and garbage enzyme.

## 5. CONCLUSION

The results show that applied RS had the opposite effects of GE. RS and GE likely have no impact on young plant biomass. However, RS and GE applications seem to decrease root height, while RS applications enhance shoot height compared with the control ( $p < 0.05$ ). The application of RS also promotes ECHs (about three times higher), while GE slightly maintains the ammonium concentration in soil ( $p < 0.05$ ). Further research for an extended period of observation is needed to confirm our results.

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## Declaration of Competing Interest

The authors declare that no competing financial or personal interests that may appear and influence the work reported in this paper.

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