Growth of cacao seedlings on application of several sawdust compost composition enriched with *Trichoderma* sp.

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Abstract. The study aimed to determine the response of the growth of cocoa seedlings in a variety of sawdust compost and *Trichoderma* sp compositions. The research was conducted in the form of a factorial experiment arranged with Randomized Block Design (RBD) with 2 factors. The first factor was the ratio of sawdust compost to manure: 1:1, 1:2 and 2:1. The second factor was the dose of *Trichoderma*, namely: 2 g per polybag, 4 g per polybag, and 6 g per polybag. The results showed that the application of compost composition of sawdust-manure 1: 2 gave the best results in several variables namely plant height (34.64 cm), number of leaves (21.24 strands), stem diameter (8.56 mm), chlorophyll index (138.76), total chlorophyll a (518.77 µmol.m$^{-2}$), amount of b-chlorophyll (347.42 µmol.m$^{-2}$), and total leaf chlorophyll (775.12 µmol.m-2). Application of *Trichoderma* sp. with a dose of 4 g per polybag gave the highest results in parameters of plant height (33.57 cm), leaf area (12.43 cm$^{2}$) and Leaf Mass Area (LMA) (0.00334 g / cm$^{2}$). There was no interaction between the sawdust compost composition treatment with addition of *Trichoderma* on all observed parameters.

1. Introduction
South Sulawesi is one main cocoa producers in Indonesia. According to Directorate General of Plantations [1] cocoa production in South Sulawesi experienced decline which was related to the reduction in the planted area. One other factor which is responsible for low cocoa productivity is the aging trees, resulting in significant yields decline. In addition, the planting area or land resources of cacao plantation might have been excessively utilized without soil improvement efforts. Consequently, it is necessary to improve the condition, one of them is by rejuvenating cocoa fields. For that purpose, cocoa seedlings need to be viable planting material that have good quality and potential high production [2].

Cacao nurseries are generally carried out in polybags, since this method has several advantages including better and uniform seedling growth and easy maintenance and transportation. Apart from quality planting materials, nurseries also need to consider appropriate planting media and fertilizer. Fertilization in the planting media is very closely related to the improvement of physical, biological and chemical properties of the soil. Growing media plays an important role in producing high quality seedlings. According to Hakim et al., [3] the physical condition of the soil largely determines root penetration in the soil, water availability, drainage, aeration and plant nutrition.
Compost has been widely known and used as natural fertilizer since it derives from organic materials [4]. Utilization of natural agricultural wastes such as leaf litter, rice straw, market waste, sawdust and so on will provide double benefits, producing natural fertilizer and reducing organic wastes [5].

Sawdust is popular waste of wood industry including the furniture industry as a sector that is in high demand in Indonesia. This industry produces a lot of sawdust waste in its processing. Sawdust waste causes problems in its handling, which mostly are left to rot, stacked, and burned all of which have a negative impact on the environment [6]. Therefore, sawdust waste from the furniture industry should be utilized for various purposes, one of which is a planting medium in plant nurseries. Utilization of sawdust as a planting medium cannot be directly applied to the soil, because the organic materials within cannot be absorbed directly by plants. Decomposition process of this organic material by composting become the solution [7]. When not properly decomposed, the cellulose and lignin will cause high C/N ratio which will adversely affect seedlings growth [8]. Therefore, sawdust must be decomposed through composting before it is applicable for plants.

One method of controlling disease in cocoa is by empowering microorganisms such as fungi [9]. Trichoderma sp. is an example of saprophytic and endophytic soil fungi in cocoa plants. Rosmana et al., [10], identified 21 Trichoderma isolates which are endophytic from cocoa plantations in Sulawesi and several isolates were potential as a disease control agent in cocoa plants. This fungus can control fruit rot, leaf blight and Vascular Streak Dieback in cocoa plants [11][12][10].

A research by Ferayanti et al., [13] on Trichoderma sp. showed that the a combination of Trichoderma species and frequency of application caused suppression to the intensity of cocoa pod rot. The application of T. harzianum, T. viren and T. asperellum in this study to all parts of the cocoa plant, was thought to provide an opportunity for Trichoderma spores to penetrate and colonize the cocoa pods, thus providing a greater defense mechanism against the pathogenic attack of Phytophthora palmivora. Ferayanti et al., [13] also observed the trichoderma colonization of stems and their hyphae came out of the tips of the trichomes after inoculation of the cocoa seedlings through the roots. This is in accordance with research conducted by Yedidia et al., [14], where Trichoderma can penetrate directly on the surface of the root hair.

2. Methodology
The study was conducted in the form of a 2-factor factorial experiment using a Randomized Block Design (RCBD). The first factor was the composition of sawdust compost on media (K), consisting of 3 levels, namely: k1=Sawdust + manure (1: 1); k2=Sawdust + manure (1: 2); k2=Sawdust + manure (2: 1). The second factor was the dose of Trichoderma (T), consisting of 3 levels, namely: t1=Trichoderma harzianum 2 g per polybag; t2 = Trichoderma harzianum 4 g per polybag; t3=Trichoderma harzianum 6 g per polybag. Each treatment combination was repeated 3 times and consisted of 3 plants making a total of 81 plant units.

Composting was prepared by mixing sawdust with manure according to treatment. The mixture was then added with Pleurotus ostreatus fungus which functions as an organic waste decomposer. The mixture was then put into a sack and doused with water evenly, then the sack was sealed and left for decomposing process for 1 month. After the compost were done, growing media was prepared by mixing soil and the sawdust compost with a volume ratio of 2: 1 before putting it into the planter bag (polybag).

Trichoderma application was conducted by first preparing the application unit by weighing each unit in accordance with the treatment (2 g, 4 g and 6 g). Then, it was applied by strewing the Trichoderma into each planting hole when planting the seedlings with the right application directly on plant roots.

3. Results and discussion
The observed variables consisted of observation on general plant growth and specific observation on the plant leaves including the leaf stomata.
3.1. Seedlings Growth

The following tables show several observations on the seedlings growth parameters:

**Table 1. Average increase in plant height (cm) with various sawdust-manure compost compositions and Trichoderma application**

| Compost composition (Sawdust : manure) | Trichoderma dosage (g per polybag) | Average | LSD 0.05 |
|---------------------------------------|-----------------------------------|---------|----------|
|                                       | 2 (t1) | 4 (t2) | 6 (t3) |          |
| 1 : 1 (k1)                           |         |        |        |          |
|                                       | 29.56   | 33.42  | 32.29  | 31.76 b  |
| 1 : 2 (k2)                           |         |        |        |          |
|                                       | 33.68   | 34.96  | 35.29  | 34.64 a  |
| 2 : 1 (k3)                           |         |        |        |          |
|                                       | 30.42   | 32.33  | 31.22  | 31.32 b  |
| Average                               |         |        |        |          |
|                                       | 31.22 b | 33.57 a| 32.93 ab|          |
| LSD 0.05                              |         |        |        | 1.86     |

Notes: The numbers followed by the same letters in the same column or row (a, b) indicates that they are not significantly different in the LSD test level of 0.05.

The variance analysis on Table 1 shows that the composition of sawdust compost and Trichoderma dose had very significant effect, while the interaction between the two factors had no significant effect on plant height increase. Table 1 shows that the 1:2 sawdust-manure composition of compost (k2) produced the highest increase in plant height of 34.64 cm and significantly different from other treatments while the lowest increase in plant height was found in the 2:1 sawdust-manure composition of compost (k3) which was 31.32 cm. Application of 4 g Trichoderma per polybag (t2) produced the highest plant height increase of 33.57 cm, not significantly different from 6 g Trichoderma per polybag (t3) dose but significantly different from 2 g Trichoderma per polybag (t1) which gave the lowest increase in plant height which was 31.22 cm.

**Table 2. Average increase in number of leaves (strands) with sawdust-manure compost compositions and Trichoderma application after data transformation to $\sqrt{x + 0.05}$**

| Compost composition (Sawdust : manure) | Trichoderma dosage (g per polybag) | Average |
|---------------------------------------|-----------------------------------|---------|
|                                       | 2 (t1) | 4 (t2) | 6 (t3) |          |
| 1 : 1 (k1)                           |         |        |        |          |
|                                       | 4.31   | 4.42   | 4.26   | 4.33 b (18.75) |
| 1 : 2 (k2)                           |         |        |        |          |
|                                       | 4.58   | 4.66   | 4.59   | 4.62 a (21.24) |
| 2 : 1 (k3)                           |         |        |        |          |
|                                       | 4.51   | 4.46   | 4.35   | 4.44 ab (19.70) |
| LSD 0.05                              |         |        |        | 0.22     |

Notes: The numbers followed by the same letters in the same column or row (a, b) indicates that they are not significantly different in the LSD test level of 0.05.

The analysis of variance showed that sawdust compost composition had very significant effect, while Trichoderma dosage and the interaction of the two factors had no significant effect on increasing the number of leaves. Table 2 shows that the 1:2 sawdust-manure composition of compost...
(k2) produced the highest number of leaves (21.24), not significantly different from the 2:1 sawdust-manure composition of compost (k3) but significantly different from the 1:1 sawdust-manure composition of compost (k1) which gave the least amount of leaf growth i.e. 18.75 strands.

**Table 3.** Average increase in number of stem diameter (mm) with sawdust-manure compost compositions and *Trichoderma* application.

| Compost composition (Sawdust : manure) | *Trichoderma* dosage (g per polybag) | Average |
|---------------------------------------|--------------------------------------|---------|
|                                       | 2 (t1)   | 4 (t2)  | 6 (t3)  |         |
| 1 : 1 (k1)                            | 7.63     | 7.58    | 7.26    | 7.49 b |
| 1 : 2 (k2)                            | 8.22     | 8.89    | 8.56    | 8.56 a |
| 2 : 1 (k3)                            | 7.95     | 7.59    | 7.50    | 7.68 b |

LSD 0.05 0.68

Notes: The numbers followed by the same letters in the same column or row (a,b) indicates that they are not significantly different in the LSD test level of 0.05.

Tabel 3 shows that the composition of sawdust-manure compost had very significant effect, while the *Trichoderma* dose and the interaction between the two factors had no significant effect on stem diameter increase. Table 3 also shows that the 1:2 sawdust-manure composition of compost (k2) produced the largest stem diameter increase of 8.56 mm, and it was significantly different from the other compost-manure compositions. The smallest stem diameter increase was produced the composition of sawdust-manure ratio 1:1 (k1) i.e. 7.49 mm.

**Table 4.** Average leaf area (cm$^2$) with various sawdust-manure compost compositions and *Trichoderma* application after data transformation to $\sqrt{(x + 0.05)}$.

| Compost composition (Sawdust : manure) | *Trichoderma* dosage (g per polybag) | Average | LSD 0.05 |
|---------------------------------------|--------------------------------------|---------|----------|
|                                       | 2 (t1)   | 4 (t2)  | 6 (t3)  |         |
| 1 : 1 (k1)                            | 11.00    | 11.81   | 11.07   | 11.29 b |
|                                       |          |         |         | (127.66)|
| 1 : 2 (k2)                            | 12.07    | 12.4    | 11.85   | 12.11 a |
|                                       |          |         |         | (146.74)|
| 2 : 1 (k3)                            | 12.28    | 13.07   | 12.21   | 12.52 a |
|                                       |          |         |         | (157.01)|
| Average                               | 11.79 b  | 12.43 a | 11.71 b |          |
|                                       | (139.19) | (154.75)| (137.46)|         |

LSD 0.05 0.56

Notes: The numbers followed by the same letters in the same column or row (a,b) indicates that they are not significantly different in the LSD test level of 0.05.

Values in brackets are the original data

The variance analysis showed that the composition of sawdust compost and *Trichoderma* dose had very significant effect, while the interaction between the two factors had no significant effect on leaf area. Table 4 shows that the 2:1 sawdust-manure composition of compost (k3) produced the largest leaf area that was 157.01 cm$^2$, and not significantly different from the composition of 1:2 sawdust-manure compost (k2), but different significantly from the 1:1 sawdust-manure compost (k1) which
produced the smallest leaf area (127.66 cm$^2$). Application of 4 g *Trichoderma* per polybag (t2) produced the largest leaf area of 154.75 cm$^2$, and significantly different from other *Trichoderma* doses. The smallest leaf area was found on the 6 g of *Trichoderma* application per polybag (t3) which was 137.46 cm$^2$.

3.2. Specific leaf components

Several specific variables on plant leaves were observed such as chlorophyll index, amount of chlorophyll and stomata components. The following tables presented the results.

Table 5. Average chlorophyll index with sawdust-manure compost compositions and *Trichoderma* application.

| Compost composition (Sawdust : manure) | *Trichoderma* dosage (g per polybag) | Average |
|---------------------------------------|--------------------------------------|---------|
|                                       | 2 (t1)                               | 4 (t2)  | 6 (t3) |
| 1 : 1 (k1)                            | 134.46                               | 134.82  | 135.04 |
| 1 : 2 (k2)                            | 137.99                               | 138.99  | 139.28 |
| 2 : 1 (k3)                            | 133.44                               | 133.93  | 135.49 |

LSD 0.05 2.66

Notes: The numbers followed by the same letters in the same column or row (a,b) indicates that they are not significantly different in the LSD test level of 0.05.

The variance analysis showed that the compost composition of sawdust-manure had very significant effect, while the *Trichoderma* dose and the interaction between the two factors had no significant effect on the chlorophyll index. Table 5 shows that the compost composition of 1:2 sawdust-manure (k2) produced the highest chlorophyll index which was 138.76, and significantly different from the other sawdust-manure compositions, while the lowest chlorophyll was produced by the composition of 2:1 sawdust-manure (k3), which was 134.28.

Table 6. Average amount of a-chlorophyll (µmol.m$^{-2}$) with sawdust-manure compost compositions and *Trichoderma* application.

| Compost composition (Sawdust : manure) | *Trichoderma* dosage (g per polybag) | Average |
|---------------------------------------|--------------------------------------|---------|
|                                       | 2 (t1)                               | 4 (t2)  | 6 (t3) |
| 1 : 1 (k1)                            | 513.21                               | 515.07  | 513.58 |
| 1 : 2 (k2)                            | 517.96                               | 518.37  | 519.96 |
| 2 : 1 (k3)                            | 511.85                               | 513.43  | 515.14 |

LSD 0.05 3.16

Notes: The numbers followed by the same letters in the same column or row (a,b) indicates that they are not significantly different in the LSD test level of 0.05.

The Results showed that the sawdust-manure composition of compost had a very significant effect, while the dose of *Trichoderma* and the interaction between the two factors had no significant effect on
the amount of a-chlorophyll. Table 6 shows that compost composition of 1:2 sawdust-manure (k2) produced the highest amount of a-chlorophyll which was 518.77 µmol.m⁻², and was significantly different from the other sawdust-manure compost compositions. The lowest amount of a-chlorophyll was produced by the 2:1 sawdust-manure composition (k3), which was 513.47 µmol.m⁻².

Table 7. Average amount of b-chlorophyll (µmol.m⁻²) with sawdust-manure compost compositions and Trichoderma application.

| Compost composition (Sawdust : manure) | Trichoderma dosage (g per polybag) | Average |
|----------------------------------------|-----------------------------------|---------|
|                                        | 2 (t1) | 4 (t2) | 6 (t3) |         |
| 1 : 1 (k1)                             | 339.16 | 341.03 | 339.76 | 339.98 b|
| 1 : 2 (k2)                             | 346.47 | 347.08 | 348.7  | 347.42 a|
| 2 : 1 (k3)                             | 337.14 | 339.53 | 341.36 | 339.34 b|

LSD 0.05 4.96

Notes: The numbers followed by the same letters in the same column or row (a,b) indicates that they are not significantly different in the LSD test level of 0.05.

The results showed that the sawdust-manure composition of compost had a very significant effect, while the treatment of Trichoderma dosage and interaction between the two factors had no significant effect on the amount of b-chlorophyll. Table 7 shows that the compost composition of 1:2 sawdust-manure (k2) produced the highest amount of b-chlorophyll (347.42 µmol.m⁻²), and was significantly different from the other compositions. The lowest amount of b-chlorophyll was given by the 2:1 sawdust-manure composition (k3) which was 339.34 µmol.m⁻².

Table 8. Total amount of chlorophyll (µmol.m⁻²) with sawdust-manure compost compositions and Trichoderma application.

| Compost composition (Sawdust : manure) | Trichoderma dosage (g per polybag) | Average |
|----------------------------------------|-----------------------------------|---------|
|                                        | 2 (t1) | 4 (t2) | 6 (t3) |         |
| 1 : 1 (k1)                             | 766.13 | 768.17 | 766.76 | 767.02 b|
| 1 : 2 (k2)                             | 774.08 | 774.76 | 776.5  | 775.12 a|
| 2 : 1 (k3)                             | 763.87 | 766.51 | 767.4  | 765.93 b|

LSD 0.05 5.59

Notes: The numbers followed by the same letters in the same column or row (a,b) indicates that they are not significantly different in the LSD test level of 0.05.

The variance analysis showed that the sawdust-manure composition in the compost had very significant effect, while the treatment of Trichoderma application and the interaction between both factors did not significantly affect total amount of chlorophyll. Table 8 shows that the 1:2 sawdust-manure composition (k2) produced the highest total amount of chlorophyll which was 775.12 µmol.m⁻², and was significantly different from the other compost compositions. The lowest total amount of chlorophyll was produced by 2:1 sawdust-manure composition (k3), which was 765.93 µmol.m⁻².
Figure 1. Average stomata density (mm\(^{-2}\)) with sawdust-manure compost compositions and *Trichoderma* application

The variance analysis showed that the treatment of sawdust-manure composition in compost, the treatment of *Trichoderma* dosage, and the interaction between the two factors had no significant effect on stomata density. Figure 1 shows that the combination of the 2:1 sawdust-manure compost composition with 4 g *Trichoderma* application per polybag (k3t2) gave the highest stomata density of 8.97 mm\(^{-2}\), while the lowest stomata density was found in the treatment combination of 1:1 sawdust-manure compost composition with a dose of 4 g *Trichoderma* per polybag (k1t2) which was 8.22 mm\(^{-2}\).

![Figure 1](image1.png)

Figure 2. Average stomata opening area (mm\(^{2}\)) with sawdust-manure compost compositions and *Trichoderma* application

The variance analysis showed that the sawdust-manure composition in compost, *Trichoderma* dose, and the interaction between both factors had no significant effect on the stomata opening area. Figure 2 shows that the treatment combination of 2:1 sawdust-manure compost composition with a dose of 4 g *Trichoderma* application per polybag (k3t2) produced the largest stomata opening area of 0.0014.
mm², while the smallest stomata opening area was found in the treatment combination of 1:1 sawdust-manure compost with a dose of 4 g *Trichoderma* application per polybag (k1t2) and sawdust-manure composition 1:2 with a dose of 2 g *Trichoderma* per polybag (k2t1), both produced 0.0008 mm² stomata opening area.

### Table 9. Average leaf mass area (g/cm²) with various sawdust-manure compost compositions and *Trichoderma* application.

| Compost composition (Sawdust : manure) | Trichoderma dosage (g per polybag) | Average LMA | LSD 0.05 |
|--------------------------------------|------------------------------------|-------------|----------|
|                                      | 2 (t1)                             | 4 (t2)      | 6 (t3)   |          |
| 1 : 1 (k1)                           | 0.0018                             | 0.00253     | 0.00190  | 0.00209 c|
| 1 : 2 (k2)                           | 0.00273                            | 0.00314     | 0.00279  | 0.00289 b| 0.00073  |
| 2 : 1 (k3)                           | 0.00324                            | 0.00435     | 0.00333  | 0.00364 a|
| Average                              | 0.00260 b                          | 0.00334 a   | 0.00267 ab|          |
| LSD 0.05                             |                                    |             | 0.00073  |

Notes: The numbers followed by the same letters in the same column or row (a,b,c) indicates that they are not significantly different in the LSD test level of 0.05.

The variance analysis showed that the sawdust-manure composition in compost composition and *Trichoderma* application had very significant effect, while the interaction between the two factors had no significant effect on plant leaf mass area (LMA). Table 9 shows that the 2:1 sawdust-manure composition (k3) produced the largest LMA of 0.00364 g/cm², and was not significantly different from the sawdust-manure composition of 1:2 (k2), but significantly different from the 1:1 sawdust-manure compost composition (k1). The smallest LMA was found in the 1:1 sawdust-manure composition (k1) that was 0.00209 g/cm².

Application of 4 g *Trichoderma* per polybag (t2) produced the largest LMA that was 0.00334 g/cm², and was not significantly different from 6 g *Trichoderma* application per polybag (t3), but significantly different from 2 g *Trichoderma* application per polybag (t1). The smallest LMA was produced by the application of 2 g *Trichoderma* per polybag (t1) which was 0.00260 g/cm².

### 3.3. Discussion

Compost composition of 1:2 sawdust-manure (k2) gave the best results in several variables. These variables are plant height increase (34.64 cm), number of leaves (21.24 strands), stem diameter (8.56 mm), chlorophyll index (138.76), amount of a-chlorophyll (518.77 µmol.m⁻²), amount of b-chlorophyll (347.42 µmol.m⁻²), and total chlorophyll (775.12 µmol.m⁻²). This composition was the best among other compositions. This was presumably because it provided the best nutrient availability for cocoa seedlings. When there are more manure than sawdust then the available nutrients are greater in the compost. Setyamidjaja [15] stated that nutrients in compost will be absorbed by plants more quickly for metabolic processes. In addition, this is caused by the role of the nitrogen element in the growing media, which are the content in manure is 2.5% [16].

It gives an indication that the amount of manure applied to the soil contributes greatly to the availability and uptake of N by plants. Suminarti [17], stated that plants with high N uptake, can stimulate overall growth and the resulting in high chlorophyll content, which in turn also affects the amount of plant's ability to carry out its metabolic activities, especially photosynthesis.

Nitrogen is one of the constituent elements of chlorophyll in plants, and is also a source of protein for plants. Barchia [18] emphasized that plants with large nitrogen uptake will increase chlorophyll and if chlorophyll increases, it will also increase the rate of photosynthesis which affects the formation
of the number of leaves in plants. The greater number of leaves allows greater capture of solar energy which will spur photosynthesis more rapidly and will ultimately result in stem growth and development. Hardjowigeno [19] added, the diameter of an increasingly larger stem is caused by the activity of division and enlargement of cells whose source of energy comes from photosynthesis.

Application of 4 g Trichoderma per polybag provided the best results compared to other Trichoderma doses. This was consistent with research by Rosmana [20] about the endophytic association of Trichoderma asperellum in Theobroma cacao which suggests that the dose of Trichoderma for good plants hrowth was 4 grams for each plant. Hakkar et al. [12] added that the application of Trichoderma asperellum to control fruit rot disease in cocoa plants at a dose of 4 grams per plant can inhibit the emergence of Phytophthora fruit rot by about 50%.

Research by Esrita, Ichwan, & Irianto [21] also revealed that Trichoderma dose of 4 g per plant gave the best growth and yield in tomato plants compared to the other doses of application. Addition of higher dose of Trichoderma sp. will not be necessary since the number of spores with 4 g application is already balanced in controlling the number of fusarium spores in the soil where the plant grows. This rate of application is also more economically beneficial.

The results showed that the interaction between the sawdust-manure compost composition with a dose of Trichoderma had no significant effect on all parameters observed. It seems like the treatment of Trichoderma dosage and composition of sawdust-manure in compost works independently. In fact, compost as an organic material with microorganisms such as Trichoderma was able to interact with one another. Hardianus, Suryantini, & Wulandari [22] confirmed that the provision of organic fertilizer can increase the population and activity of antagonistic microorganisms that are beneficial for plants such as Trichoderma sp. Trichoderma sp. as a biofertilizer and decomposer from organic fertilizer will also increasingly support the growth of seedlings, so the need for quality seedlings will be met. Apzani, Sudantha, and Fauzi [23] added that to further increase the effectiveness of compost, organic fertilizer should be fermented again with useful microbes (Effective Microorganism) such as Trichoderma sp. which can act as a decomposer and be able to stimulate plant growth. The combination of compost with useful microbes such as Trichoderma sp. will produce biocompost.

4. Conclusion

Application of compost composition with a ratio of sawdust and manure 1: 2 gave the highest results in increasing plant height (34.64 cm), increasing number of leaves (21.24 strands), increasing stem diameter (8.56 mm), chlorophyll index (138.76), amount of a-chlorophyll (518.77 µmol.m⁻²), amount of b-chlorophyll (347.42 µmol.m⁻²), and total leaf chlorophyll (775.12 µmol.m⁻²). Application of 4 g Trichoderma sp. per polybag gave the highest yields in increasing plant height (33.57 cm), leaf area (154.75 cm²) and Leaf Mass Area (LMA) (0.00334 g/cm²). There was no interaction between the treatment of compost composition with Trichoderma dose on all observed variables.

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