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Application of *Trichoderma* sp in pineapple biomass composting

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Abstract

Background: Pineapple litter has great potential as an organic source and if it can be decomposed properly it can turn into organic fertilizer. Pineapple biomass is difficult to decompose due to high lignin and cellulose content which slow down the decomposition process. Thus the addition of auxiliary microbes such as decomposer fungi is thought to increase the rate of decomposition.

Aim: The aim of this research is determine the effect of *Trichoderma* sp. inoculum grown on rice substrate on pineapple litter composting

Method: This study was in a completely randomized design (CRD) method consisting of 6 treatments with two stages of testing, there are fungal inoculum productivity test and application on pineapple litter. The spore density and viability (CFU) values were calculated for the inoculum that was incubated for 14 days to determine the productivity level of the inoculum. The pineapple litter given the inoculum is then subjected to a decomposition process for seven weeks. Pineapple litter compost was analyzed at the fourth and seventh week with the parameters observed were C-organic content and C/N ratio.

Result: The results showed the spore density was 2.0 $x10^7$ spores / ml and the CFU value was $10.6x10^8$ cfu / ml with 100% viability. The best compost had the lowest C-organic and C/N ratio value, there are P3 compost obtained 12,5% C-organic and P2 compost obtained 9,6 C/N ratio.

Conclusion: The use of rice media as an inoculum medium for the Fungi *Trichoderma* sp. can increase the level of productivity of Fungi *Trichoderma* sp. with a viability percentage of 100% at 14 days of inoculum age.

Keywords: *Trichoderma* sp; Compost; Ligninolytic; C/N ratio; Pineapple litter

1. Introduction

Pineapple biomass in the form of litter is rarely reused as compost, because the lignin content in pineapple litter slows down the decomposition process. Pineapple litter consists of pineapple leaves, nodules, and skins. The polymer content in pineapple litter on pineapple leaves contains 43.53% cellulose, 21.88% hemicellulose, and 13.88% lignin. In the tuber pineapple contains 24.53% cellulose, 28.53% hemicellulose, and 5.78% lignin. And the pineapple skin contains 28.69% hemicellulose, 40.55% cellulose, and 10.01% lignin [1]. Lignin polymers are composed of phenylpropanoid compounds and aromatic compounds that have a heterogeneous structure that is recalcitrant (difficult to degrade) and the lignin polymer acts as a plant structure reinforcer that makes up ± 30% of plant material. The chemical structure of lignin is complex, heterogen, hydrophobic and aromatic [2]. To accelerate the decomposition process of pineapple litter, bio activators from microorganisms are needed that are capable of producing extracellular ligninolytic enzymes and degrade polysaccharide components contained in pineapple litter. The microorganisms that have been tested and

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known to have the ability to produce ligninolytic enzymes are *Trichoderma* sp. [3]. Bio- activators can be grown as inoculum on media that contain lots of carbohydrates such as rice media containing starch compounds which are useful as carbon sources for decomposer microorganisms. So that it is expected the *Trichoderma* sp fungal inoculum grown on rice media can be used as a compost bio-activator.

2. Material and methods

Pineapple biomass was obtained from the Great Giant Pineapple Company, Terbanggi Besar, Lampung, Indonesia. This research use *Trichoderma* sp also came from isolation on pineapple substrate which furthermore the isolate has also been characterized as a ligninolytic isolate.

This study was on completely randomized design which was carried out in two stages of testing, that is the spore productivity test of the *Trichoderma* sp fungal grown on rice media and application of inoculum on pineapple litter.

Inoculum application test for pineapple litter composting was carried out using a modification of the Takakura Home Method (THM) method which was carried out for 7 weeks. The composition of the raw material, that are pineapple leaf litter, pineapple hump, and a mixture of both was made 6 treatments (KP1, KP2, KP3, P1, P2, P3) then carried out 3 repetition for each treatment.

K: Control (Without Inoculum)
KP1 = pineapple leaf litter + cow manure (2:1).
KP2 = pineapple hump litter + cow manure (2:1).
KP3 = pineapple leaf litter + pineapple hump litter + cow manure (1: 1: 1).
P1= pineapple leaf litter + cow manure (2:1) + 1% of *Trichoderma* sp. inoculum.
P2= pineapple hump litter + cow manure (2:1) + 1% of *Trichoderma* sp. inoculum.
P3= pineapple leaf litter + pineapple hump litter + cow manure (1: 1: 1) + 1% of *Trichoderma* sp. inoculum.

Inoculum media was prepared using pounded rice, 4% CaSO4 solution and 2% CaCO3 solution. Then it is mixed with 60 g of rice and put in a 250 ml sterile glass bottle. The media is sterilized for 15 minutes, then the media is waited until the temperature drops to near room temperature. Inoculation of *Trichoderma* sp Fungi was carried out on inoculum media, then incubated at room temperature for 14 days [4]

The compost quality testing was carried out by analyzing the levels of carbon (C), nitrogen (N), and C/N ratio. Total organic carbon was determined using wet digestion method [5]. Nitrogen totals were calculated by the Kjeldahl method [6]. Compost quality testing is performed by analyzing carbon content (C-Organic) and compost C/N ratio, Then the ANOVA (*Analysis of Variance*) test was performed on the data from the analysis with the LSD (Least Significant Difference) test using the $\alpha = 5\%$ level.

3. Results

3.1. Spore Density and Viability of *Trichoderma* sp. Inoculum

Spore count and inoculum viability grown on rice inoculum media showed interesting results (Table 1).

Table 1 Spore Density and viability of *Trichoderma* sp

Parameters				
Spore Density	**Spore Viability**	**Spore Density Log**	**Spore Viability Log**	**Percentage of Spore Viability**
2,2x107 Spora/ml	1.1x109 CFU/ml	7.34	9.03	>100%

Table 1 shows that the density of inoculum *Trichoderma* sp. in rice media was 2.2×10^7 spores/ml and the viability of the inoculum of the fungus *Trichoderma* sp. is 1.1×10^9 CFU/ml with a viability percentage of 100%. This indicates that the spores produced by the inoculum of the fungus *Trichoderma* sp. those grown on rice media were able to germinate optimally with a relatively high level of spore density.

3.2. C Organic of Compost

The results of the analysis of the carbon content of the compost at the 4th and 7th week of composting showed varied results.

Table 2 C-Organic of the 4th and 7th Weeks of Composting

Treatments	C-Organic (%)	
	Week-4	**Week-7**
KP1	24.5	20.5 cd
KP2	22.4	23.0 d
KP3	20.8	15.7 ab
P1	26.6	17.5 bc
P2	21.0	13.2 a
P3	22.0	12.5 a

Numbers in row with different superscripts are significantly different ($p<0.05$)

In table 2, the 4th week of composting did not show significantly different results, whereas in the 7th week there were significant differences between treatments. 7th week of composting results. In week 7, the highest C-organic content was obtained at KP2 (23%), and the lowest at P3 (12.5%). According to [7] the lower the C-organic content in the compost, the better the maturity level of the compost. So that P3 is the compost treatment that has the best quality and level of maturity because it has the lowest C-organic content.

3.3. Nitrogen (N) total

The results of the analysis of total nitrogen content in the 4th and 7th week treated compost are shown in Table 3

Table 3 Total Nitrogen for Each Treatment in the 4th and 7th Weeks of Composting

Treatments	Nitrogen Total (%)	
	Week-4	**Week 7**
KP1	2.12 bc	1.75 cd
KP2	2.29 c	1.81 cd
KP3	2.05 b	0.79 a
P1	1.55 a	1.18 ab
P2	1.70 a	1.36 bc
P3	1.60 a	1.26 ab

Numbers in row with different superscripts are significantly different ($p<0.05$)

Table 3, composting results of N total at week 4 and week 7 both showed significant differences between treatments. The results showed that the lowest total nitrogen content in the 4th week was obtained in treatment P1 (1.55%), and the highest in treatment KP2 (2.29%). Whereas in the 7th week, the lowest total nitrogen content was obtained in the KP3 treatment (0.8%), and the highest level was in the KP2 treatment (1.81%). In the final results of composting, it can be seen that the KP2 treatment has the highest total nitrogen content compared to the other treatments, so that KP2 compost is the best compost based on the N-Total content.

3.4. C/N Ratio Value

The results of the analysis of the C / N ratio value for 4 and 7 weeks of the composting process is presented in **Figure 1.**

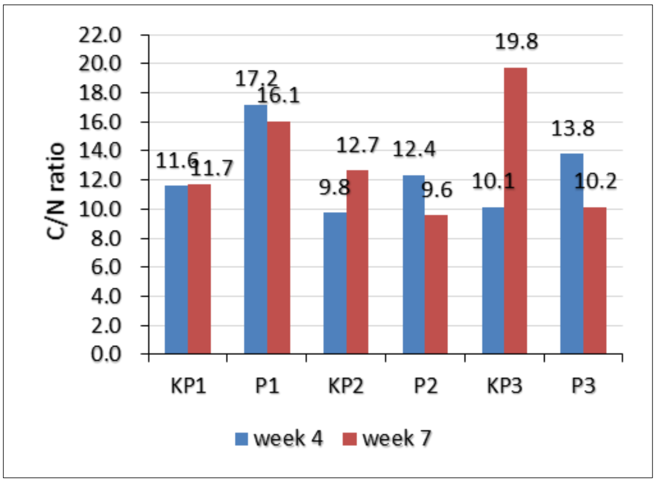

Figure 1 C / N ratio of compost of week 4 and week 7 of composting

Figure 1 showed that at week 4, the lowest C/N ratio was obtained in the KP2 treatment (9.8%) and the highest was in P1 treatment (17.2%). Whereas at week 7, the lowest C/N ratio value was obtained in the P2 treatment (9.6%), and the highest value was in KP3 treatment (19.8%). The treatments that experienced an increase in the C/N ratio during the seven weeks of the composting process were KP3, KP2, and KP1. Whereas in the P1, P2, and P3 treatments, there was a decrease in the C / N ratio during the seven weeks of the composting process. According to [8] the lower C / N ratio in compost will affect the decomposition process of organic matter in the compost, so this will cause the decomposition process to be more optimal. The optimal composting process will produce better quality compost. A treatment that produces compost with the best level of maturity is compost treatment P2. Physical characteristics of mature compost are blackish brown to black, odorless, and have a texture like humus soil.

4. Discussion

The value of the spore density of the fungus *Trichoderma* sp is said to be high if the value is more than 10^7 spores/ml, so it can be concluded that the spore density value of the inoculum of the fungus *Trichoderma* sp in this study was very high if it was grown on rice media. The high spore density value indicates the Fungi *Trichoderma* sp. can grow optimally on rice media which is rich in starch. Fungi *Trichoderma* sp. has the ability to produce enzymes amylase, ligninase, cellulase endoglucanase, and cellulase ectoglucanase which can break down starch in rice into simple carbohydrates needed by the fungus *Trichoderma* sp. as a carbon source [9].

In the 7th week, the analysis of C-organic content in the compost showed significantly different results between treatments, this shows that the seven-week composting process was much more effective and produced much more mature compost. The drastic reduction in C-organic levels was triggered by the involvement of the fungus *Trichoderma* sp. which helps other microbial decomposers to decompose more organic matter in the compost, resulting in a drastic increase in decomposer activity. During the composting process, CO2 will be released, so that the C-organic content will decrease along with the decomposition process that takes place in the compost. Carbon changes that occur in the composting process due to carbon biodegradation allow the release of C into the environment as a result of hydrolysis by extracellular enzymes from decomposer microorganisms; and the carbon becomes a source of energy for microorganisms in the environment [10].

The addition of *Trichoderma* sp. the compost treatment P1, P2, and P3 did not have an effect on increasing nitrogen levels in the compost, instead the addition of *Trichoderma* sp. compost has a very drastic reduction in total nitrogen content. Nitrogen is the main component chlorophyll and amino acids (building blocks of protein), component compounds energy transfer (ATP) and components of nucleic acids (DNA). Besides that research by [11] also confirmed the presence of nitrate ($NO3^-$) and ammonium ($NH4^+$) in the cytosolic stream as a nitrogen source; Thus the occurrence of biodegradation of plant tissue by microorganisms allows the release of N into the environment. At the beginning of the decomposition process, the C-organic content is still too high so that decomposer microbes need more nutrients such as nitrogen to produce enzymes and break down more C-organic, and this will trigger competition for sources of nitrogen and other nutrients in the compost which causes fungi. These must compete with other decomposer organisms to survive and to be able to carry out metabolism to produce enzymes that decompose organic matter in compost [12].

The change in the value of the C / N ratio describes the level of compost maturity. Based on the rules of SNI 19-7030-2004, the C / N ratio of compost is meet the minimum requirements for mature compost if the value range is 10-20%. Based on the SNI Standard, if the C / N ratio of compost exceeds the maximum limit (> 20%), the compost will not be effective in releasing nutrient minerals because the decomposition process is still not perfect and the decomposer organisms still need more nitrogen and other nutrients to break down organic carbon in compost. Meanwhile, if the compost C / N ratio value is below the minimum limit value set by SNI (<10%), then this will trigger nitrogen accumulation in the form of ammonia (NH_3) which is very volatile, so that nitrogen compounds in the compost will decrease faster. The value of the C / N ratio of KP3 composts increased drastically due to a decrease in the total nitrogen content in the compost, so that the final composting results in the KP3 treatment showed a lower total Nitrogen content than C-Organic content. Based on [13] that microbes need nitrogen as a metabolic material and building blocks of cells, and also as a source of energy.

The high level of C-Organic in compost will cause the nitrogen content in the compost to decrease faster. This is because more nitrogen is absorbed by decomposer microorganisms to carry out metabolic processes and produce enzymes to decompose organic matter. so this will have an impact on the high levels of C-Organic in the compost material. Normally the C / N ratio will decrease along with the decomposition process of organic matter until mature compost is formed, and this happened in the compost treatment P1, P2, and P3 whose C / N ratio values decreased during the seven weeks of the composting process. Treatment P2 is a treatment that has the lowest C / N ratio value than treatment P3 and P1. The decrease in the value of the C / N ratio in compost during the decomposition process is caused by the conversion of organic carbon to CO_2 by decomposer organisms. During the decomposition process, organic carbon is converted into CO_2 + H_2O + nutrients + humus + energy. During the decomposition process, CO_2 from the results of microbial metabolism will be released into the air and this will reduce carbon levels so that the C / N ratio of compost can decrease to a stable condition. This is due to the low levels of C-organic in the compost, thus nitrogen levels become higher and have an impact on decreasing the value of the compost C/N ratio so that more decomposer microorganisms will break down nitrogen into the form of NH_3 compounds which are volatile and this will lead to lower nitrogen levels in the compost.

5. Conclusion

The conclusion from the results of this study is that rice can be used as a medium for growth and multiplication of *Trichoderma* sp fungi inoculums because rice media can increase the productivity of *Trichoderma* sp fungi until it reaches a 100% viability level. The application of *Trichoderma* sp inoculum in the composting process of pineapple litter has a significant effect on reducing levels of C-organic and C / N ratio in compost resulting from the composting process of pineapple litter. P2 treatment had the lowest C / N ratio. Meanwhile, P3 treatment had the lowest C-organic content. So it can be said that the two compost treatments are the most ideal combination of compost materials for making compost.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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