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Viability of *Trichoderma harzianum* Grown on Different Carrier Formulation

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Abstract

The suitable carrier composition is needed to ensure the effectiveness of *Trichoderma harzianum* as inoculant of biocontrol, plant growth promotion and decomposer fungus. The research aimed to investigate formulation of carrier to enhance of *Trichoderma harzianum* viability was conducted from January till June 2015 in Laboratory. Testing of the compotition of carrier materials on viability of *Trichoderma harzianum* using a completely randomized design consisting of 9 treatment compotitions of peat soil, cow manure, biocharcoal and nutrient. The observed variables were the population of *Trichoderma harzianum* (cfu/g) on a regular basis *i.e* 2, 4, 8, 12, 16, 20, and 24 weeks after production, pH and moisture content (%) of media. The result showed that the different carrier formulations resulted number of spore varried, from 1.33×10^5cfu/g to 7.98×10^6cfu/g. The best formulation of *Trichoderma harzianum* was peat soil 40% + cow manure 40% + biocharcoal 10% + nutrient 10% with the maximum population count achieved 7.98×10^6cfu/g after 24 weeks of storage.

Keywords: Trichoderma harzianum, carrier formulation, viability, biocharcoal, nutrient.

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1. Introduction

The sustainability of rice production is facing a seriously problems of soil fertility degradation. Currently, about 70% of paddy soil has a low low organic content (<2%) and it categorized as unhealthy soil [1]. In contrast, rice straw biomassa as a locally mainly source of organic material are normally remove or simply burning. The burning rice straw is not only lead to loss of nutrients (carbon, nutrient of nitrogen, potassium, phosphor and sulphure) and also release about 5.4 ton of carbon dioxide as greenhouse gases (GHGs) and environmental hazard that produce [2-3]. Returning the rice straw or applied composted straw increase the efficiency of fertilizer, and supply of the majors nutrients for rice (Si, K, N, P, Ca, Mg) and micro nutrients [1]. The composting of rice straw is needed to control the pathogenic microbes in straw that may endangers to

the next cropping and to accelerate the composting process and as well as to improve the quality of composted straw [4].

Trichoderma spp. play an important roles in biologically decomposition and also are known to be producer of cellulose hemicellulose enzymes for lignocellulosic biomass biodegradation and biocontrol agent [5]. Application of *Trichodermaspp* was enable to decrease carbon content and increase nitrogen concentration of palm oil empty fruit bunches in 3-6 weeks after incubation and decrease the C/N ratio significantly [6, 7]. The efficacy of *Trichoderma spp* inoculant are highly correlated the composition and quality of the carriers in supporting its viability. Therefore, the suitable and effective carriers composition is required for mass production of decomposer for commercially purpose. The carrier is normally enrich with organic manures [8, 9] used farmyard manure, vermi-compost, spent mushroom compost, broken maize grain and sorghum grain for multiplication of *Trichodermaharzianum.* Adan *et al.* [10] reported that formulation black gram bran + peat soil and mixed with some water resulted the high number of spore reached 24×10^7 spores/g. In order to select the appropriate material for mass production of *Trichoderma harzianum,* different material formulation were tested. The aim of this research was to find the best formulation as carrier to enhance the viability of *Trichoderma harzianum.*

2. Materials and Methods

2.1. Laboratory Experiment

The research was conducted from January till June 2015 at Microbiology Laboratory of CV. BintangAsriArthauly Bandung. The experiment was set up as randomized completely design, consisted of nine treatments and provide with three replications.

2.2. Preparation of Fungal Spores

The fungal inoculum (*Trichodermaharzianum*) was grown on Potatos Dextrose Agar (PDA) medium until heavy growth of conidia. Conodia was harvested by scrapping the surface of the coloni by using spatula, and transferred to sterile Potato Dextrose Liquid (PDL) medium and shaked at 120 rpm for three days (Thermolinescientific). The initial spore concentration counted by using Total Plate Count (TPC) technique was 10^8 spores/mL.

2.3. Formulation of Fungal Carriers

Different carriers formulation for decomposer bioagent were used as follows:

A = peat soil 50% + manure 50% + biocharcoal 0% + nutrient 0%,
B = peat soil 47.5% + manure 47.5% + biocharcoal 0% + nutrient 5%,
C = peat soil 45% + manure 45% + biocharcoal 0% + nutrient 10%,
D = peat soil 45% + manure 45% + biocharcoal 10% + nutrient 0%,
E = peat soil 42.5% + manure 42.5% + biocharcoal 10% + nutrient 5%,
F = peat soil 40% + manure 40% + biocharcoal 10% + nutrient 10%,
G = peat soil 40% + manure 40% + biocharcoal 20% + nutrient 0%,
H = peat soil 37.5% + manure 37.5%+biocharcoal 20% + nutrient 5%,
I = peat soil 35% + manure 35% + biocharcoal 20% + nutrient 10%.

All the materials were mixed to obtain the proposed stated different formulation, and were packed into aluminium foil bag (@100 g). The carrier material was sterilized by using outoclave at 120 °C and 15 Psi. Fungal spores suspension was added to each pack by injecting at the rate 30% (v/w), than stored at room temperature.

2.4. The Observed Variables

The enumeration of fungal spore concentration was done at 2, 4, 8, 12, 16, 20, and 24 weeks after production. The plate count technique was followed for fungal count. Ten grams from each formulation was added to sterile bottle 90 ml of sterilized distilled water, shaked for 15 minutes using orbital shaker (120 rpm/min). Serial dilution (10^{-4} – 10^{-5}) of each stored formulation was made up. A volume of 1.0 mL of 10^{-4} and 10^{-5} dilution of store formulation of fungi was poured into Petridish with 10 mL of PDA. The plates were incubated at room temperature for three days. Formed fungal colonies were counted and the number of colony unit form (cfu/g) of *Trichoderma harzianum*. The formulated fungal carriers were taken to laboratory for determination of pH and moisture content. All data were analysed by one-way ANOVA, and continued by Duncan Multiple Range's Test at 5%.

3. Result and Discussion

The pH value of the carrier shows the range 7-8 (Table 1.). The highest pH value is shown in formulation D at 8.28, and lowest in the formulations I of 7.96. Differences in pH values strongly associated with moisture media, where the change in pH caused

Table 1: pH and water content of formulated carriers after six months storage.

Treatments	pH	Water Content (%)
A = peat soil 50%+ manure 50%	8.11 b	26.73 a
B -= peat soil 47.5% + manure 47.5% + nutrient 5%	8.16 ab	30.96 a
C= peat soil 45% + manure 45% + nutrient 10%	8.12 b	28.54 a
D= peat soil 45% + manure 45% + biocharcoal 10%	8.28 a	26.03 a
E = peat soil 42.5% + manure 42.5% + biocharcoal 10% + nutrient 5%,	8.24 a	32.65 a
F = peat soil 40% + manure 40% + biocharcoal 10% + nutrient 10%,	8.09 b	26.19 a
G= peat soil 40% + manure 40% + biocharcoal 20%	8.07 bc	25.19 a
H = peat soil 37.5% + manure 37.5% + biocharcoal 20% + nutrient 5%	8.07 bc	27.01 a
I = peat soil 35% + manure 35% + biocharcoal 20% + nutrient 10%	7.96 c	29.83 a
Note: Value followed by same letter in each column do not different significantly.		

by the reaction between CO_2 and water [11]. The availability of water strongly affects microbial growth, so moisture content and water activity are the key factor in solid formulation [12]. Although the results showed that the water content of the carrier did not significant differences among the carrier material formulations. [13] stated that moisture of the solid-substrate are not the only factor that affected *Trichoderma* conidia production, but in the solid state fermentation is also affected by the solid composition and structure, as well as by the cultivated strain.

Sargin *et al.* [12] stated that the production of fungal strains optimum at the pH range of 3.50-6.0. The results showed pH values above pH 6.0, however Motta *et al.* [14] stated that*Trichoderma*spp. is able to grow under wide range of pH (4-9). Agosin *et al.* [15] stated that spore yield and volumetric productivity of *Trichoderma harzianum* were greater at pH 7.0 than pH 4.0. Increasing the pH value to the alkaline conditions tend to suppress the availability of some nutrients for fungus germination.

The viability of *Trichoderma harzianum* was studied during 24 weeks storage at different carriers formulated (Table 2). The highest population of *Trichoderma harzianum* about7.98×10^6cfu/g after 24 weeks storage was achieved by formulation F, followed by formulation B (3.57×10^6cfu/g), C (2.73×10^6cfu/g), E (1.78×10^6cfu/g), G (1.07×10^6cfu/g), H (2.00×10^6cfu/g), and I (1.85×10^6cfu/g). The initial population of *Trichoderma harzianum* of formulation F was 4.67×10^5 CFU/g at 2 weeks after inoculation, gradually increased to 5.13×10^6 CFU/g at 12 weeks storage, but decline at 16 and 20 week storage. The lowest population of *Trichoderma harzianum* after 24 weeks storage was obtained by formulation D (1.33×10^5cfu/g), and formulation A (6.67×10^5cfu/g).

The results showed that the addition of biocharcoal (10% and 20%), and nutrients (5% and 10%) was able to improve and retain the viability of *Trichoderma harzianum* on different formulations of carrier material. These results were similarly with Kumar

Table 2: Viability of *Trichoderma harzianum* at different formulated 'carriers.

Treatments	Population of *Trichodermaharzianum*(cfu/g) after						
	2 weeks	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks
A	2.17×10^5 a	9.00×10^5 a	8.00×10^5 abc	1.58×10^6 a	1.17×10^5 d	8.00×10^5 bc	6.67×10^5 cd
B	2.33×10^5 a	9.83×10^5 a	1.53×10^6 ab	2.05×10^6 a	1.70×10^6 ab	1.87×10^6 a	3.57×10^6 ab
C	2.33×10^5 a	1.13×10^6 a	9.00×10^5 abc	1.43×10^6 a	1.27×10^6 abc	2.35×10^6 a	2.73×10^6 ab
D	1.17×10^5 a	7.00×10^5 a	5.00×10^5 c	1.13×10^6 a	4.50×10^5 dc	2.17×10^5 c	1.33×10^5 d
E	2.17×10^5 a	8.67×10^5 a	1.42×10^6 ab	2.25×10^6 a	1.13×10^6 abc	2.12×10^6 a	1.78×10^6 ab
F	4.67×10^5 a	1.85×10^6 a	2.65×10^6 a	5.13×10^6 a	3.55×10^6 a	1.90×10^6 a	7.98×10^6 a
G	2.83×10^5 a	5.83×10^5 a	6.17×10^5 bc	3.25×10^6 a	7.50×10^5 bc	1.00×10^6 ab	1.07×10^6 bc
H	3.83×10^5a	1.55×10^6 a	1.83×10^6 a	3.18×10^6 a	9.00×10^5 bc	2.00×10^6 a	2.00×10^6 ab
I	3.83×10^5 a	1.13×10^6a	2.05×10^6 a	2.00×10^6 a	1.80×10^6 ab	1.68×10^6 a	1.85×10^6 ab

Note: A: peat soil 50% + manure 50% + biocharcoal 0% + nutrient 0%, B: peat soil 47.5% + manure 47.5% + biocharcoal 0% + nutrient 5%, C: peat soil 45% + manure 45% + biocharcoal 0% + nutrient 10%, D: peat soil 45% + manure 45% + biocharcoal 10% + nutrient 0%, E: peat soil 42.5% + manure 42.5% + biocharcoal 10% + nutrient 5%, F: peat soil 40% + manure 40% + biocharcoal 10% + nutrient 10%, G: peat soil 40% + manure 40% + biocharcoal 20% + nutrient 0%, H: peat soil 37.5% + manure 37.5% + biocharcoal 20% + nutrient 5%, and I: peat soil 35% + manure 35% + biocharcoal 20% + nutrient 10%. Value followed by same letter in each coloumn do not differ significantly

et al. [16], which is a carrier-based biocharcoal formulation capable of suppressing a decrease in viability of *T. viridae* gradually, and remained viable up to 4 months of storage. Baghel *et al.* [17] reported that the formulation using 15% biocharcoal was able to maintain the viability of *Trichodermaviridae* to 260 days of storage. Moreover, in the formulation F, wherein the ratio of nutrients 10% and 10% showed stable viability of *T. harzianum* when compared to other formulations. The addition of nutrients was able to increase the availability of nutrients for the fungus. In contrast, the increase of biocahrcoal content up to 20% without nutrients causing decline of *Trichoderma harzianum*viability. This can be seen in the formulations G, wherein the formulation without the addition of nutrients may result in nutrient limitation. According Kresnawaty *et al.* [11] on biocharcoal carbon available in the form of crystals and aromatic making it difficult to use by the fungus, while the germination of the fungus requires C and N. Elad *et al.* [18] reported that biocharcoal in low doses (1%-5%) contain residual tars compound which have been found to aid seeds germination and trigger the growth of microorganisms and have biocidal properties.

4. Conclusion

The formulated carrier for *Trichoderma harzianum* consisted of mixture of peat soil 40% + cow manure 40% + biocharcoal 10% + nutrient 10% resulted the best viability of *Trichodermna harzianum* (7.98×10^6cfu/g) at moisture content of 26.19%, and pH value of 8.09 after six months of storage.

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