Conference Paper

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 composition 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 varied, from $1.33 \times 10^5$ cfu/g to $7.98 \times 10^6$ cfu/g. The best formulation of *Trichoderma harzianum* was peat soil 40% + cow manure 40% + biocharcoal 10% + nutrient 10% with the maximum population count achieved $7.98 \times 10^6$ cfu/g after 24 weeks of storage.

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

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 biomass 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...
thenextcroppingandtoacceleratethecompostingprocessandaswellastoimprove
thequalityofcompostedstraw[4].

*Trichoderma* *spp.*playanimportantrolesinbiologicallydecompositionandalsoare
knowntobeproducerofcellulosehemicelluloseenzymesforlignocellulosicbiomass
biodegradationandbiocontrolagent[5].Applicationof*Trichodermaspp*wasenable
todecreasecarboncontentandincreasenitrogenconcentrationofpalmoilemptyfruit
bunchsin3-6weeksafterincubationanddecreasetheC/Nratiosignificantly[6,7].
Theefficacyof*Trichoderma* *spp*inoculantarehighlycorrelatedthecompositionand
qualityofthecarriersinsupportingitsviability.Therefore,thesuitableandeffective
carrierscompositionisrequiredformassproductionofdecomposerforcommercially
purpose. The carrier is normally enrich with organic manures [8, 9] used farmyard
manure, vermi-compost, spent mushroom compost, broken maize grain and sorghum
grainformultiplicationof*Trichodermaharzianum*. Adan et al. [10] reportedthatformu-
lationblackgrambran+peatsoilandmixedwithsomewaterresultedthehighnumber
ofsporereached24×10⁷spores/g. Inordertoselecttheappropriatematerialformass
productionof*Trichoderma* *harzianum*, differentmaterialformulationweretested. The
aimofthisresearchwastofindthebestformulationascarriertoenhancetheviability
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. Bintang Asri Arthauly Bandung. The experiment was set up as randomized com-
pletely design, consisted of nine treatments and provide with three replications.

2.2. Preparation of Fungal Spores

The fungal inoculum (*Trichoderma* *harzianum*) was grown on Potatos Dextrose Agar
(PDA) medium until heavy growth of conidia. Conidia was harvested by scrapping
thesurfaceofthecoloniabyusingspatula,andtransferredto steriley Potato Dextrose
Liquid (PDL) medium and shaked at 120 rpm for three days (Thermolinescientific). The
initialsporeconcentrationcountedbyusingTotalPlateCount (TPC) technique was 10⁸
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 autoclave at 120 °C and 15 Psi. Fungal spores suspension was added to each pack by injecting at the rate 30% (v/w), then 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>Water Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = peat soil 50% + manure 50%</td>
<td>8.11 b</td>
<td>26.73 a</td>
</tr>
<tr>
<td>B = peat soil 47.5% + manure 47.5% + nutrient 5%</td>
<td>8.16 ab</td>
<td>30.96 a</td>
</tr>
<tr>
<td>C = peat soil 45% + manure 45% + nutrient 10%</td>
<td>8.12 b</td>
<td>28.54 a</td>
</tr>
<tr>
<td>D = peat soil 45% + manure 45% + biocharcoal 10%</td>
<td>8.28 a</td>
<td>26.03 a</td>
</tr>
<tr>
<td>E = peat soil 42.5% + manure 42.5% + biocharcoal 10% + nutrient 5%</td>
<td>8.24 a</td>
<td>32.65 a</td>
</tr>
<tr>
<td>F = peat soil 40% + manure 40% + biocharcoal 10% + nutrient 10%</td>
<td>8.09 b</td>
<td>26.19 a</td>
</tr>
<tr>
<td>G = peat soil 40% + manure 40% + biocharcoal 20%</td>
<td>8.07 bc</td>
<td>25.19 a</td>
</tr>
<tr>
<td>H = peat soil 37.5% + manure 37.5% + biocharcoal 20% + nutrient 5%</td>
<td>8.07 bc</td>
<td>27.01 a</td>
</tr>
<tr>
<td>I = peat soil 35% + manure 35% + biocharcoal 20% + nutrient 10%</td>
<td>7.96 c</td>
<td>29.83 a</td>
</tr>
</tbody>
</table>

Note: Value followed by same letter in each column do not differ 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* about 7.98 × 10\(^6\)cfu/g after 24 weeks storage was achieved by formulation F, followed by formulation B (3.57 × 10\(^6\)cfu/g), C (2.73 × 10\(^6\)cfu/g), E (1.78 × 10\(^6\)cfu/g), G (1.07 × 10\(^6\)cfu/g), H (2.00 × 10\(^6\)cfu/g), and I (1.85 × 10\(^6\)cfu/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 3.13 × 10\(^5\) 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\(^5\)cfu/g), and formulation A (6.67 × 10\(^5\)cfu/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.'

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Population of <em>Trichoderma harzianum</em> (cfu/g) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td>A</td>
<td>2.17×10^5 a</td>
</tr>
<tr>
<td>B</td>
<td>2.33×10^5 a</td>
</tr>
<tr>
<td>C</td>
<td>2.33×10^5 a</td>
</tr>
<tr>
<td>D</td>
<td>1.17×10^5 a</td>
</tr>
<tr>
<td>E</td>
<td>2.17×10^5 a</td>
</tr>
<tr>
<td>F</td>
<td>4.67×10^5 a</td>
</tr>
<tr>
<td>G</td>
<td>2.83×10^5 a</td>
</tr>
<tr>
<td>H</td>
<td>3.83×10^5 a</td>
</tr>
<tr>
<td>I</td>
<td>3.83×10^5 a</td>
</tr>
</tbody>
</table>

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 5%; 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 column 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 *Trichoderma viridae* 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 *Trichoderma harzianum* \((7.98 \times 10^6 \text{ cfu/g})\) at moisture content of 26.19%, and pH value of 8.09 after six months of storage.

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References


