Trichoderma asperellum Cell Density in Several Carriers

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Keywords: Biochar, Carrier materials, Cell density, Storage period, *Trichoderma asperellum*.

Abstract: Biofertilizers was a carrier-based inoculant containing latent microorganisms. The incorporation of microorganisms in the carrier allows easy long-term storage and high effectiveness of biofertilizers. The aims of this study were to obtain the best carrier in maintaining population of *Trichoderma asperellum*. The research used completely randomized design, consist of six carrier material. The materials tested for its ability as a carrier were the mixed of empty fruit bunches compost + azolla + chicken egg shell + poultry manure; the mixed of rice straw + azolla + chicken egg shell + poultry manure; the mixed of rice husk + azolla + chicken egg shell + poultry manure; empty fruit bunches biochar; rice husk biochar and cow bone biochar. Research was conducted in soil biology laboratory, Agriculture, Universitas Sumatra Utara, Medan from April 2018 until May 2018.The result showed the cell density of *T. asperellum* after 8 weeks of storage on all carrier treatments was increasing steadily. The carriers with the highest population *T. asperellum* after 8 weeks of storage was the mixed of rice straw + azolla + chicken egg shell + poultry manure. The carbon and nitrogen content of the carrier affects the cells density of *T. asperellum*.

1 INTRODUCTION

Fertilizer is a material that is given into the soil to fulfill the requirement nutrients for plants to growth and production. Inorganic fertilizer is the choice of farmers to meet the nutrient needs of the plant. Since the green revolution, fertilization is doing intensively in the most agricultural system. However, unfortunately the productivity of the plants is not significant increase economically now (Parman, 2007).

Biological fertilizer (biofertilizer) is an alternative way to reduce dependence on inorganic fertilizers. Biological fertilizer made from carrier containing living cells or microbes (Rao, 1982). Inoculated microbes into biological fertilizers can serve as nutrient providers as well as a remodel of soil organic matter. Biological fertilizers have been shown to increase the ability of plant nutrient uptake, to accelerate composting process, and to improve soil structure (Tombe, 2008).

Biological fertilizers are generally packed in the liquid form. The formulation of biofertilizer in liquid form has several drawbacks, like: the difficulty in packaging, distribution, application, storage, and quality of fertilizer will be reduced if stored in the longer period. The quality of the fertilizer is reduced due to the decline of microbial population and the low resistance of microbes contained in the biological fertilizer. Carrier is considered as the best solution to overcome the deficiency (Putri, 2010). Combination materials formed carrier is a new alternative medium that can be used to grow, pack, and extend microbial storage time. The carrier composition must contain important components (organic nutrients) that can support microbial growth (Firdausi, 2016).

One type of microbe often inoculated into the biological fertilizer is *Trichoderma* sp. because *Trichoderma* sp. can grow in various propagation media. *Trichoderma* sp. is one of the soil saprophytic fungi which is advantageous to the plant due to its antagonistic properties with the pathogen (Gusnawaty, 2017). *Trichoderma* sp. can also be used to accelerate the decomposition process of organic materials such as carbohydrates (cellulose) with the help of cellulose enzyme (Rinata, 2016). Microbe carrier medium used in this study was avoided from materials that can be used as animal feed; as is usually done inoculum local seller are using bran, corn flour or rice.

Sari, R., Sabrina, T. and Mukhlis, .
Trichoderma asperellum Cell Density in Several Carriers.
DOI: 10.5220/0010073800200025
In *Proceedings of the International Conference of Science, Technology, Engineering, Environmental and Ramification Researches (ICOSTEERR 2018) - Research in Industry 4.0*, pages 20-25
ISBN: 978-989-758-449-7

Table 1: Carbon, Nitrogen, Phosphate and Potassium Analysis of Carrier Medium

Carrier	Water content (%)	C-organic (%)	Nitrogen (%)	Phosphate (%)	Potassium (%)
A	18.81	18.78	1.92	0.27	0.35
B	17.27	27.95	2.26	0.30	1.12
C	9.56	33.85	0.89	0.24	0.55
D	7.48	1.45	0.07	0.66	49.70
E	51.67	6.20	0.72	0.13	0.54
F	13.74	4.96	1.88	0.83	0.04

2 MATERIALS AND METHODS

This research was conducted at Soil Biology Laboratory, Agriculture, Universitas Sumatra Utara, Medan on April 2018 until May 2018.

The materials used as carrier of *T. asperellum* were empty fruit bunches compost, rice straw, rice husk, azolla, chicken egg shell, poultry manure, biochar of empty fruit bunches, biochar of rice husk, biochar of cow bone.

This study was used: completely randomized design, consisting of six treatments and repeated four times. Treatmentswere:

A : Mixture of empty fruit bunches compost (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%)
B : Mixture of rice straw (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%)
C : Mixture of rice husk (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%)
D : Biochar of empty fruit bunches (100%)
E : Biochar of rice husk (100%)
F : Biochar of cow bone (100%)

2.1 Preparation of Medium Carrier

Carrier materials were milled to pass through <2 mm mesh sieved, then mixed according to the composition of each treatment. Each 10 g carrier were packed in an autoclaveable plastic bag. Then all the carrier medium was sterilized using an autoclave for two consecutive days (121^{o}C and 1 atm pressure for 15 minutes). After completion of the autoclave process on the second day, water vapor in the plastic was allowed to dry first, then the plastic containing the carrier material sealed tightly using a sealer.

The carbon, nitrogen, phosphate and potassium content of each carrier medium were analyzed (Table 1).

2.2 Preparation of *T.asperellum*

Trichoderma asperellum used was a collection of soil biological laboratory, Agriculture, Universitas Sumatra Utara, Medan, that was isolated from the chipping trunk of oil palm tree (Sabrina, *et al*., 2017). Brooth culture of *T. asperellum* having a cell density 10^8cells / mL was used to inoculate. All bags stored in room temperature (25^{o}C).

2.3 Cell Density Observation

Observation of cell density was taken every 2 weeks during the storage period. Cell density was calculated using *haemocytometer*, with the formula (Gabriel & Riyatno, 1989):

$$K = \frac{t}{n \times 0{,}25} \times 10^6 \quad (1)$$

Description:
K : Number of cells / mL of solution
t : Number of cells in the sample box observed
n : Number of sample boxes
0.25 : Correction factor for use of small sample boxes on *haemocytometer*

3 RESULT AND DISCUSSION

The growth of *T. asperellum* in different carrier medium during the 2nd, 4th, 6th, and 8th week stored time is showed at Table 2.

Table 2: The Cell Density of *Trichoderma asperellum* in Several Carrier During Storage Period

Carrier	Storage Period (Week)			
	2	4	6	8
	10^7cells / g carrier			
A	12.18 a	21.73 b	53.13 b	242.50 b
B	11.68 b	29.08 a	119.75 a	329.00 a
C	5.29 c	9.79 c	45.63 c	232.75 c
D	0.30 f	0.61 f	12.94 f	75.63 f
E	0.53 e	0.66 e	18.13 e	91.25 e
F	0.62 d	0.71 d	23.00 d	139.38 d

Description: The number followed with different letters in each storage period (same column) are significant different at 5% level based on LSD test.

Observation at 2-week storage period showed the highest cells density of *T. asperellum* was obtained from the mixed carrier of empty fruit bunches compost (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (A). While at 4, 6, and 8 week storage period, the highest cells density of *T. asperellum* was obtained from the mixture carrier that consist of rice straw (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (B). The cells density of *T. asperellum* in all carrier medium increased during storage time. It may be due to the carrier contain the suitable organic materials that can ensure the survival of microbes during the storage (El-Fattah, *et al*., 2013). However, the cell number in the carrier formulated from the mixture of materials was higher than the cell density of the carrier formulated from biochar (Figure 1).

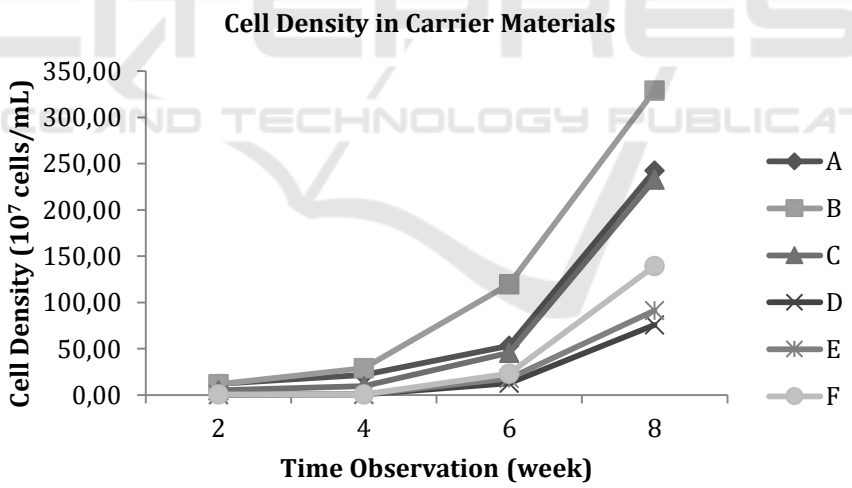

Figure 1: Cell density of *Trichoderma asperellum* in each carrier materials

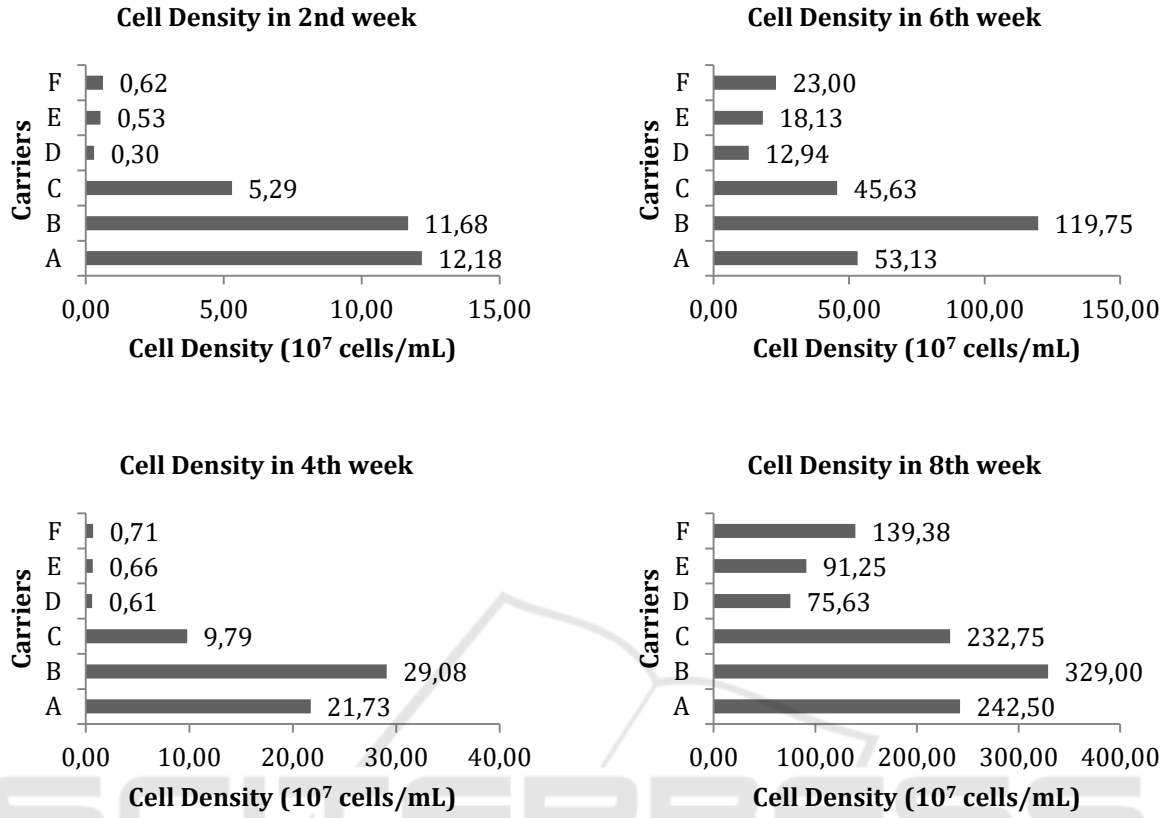

Figure 2: Cell density of *Trichoderma asperellum* during storage period

Increased cell density of *T. asperellum* in the carrier medium consist of the mixture of empty fruit bunches compost (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (A), the mixed of rice straw (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (B), and the mixed of rice husk (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (C) between observations ranged between 4.50 x 10^7 - 209.25 x 10^7 cells/g, while on treatment of the biochar of empty fruit bunches (100%) (D), the biochar of rice husk (100%) (E), and the biochar of cow bone (100%) (F) is ranging from 0.09 x 10^7 - 116.38 x 10^7cells/g (Table 3). *Trichoderma asperellum* is able growth in a variety of habitats and environments (Prabowo, 2006). *Trichoderma asperellum* also has a role as a biodecomposer and able to utilize organic materials containing cellulose (Widyastuti, 2001). To accelerate the availability of nutrients for its growth, *T. asperellum* produces cellulase enzymes that can degrade cellulose (Ratanaphadit, 2010). Increased higher cell density on carrier from mixture materials (A, B, and C) was possibly caused by the higher organic carbon (18.78-33.85%) and nitrogen (0.89-2.26%) content of the medium (Tabel 1).

Table 3: Increased Cell Density of *Trichoderma asperellum* in Carrier Materials per 2 weeks Storage Period

Carrier	Increased Cell Density per 2 Weeks Storage Period (10^7cells / g carrier)		
	2 – 4	4 – 6	6 – 8
A	9.55	31.40	189.38
B	17.40	90.68	209.25
C	4.50	35.84	187.13
D	0.31	12.33	62.69
E	0.13	17.46	73.13
F	0.09	22.29	116.38

4 CONCLUSIONS

Cell density *T. asperellum*in a different carrier increased to 8 weeks of storage. Cell density on the treatment of empty fruit bunches compost (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (A), the mixed of rice straw (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (B), and the mixed of rice husk (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) (C) higher than in the treatment of biochar of empty fruit bunches (100%) (d), biochar of rice husk (100%) (e), and biochar of cow bone (100%) (F). The mixed of rice straw (70%) + azolla (10%) + chicken egg shell (10%) + poultry manure (10%) was the best carrier to increase population of *T. asperellum* up to 8 weeks of storage. The carbon and nitrogen content of the carrier medium influenced the increase cell density of *T. asperellum*.

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