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Effect of *Trichoderma harzianum* in Different Media and Incubation Time on Color, pH and Conidia Density

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Abstract— *The purpose of this study was to determine effect of Trichoderma harzianum in different media and incubation time on color, pH and conidia density. The method used was laboratory experiment using nested Completly Random Design (CRD). Twelve treatments were deferentiated on four media for Trichoderma harzianum growth namely was PDA or Potato Dextrose Agar (P_1), molasses (P_2), Panicum maximum cv. mombaca (P_3) and Panicum maximum cv. mombaca + 5% molasses (P_4) nested to three incubation times namely 4, 7, and 10 days. The incubation time was 0 days for comparison before the inoculation. The variables observed were color, pH and Trichoderma harzianum conidia density. The color was analized as descriptive analysis, while pH and conidia density were analized using Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The results showed that Trichoderma harzianum had different color based on different media and incubation time. Trichoderma harzianum inoculated in different media and incubation time gave highly significant effect ($p<0.01$) on pH and conidia density. The conclusion of this study were : 1) Panicum maximum cv. mombaca + 5% mollases gave the best result for Trichoderma harzianum growth media and 2) The increasing of incubation time could increase color, pH and conidia density.*

Keywords— *Trichoderma harzianum, Panicum maximum cv. mombaca, Inoculan, Conidia density.*

I. INTRODUCTION

Indonesia have two season of the year, there is a problem in availability of forage in dry seasion. This problem can be overcome by utilizing feed preservation technology like silage. Silage is a forage preservation process by utilizing a lactic acid bacteria processed in a silo. A good ensilage is characterized by a rapid decrease in pH for good silage quality. Inoculans in the ensilage had an important role to produce good quality silage (Zakariyah, *et al*., 2015).

The research of Chalisty *et al*. (2017) using *Trichoderma veride* and *Lactobacillus Plantarum* can be reduced pH and ammonia concentration in forage mixture silage. Addition of *Trichoderma harzianum* also had an effect on hemicellulose degradation in sugar cane shoots (*Saccharum officinarum* L) fermentation (Suryadi, *et al*., 2018). Rizali *et al*. (2018) also find that *Trichoderma sp.* can be increasing the quality of nutrition like crude protein content, reduce crude fiber content and increase Total Digestible Nutrient (TDN) in leaves and palm fronds (*Elaeis oleifera*) fermentation. *Trichoderma sp.* is saprophyte which naturally can attack pathogenic fungi making it beneficial for plants. This microorganisms will develop rapidly in the soil rooting area and can be used as biological agents controlling soil pathogens. Some species are *Trichoderma veride*, *Trichoderma koningii*, *Trichoderma harzianum* etc. (Gusnawaty, *et al*., 2014).

Morphologically, *Trichoderma harzianum* has branched upright conidiophores, thicker and shorter phialids and oval conidial forms (Gusnawaty *et al*., 2014). The growth of *Trichoderma harzianum* is much influenced by the media (Likur *et al*., 2016 ; Gusnawaty, *et al*., 2017 ; Rulinggar, *et al*., 2016 and Urulial, *et al*.,2012). The growth properties of these spores are strongly influenced by saveral factor as humidity (moisture content about 14 – 15%), pH and nutrient composition of media. *Trichoderma sp*. has an optimum temperature for growth and maximum temperature are 30 - 36 ^{0}C (Urulial, *et al*., 2012).

Panicum maximum cv. mombaca is a forage used as a source of fiber in ruminant feed. The Nutrient composition based on Dwinarto *et al*. (2018) are dry matter 25.41%, Ash 12.85%, crude protein 12.89%, crude fiber 33.19%, crude fat 1.34%, TDN 38 – 61%, and nitrogen free extract 40 – 50%. *Trichoderma sp.* is a type of fungi that can utilize materials that contain crude fiber. As we know *Panicum maximum* cv. *mombaca* has high crude fiber content and it can be used by Trichoderma harzianum for its growth. Elad and Freeman (2002) in Syahni and Tamrin (2011) explain that *Trichoderma sp.* can produce enzymes β 1,3-*Glucanase*, *cellulase*, *chitinase*, *proteinase* and *phosphatase*. Based on this description, a study was carried out on the effect of *Trichoderma harzianum* in different media and incubation time, and the best treatment can be used as silage inoculant.

II. MATERIALS AND METHODS

Materials

The material used was pure culture *Trichoderma harzianum*, PDA, molasses (*Saccharum officinarum* L), and sun-dried *Panicum maximum cv. mombaca* grass were finely grinded 1 mm, aquadest, alcohol 70%. Equipment used includes autoclaves, laminar air flow, bunsen, glass bottles, plastic bags, ose needles, scales, heating pans, stoves, aluminium foil, cotton, incubators, 100 x magnification microscopes, haemocytometer, measuring pippete and pH meter. The nutrient composition *Panicum maximum* cv.

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mombaca and molasses showed in Table 1 and PDA composition were 15 g/L agar, 4 g/L potato extract, and 20 g/L dextrose (Anonymous[1], 2019)

TABLE 1. Nutrient Composition Treatment

Nutrient	*Panicum maximum cv. mombaca*	Molasses
Dry matter (%)	32.00	73.16
Ash* (%)	11.68	11.19
Crude Protein* (%)	8.75	3.40
Crude Fiber* (%)	33.85	0.39
Crude Fat* (%)	2.13	0.30
Neutral Detergent Fiber* (%)	65.24	-
Acid Detergent Fiber* (%)	42.03	-
Hemicellulose* (%)	23.22	-
Selulose* (%)	35.60	-
Silica* (%)	2.24	-
Lignin* (%)	4.19	-
Nitrogent Free Extract* (%)	43.59	84.72
Carbohydrate* (%)	77.44	85.11
C/N Ratio	8.85	25.30

Notes : *) Based on dry matter basis
Analysis result from Nutrition Laboratory, Faculty of Animal Science, University of Brawijaya.

Methods

The research method was a laboratory experiment using nested Complately Randomized Design (CRD). Twelve treatments were deferentiated on four media for the growth of *Trichoderma harzianum* including PDA (P_1), molasses (P_2), *Panicum maximum cv. mombaca* (P_3), and *Panicum maximum* cv. *mombaca* + 5% molasses (P_4) nested to three incubation time namely 4, 7, and 10 days, each treatment was replicated 4 times. The incubation time is 0 day for comparison before the inoculation process.

All media weighed as much as 30 grams, PDA and molasses were put into bottle glass then covered with aluminium foil, and others media put into a heat resistant polypropylene plastic bag. The media then sterilized in an autoclave at 121^0C for 15 menuts and cooled to room temperature. *Trichoderma harzianum* inoculated in the laminar air flow and given to all media as much as 5% from media weight then incubated and observed.

Variables Observed

The variables observed were color, pH and conidia density based on different media and incubation time. The time of *Trichoderma harzianum* incubation is determined as the time required to it's multiply. The amount of conidia density was calculated using formula :

$$K = \frac{t \times d}{n \times 0.25} \times 10^6$$

Note :
K = conidia density gr/mL sovent
t = number of conidia in all sample boxes
d = dillution factor
n = total the sample boxes calculated
0.25 = correction factor

Data Analysis

Data obtained from colour were analized as descriptive analysis. Conidia density and pH were processed using Microsoft Excel program and analyzed using Analysis of Variance (ANOVA) of nested Complately Randomized Design (CRD), if it showed differences then continued with Duncan's Multiple Range Test (DMRT).

III. Result and Discussion

A. The Color

Physical observation result based on colors in different media are presented as Table 2.

TABLE 2. Effect Treatment and incubation time on the color

Treatments	Incubation Time (day)			
	0	4	7	10
P_1	yellow	white	light green	dark green
P_2	brown	brown	brown	brown
P_3	green	little white	more white	more light green
P_4	green brown	verry little white	white to light yellow	green to dark grey

The growth of *Trichoderma harzianum* shown by the presence of white in some parts after the 4th incubation day. The research of Prayuwidayita (2009) found that the growth of *Trichoderma sp.*, firstly the surface part will look clean white, and has a dull mycelium, after adulthood then will have a yellowish green color. As Novianti (2018) states that the color is obtained after 4th and 7th incubation day.

Incubation after 10 days shows both P_1, P_3 and P_4 had a light-green to dark green color while the P_2 color remained brown. Gusnawaty *et al*. (2014) states on 7th day after incubation showed a deep green color but explained different species have different color during different incubation periods.

B. The pH

Trichoderma harzianum requires optimal conditions for growth, one of which is showed from the pH. The results showed a different highly significant effect ($p<0.01$) in Table 3.

TABLE 3. Effect Treatment and Incubation Time on pH

Treatments	Incubation (day)			Mean
	4	7	10	
P_1	4.23 ± 0.287^a	4.23 ± 0.330^a	5.05 ± 0.705^b	4.50 ± 0.594^{ab}
P_2	3.74 ± 0.126^a	3.78 ± 0.050^a	5.25 ± 0.238^b	4.26 ± 0.748^a
P_3	4.78 ± 0.287^a	4.60 ± 0.271^a	5.63 ± 0.222^b	5.00 ± 0.524^b
P_4	4.80 ± 0.583^a	4.75 ± 0.520^a	5.30 ± 0.688^b	4.95 ± 0.602^b

Notes : ^{ab}) The different superscripts in the same colomn and row showed highly significant differences ($p<0.01$)

Molasses media (P_2) had different highly significant effect ($P<0.01$) compare with P_1, P_3 and P_4 respectively 4.26, 4.5, 5.00 and 4.95 while P_3 and P_4 had not significant effect and P_2 had lower value. The pH of treatment P_1 and P_2 almost the same but lower in P_2. Treatment P_3 and P_4 was relatively higher in pH. It could be explained that growth of *Trichoderma harzianum* on P_2 treatment less than optimal

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because the environmental conditions were not optimum, especially for the pH in 4 and 7 days after incubations. While the P_3 and P_4 treatments gave an indication that the environmental condition were optimal with a pH range of 4.95 – 5.00.

There were different highly significant effect ($p<0.01$) between incubation time for each treatment but had not significant effect between treatment for each incubation. The pH tend to increase up to 10 days after fermentation for all treatment. The pH on 10 day after incubation had lower in P_1 treatment than other treatment and the highest was P_3 respectively 5.05 and 5.65.

The growth of Trichoderma besides requiring food substances for its growth also need a suitable environment such as temperature and pH. Trichoderma can grow well at the temperature of 7^0C – 41^0C. The pH requirements that suitable for Trichoderma's growth tend to low at 4.5 – 5.6 (Capucino and Sherman, 2014 in Pasaribu, 2017). Other studies suggest that Trichoderma is able to live within a fairly long pH range of 2 – 6 with maximum growth at pH 4 (Singh, *et al*., 2014). Based on the pH value of *Trichoderma harzianum* growth in this study is still in the optimal pH range.

C. The Conidia Density

The results of the conidia density calculation using *haemocytometer* obtain different highly significant effect ($p<0.01$) as presented in Table 4. The conidia density of molasses media (P_2) had different highly significant effect compare with P_1, P_3 and P_4 respectively 9.53, 10.16, 10.37, 10.30 log cfu/ml. Otherwhile P_1, P_3 and P_4 had no significant effect.

TABLE 4. Effect Treatment and Incubation Time on Conidia Density

Treatments	Incubation (day)			Mean
	4	7	10	
	(log cfu/ml)			
P_1	9.81 ± 0.306^a	10.05 ± 0.239^a	10.63 ± 0.288^b	10.16 ± 0.437^b
P_2	8.97 ± 0.316^a	9.56 ± 0.217^b	10.05 ± 0.364^b	9.53 ± 0.537^a
P_3	10.08 ± 0.310^a	10.32 ± 0.273^{ab}	10.72 ± 0.123^b	10.37 ± 0.353^b
P_4	9.79 ± 0.241^a	10.02 ± 0.225^a	11.09 ± 0.445^b	10.30 ± 0.660^b

Notes : ^{ab}) The different superscripts in the same colomn and row showed highly significant differences ($p<0.01$)

Each treatment based on incubation time had different highly significant effect on conidia density but between treatment there were not significant effect on 4 and 10 days after incubation. The conidia density tend to increase with the increasing time of incubation and 10 days after incubation relatively high. The highest conidia density on 10 days after incubation was P_4 and the lower was P_2 respectively 11.09 and 10.05 log cfu/ml.

Trichoderma harzianum can grow by utilizing nutrients from the media culture. Culture media are sterile media used to grow microorganism by providing a place and conditions that support the growth of microorganisms. This culture media consists of energy sources (carbon), nitrogen, vitamins and growth regulators, can also be added organic compounds or other complex compounds. The mcro elements needed for Trichoderma's growth include sulfur, phosphorus, Ca, Zn, Na, K, Cu, Mn, Mg and Fe (Cappucino and Sherman, 2014 in Pasaribu, 2017).

Trichoderma sp. is a fungus that commonly used to ferment and produce cellulase enzymes to break down crude fiber in feed ingredients. The availability of energy source substrates also has an important role in good fermentation because it provides glucose as an energy source for the growth and development of fungi (Sukaryana, 2013). *Panicum maximum* cv. *mombaca* is known have the higest crude fiber content (P_3 and P_4) compare to other media (Table 1) and *Trichoderma harzianum* is able to utilize the crude fiber content and other nutrients in this grass to support their growth. While crude fiber content of the molasses (P_2) was quite low so that the growth of fungus was not optimal as well as PDA (P_1), eventhough PDA had lower crude fiber content compare to *Panicum maximum cv. mombaca*. It can be count that the fiber content in PDA was below 1% (Anonymous[2], 2019).

The texture of P_3 and P_4 compare to P_1 and P_2 were more crumb so that *Trichoderma harzianum* can grow well as the results of Rulinggar *et al*. (2016) that the growth of rice bran media is better than the media of corn rice and compost because the texture of rice bran is more crumb. The conidia density of P_4 treatment at 10 days after incubation was slightly higher than P_3, it was possible to add C (carbon) element due to the addition of molasses. As Gusnawaty *et al*. (2017) states that inadequate C content in the media causes the Trichoderma growth to be uneventful.

The increasing *Trichoderma harzianum* conidia physically marked by an increase the colony diameter as Urulial *et al*. (2012) states and the count on 4 days after incubation about 10.45 log almost the same in this study between 7 and 10 days after incubation.

IV. CONCLUSION

The results of the study can be concluded that *Panicum maximum cv. mombaca* + 5% molasses were the best media for *Trichoderma harzianum* growth according color, pH and conidia density. While The best incubation time was 10 days.

V. RECOMMENDATION

The best result from this research can be used as silage *Panicum maximum cv. mombaca* inoculan.

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