he_Effect_of_Organic_Medium_Use_in_Formulation_of_Trchoderma.pdf

by

Pak. J. Biotechnol. Vol. 15 (1) 95-100 (2018)
www.pjbt.org

ISSN Print: 1812-1837
ISSN Online: 2312-7791

THE EFFECT OF ORGANIC MEDIUM USE IN FORMULATION OF *TRICHODERMA HARZIANUM* AND *PLEUROTUS OSTREATUS* IN VIABILITY AND DECOMPOSITION OF CACAO POD HUSKS WASTE

Suherah[1*], Tutik Kuswinanti[1,2**], Ade Rosmana[1,2], Burhanuddin Rasyid[1,3]

[1]Post-Graduated Program, Hasanuddin University, Makassar, Indonesia, [2]Department of Plant Protection, Agriculture Faculty, Hasanuddin University, Makassar, Indonesia, [3]Department of Soil, Agriculture Faculty, Hasanuddin University, Makassar, Indonesia. Email: **koeswinanti@yahoo.com; *hela_317@yahoo.co.id

Article received 29.11.2017, Revised 9.2.2018, Accepted 17.2.2018

ABSTRACT

Biomass waste can be processed into high nutritious organic fertilizer and improve soil structure. This study aims to assess viability of *Trichoderma harzianum* TH03 and *Pleurotus ostreatus* PO2 isolates that propagated in different growth mediums: rice, corn and sawdust medium, followed by formulation in powder form and stored in different packaging after 2-24 weeks of storage periods under room temperature. This study used Completely Randomized Design with two factors : mediums and packaging types with three replications. The highest viability was observed in *T. harzianum* TH03 derived from rice medium and *P. ostreatus* PO2 from corn medium. Aluminum foil and plastic bag provided highest viability on *P. ostreatus* PO2 and *T. harzianum* TH03. *P. ostreatus* PO2 and *T. harzianum* TH03 treatment was appropriate with mature compost criteria. The lowest C/N content was found in cacao pod husk waste treated with *T. harzianum* TH03 (4.85%) whereas the control (23.20%). The nutrient of the compost treated with *P. ostreatus* PO2 is higher than control.

Keywords: *rot fungi, formulation, viability, packaging variation, compost*

INTRODUCTION

Cacao produces a huge volume of biomass either from leaves or twigs which reached 6.85 ton/ha/year without shade and reached to 11.88 ton/ha /year under shade. Each harvest season, cacao produces 2178kg of wet beans and 6200kg of pod husks waste. This biomass can be processed into a good high nutritious organic fertilizer and returned it back into the soil to improve soil structure.

Utilization of microorganisms such as bacteria, fungi and actinomycetes in decomposition of organic material have been widely used. According to Eriksson *et al.*, (1989), fungi has the most significant decomposition activity can immediately break down the soil organic matter into a simple organic compounds, used as basic ion exchanger and release nutrients around the plant. The Genus Trichoderma and Pleurotus are the microorganisms widely spreaded in the nature and known as producer of hydrolytic enzyme, cellulase, pectinase and xylanase which can degrade polysaccharides complex such as cellulose, pectin, hemicellulose, and xylan. The use of *Trichoderma harzianum* has several advantageous due of easily cultivation and it rapidly growth rate (Selby, 1[illegible]). Fungi also can serve as antagonist toward plant pathogens (Altomare *et al.*, 1999; Hanson and Howell, 2004; Harman 2006) and according to Baloch et al., (2017), invitro evaluation of the bio-control agent T. harzianum against B. theobromae was found to be very effective and the maximum inhibition is 40.34% in the colony growth of the test fungus within seven days.

T. harzianum and *P. ostreatus* often cultivated in the many form of substrates, like rice bran, sawdust, corn, rice husks and a mixture of several substrates. Application of both *T. harzianum* and *P. ostreatus* in the form of these substrates is not practical because it requires a lot of container and labourous, often encounters obstacles when taken and applied in the field. Therefore, it is necessary to find the best solid formulation, that more practical, effective, and efficient. Biological agents formulation can be made by using of cheap and widely available ingredients such as agricultural waste (Soesanto, 2008). Various substances like talc (Jayaraj *et al.*, 2007) and alginate pellets have been used to formulate the biocontrol agents by different researchers. Talc is a natural mineral referred as steatite or soapstone composed of various minerals in combination with chloride and carbonate. Talc was found as the best carrier material to retain and support maximum propagules of *T. harzianum* up to 180 days Gade *et al.*, (2008).

Apart of the carrier material in the formulation, it is also important to concern the use of product packaging. Commercialized biological product requires ideal packaging for its growth, that can affect their viability (Wigati, 2009). It is nessesary to assess the effect of propagation culture media, type of packaging after storage on the viability of the product and their effectiveness in decomposition of cacao pod husks in the field condition.

MATERIALS AND METHODS

Cultivation, Formulation and Packaging of Fungal Isolates: *Trichoderma harzianum* TH03 and *P. ostreatus* PO2 isolate were cultivated on rice, corn, and sawdust medium. Due of unoptimal growth, *P. ostreatus* was grown only on corn and sawdust medium, while *T. harzianum* cultivated on all three types of mediums. After 14 days of incubation, culture of each isolate was harvested and air dried. Inoculum was then grinded using a blender and mixed evenly with sterile talc powder and tapioca flour in ratio of 1:2:0.005. The formulation was filtered and air dried untill a moisture level reached less than 12% then packed 100g in three packaging variations: clear plastic, aluminum foil and plastic bag and plastic bottle and stored at room temperature. The effect of temperature (25℃-35℃) was checked on the growth and production phase of metabolite by P. ostreatus. And highest production of metabolites were achieved at 30℃ (Rana and Dahot, 2017).

Viability Test: The viability of fungal spores in powder formulation derived from three different growth mediums and packaging variation was observed every two weeks until 24 weeks of storage periods. Each of 1g formulated isolate diluted with sterile water to 10-6 level, taked 100 µL and spread out on PDA medium then incubated at room temperature. The number of colonies on the medium were observed after 2-3 days of incubation. The population (cfu/g) was counted by average number of colonies formed in each treatment multiplied by dilution factor of sample. Completely randomized design with 2 factors and 3 replications was used in this experiment. Formulation still considered as feasible if the microbial population is still above of 106 cfu/ml.

Percentage of viability: Percentage of viability was measured comparing the number of colonies that grew before packaging and the number of colonies that grew after packaging in several storage periods.

Decomposition of Cacao pod husks: For decomposition test, it used fresh formulated *P. ostreatus* PO2 and *T. harzianum* TH03 in ratio of 1.25% (w/w). The composting process was conducted in a wooden tub that previously coated with plastic. The first layer consist of ± 1cm thick layer of bran, cacao pod husks and sprinkled with dolomite lime then sprayed with a formulation of both fungal product that has been diluted in water. It was repeated in four layers then covered with plastic. The observation of decomposition process consisted of organoleptic tests to determine the maturity of compost: changes of color, texture and odor according to Asngad and Suparti (2005). Nutrient content and C/N ratio was revealed 60 days after incubation using 100 g of sample in each treatment. This study used a non-factorial Completely Randomized Design (CRD) design consisting of 4 treatments. The treatments were formulation of *T. harzianum* TH03, formulation of *P. ostreatus* PO2, formulation of *T. harzianum* TH03 and *P. ostreatus* PO2, and Control, with 3 replications.

RESULT AND DISCUSSION

Viability of *T. harzianum* TH03 and *P. Ostreatus* PO_2 Formulation: *Trichoderma harzianum* TH03 showed best growth using rice medium, indicated by highest colony forming unit after a series of dilution plating method (217 x 10^6 cfu/g), followed by corn medium (16 x 10^6 cfu/g) and sawdust medium (1.1 x 10^6 cfu/g). In other hand *P. ostreatus* PO2 growth most optimal using corn medium (17 x 10^6 cfu/g). However, viability of *T. harzianum* TH03 formulation using rice, corn and sawdust media after 24 weeks of storage on three packaging variations continued to decrease. The percentage of viability can be seen in Figure 1. *T. harzianum* TH03 derived from rice medium can survive until the 12th weeks with viability percentage of 83.3% while using corn medium it viability hold until the weeks 12 indicated with viability percentage of 83.7%. Using sawdust medium the formulation of *T. harzianum* can only survive until 2 weeks with viability percentage of 3.03%.

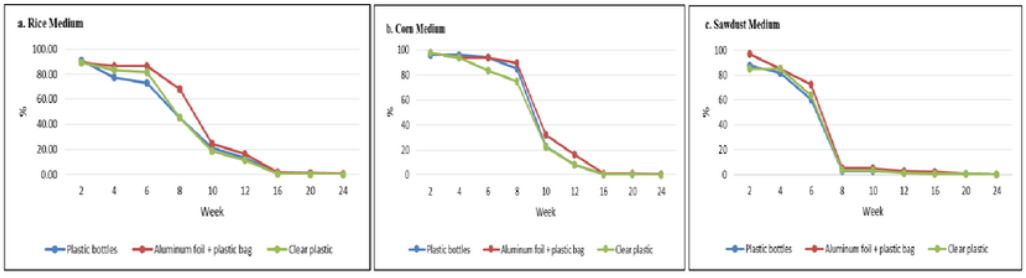

Figure 1: Percentage of *T. harzianum* TH03 viability using rice, corn, and sawdust on 2-24 weeks of storage periods in 3 packaging variations.

The nutrients contained in the carrier is an important factor that can affect the growth of *T. harzianum* and also affect its viability (survival). According to Papavizas (1986), Trichoderma need nutrients as a source of energy for its growth. The most important elements that must be present in natural or artificial media are carbon (C) and nitrogen (N) elements. Lopez (2003) stated that substrate conditions with excess C and less N sources would be a factor in growth of fungi mycelium. The carbon element is needed in the growth process as a source of energy and the synthesis of cell components to repair damaged cells. According to Aiman (1999), the C/N ratio contained in good mushroom growth substrate is relatively low or almost the same as the soil has that is between 10-20. The ratio of C/N ranges from 10 to 20 can spur the growth of fungi mycelium and increase production of conidia, whereas an overly high of C/N ratio may inhibit mycelium growth and decrease conidia production. Nitrogen element is needed to support the process of vegetative growth and cell organelle formation.

Different result was observed in *P. ostreatus* PO2 formulation. The viability of *P. ostreatus* PO2 propagated using sawdust medium and corn medium decreased significantly after 4 weeks of storage periods in room temperature (Figure. 2). Percentage of viability in formulation using corn medium was 88.47% while formulation using sawdust medium was 15.69%.

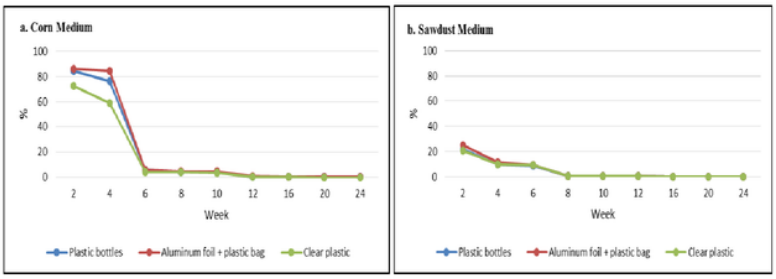

Figure 2: Percentage of *P. ostreatus* PO2 viability using corn and sawdust powder on 2-24 weeks of storage periods in 3 packaging variations.

This is because the ability of the fungus in maintaining its life longer requires sufficient energy at the growth growth, where the source of energy is obtained from substrate rich in nitrogen and carbohydrates. An ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth (Khare, 2010), Corn medium contains a lot of sugar (monosaccharide) is a carbon source for fungal growth compared to sawdust. Corn media contains carbohydrates that are the source of C and protein as the source of N to obtain the optimal C/N values required for growth of mycelium (Cappucino et al., 2014). *P. ostreatus* mushroom can grow well on KanAyodele and Akpaja sawdust medium, that was reported that supplementation of sawdust with oil palm fibres can enhance mycelia growth and sporophore yield of wood ear mushroom but at the time of formulation into flour turned out the good viability at age 14days, this is because protein and carbohydrate content in sawdust media was not as good as on corn medium (Ayodele and Akpaja, 2007).

In general, the antagonists multiplied in an organic food base have longer shelf life than the inert or inorganic food bases. Jayaraj *et al.* (2005) prepared formulations of *B. subtilis* AUBS-1 including talc based powder and reported that populations of bacteria in the formulations were stable up to 2 years of storage at room temperature but was significantly reduced beyond one year of storage. Talc, peat, lignite and kaolin based formulation of Trichoderma, have a shelf life of 3-4 months. The viable propagules of Trichoderma in talc formulation were reduced by 50% after 120 days of storage (Sankar and Jeyarajan, 1996).

The proper packaging is necessary to maintain the viability of the inoculum and protect the product until it is ready to be marketed and applied. The efforts to maintain probiotic inoculum viability can be done by packaging using several packaging types such as aluminum foil, cover paper, zipact plastic and heat resistant plastic so that the growth and damage of fungi in the packaging can be suppressed by preventing the entry of microorganisms. After the three variations of packaging were used, only the packaging using aluminum foil and plastic bag showed the best result in comparison with clear plastic and plastic bottle, this is because the packaging of aluminum foil and plastic bag thicker and when pressed

very tight and not easily torn, in contrast to the plastic bottle packaging where the lid was easily passed through the air which can be the entry of microbial contaminants, as well as on clear plastic packaging where the material was thin and easily damaged.

Jayaraj *et al*. (2006) evaluated the shelf life of formulations of *T. harzianum* under storage at 24ºC up to 11 months and reported that the population of propagules was optimum in all formulations up to 3 months of storage. According to Astiko (2008) good packaging in addition to inhibit the viability decreasing also to facilitate the storage, transportation and distribution. Viability decreasing for 24 weeks of storage is not only caused by the self-defense from biological controlling agent viability itself, but is also strongly influenced by external factor which is microbial contamination either from both fungi and bacteria. Inhibition of *T. harzianum* TH03 and *P. ostreatus* PO2 growth on contaminant fungi and bacteria is suspected due to secondary metabolite activity in the form of antibiotic compound produced by each microbial contaminant that decreased the viability of *T. harzianum* TH03 and *P.ostreatus* PO2, these microbial contaminants can infect the formulation due to poor packaging.

Decomposition of cacao pod husks: Maturation of compost was indicated by color change, texture, and smell that was observed organolep tically according to Asngad and Suparti (2005). Compost color was generated ranging from bright brown to black, with crumbly texture and unique compost odor. This is be in accordance of Budihardjo (2006). The observation results of color, texture and odor of composted cacao pod husk waste are shown in Table 2.

Table 2: Color, texture and odor of composted pod husks waste, 60 days after fermentation.

Treatment	Organoleptic		
	Color	Texture	Odor
P. ostreatus PO2	2B	2.67B	2.67B
T. harzianum TH03	1A	2.00A	2.00A
P. ostreatus PO2+ *T. harzianum* TH03	1A	2.00A	2.00A
Control	3.67C	4.67C	4.00C

Numbers followed by the same letter in the same column are not significantly different (DMRT test, α= 0.05).

Application of *T. harzianum* TH03 and *P. Ostreatus* PO2 and *T. harzianum* TH03 gave bet-ter result in decomposition of cacao pod husk waste than *P. ostreatus* PO2 in single application. Organoleptic observation in control without rot fungi, smell and texture did not analogous with the criteria of mature compost. Applications of *T. virens* and manure on cacao pod husks that contaminated with *P. palmivora* showed that color, odor, and texture was appropriate with mature compost criteria (Sriwati *et al*., 2013). In general, the composting proses will gradually change the color of the compost material toward the blackish brown resulting from the transformation of organic matter from forming humus substances and compost color changes due to differences in material moisture, but also due to changes in CO_2 or organic acids of a nature Volatile (Brinton and Droffner, 1994).

Nutrient content and C/N ratio of cacao pod husk compost: The nutrient content of cacao pod husk compost treated with *P. ostreatus* PO2 formulation and *T. harzianum* TH03 formulation was analyze after 60days. Table 3 showed on all four treatments was significantly different. The lowest C/N content was found in the treatment of *T. harzianum* TH03 formulation (4.85%) compared with control that had the highest C/N ratio (23.20%).

Table 3: The total of N content, P_2O_5, K_2O, C-organic and C/N ratio of Cacao pod husk compost fermented with *P. ostreatus* PO2 and *T. harzianum* TH03 formulation and control, 60 days after fermentation.

Treatment	Nutrient				
	C (%)	N total (%)	P_2O_5 (%)	K_2O (%)	C/N (%)
P. ostreatus PO2	5.88B	0.48B	6.2D	7.65D	12.25C
T. harzianum TH03	5.80B	0.54C	3.45B	6.59C	10.74A
P. ostreatus PO2+ *T. harzianum* TH03	4.85A	0.45B	4.65C	6.39B	10.77B
Control	5.80B	0.25A	3.28A	5.61A	23.20D

Numbers followed by the same letter in the same column are not significantly different (DMRT test, α = 0.05)

C/N ratio meets the SNI standard of compost was 10-20 (Dirjen Cipta Karya, 2004). This result indicates that the decomposition process occurs in the composting process on formulation of *T.*

harzianum TH03 treatment. One indicator that showed the decomposition process in composting is the decomposition of C/N substracts by microorganisms or other decomposer agents. The destruction of lignin and cellulose causes the carbon content will be decreased and the nitrogen level will be increased so that the C/N value becomes small (Mey, 2013).

The nutrient content of cacao compost inoculated with the fungal rot showed nutrient content capable of being released by microbes. The result of cacao pod husk compost using *T. harzianum* TH03 isolate formulation fulfilled SNI standard which determine parameter minimum N 0.40%, P 0.10% and K 0.20% is the best used for re-fertilization in cacao plant. The DMRT test results in the analysis of the macro nutrient content average from Cacao pod husk compost given treatment at total N content showed *T. harzianum* TH03 formulation treatment was the highest N content which is 0.54% while the highest P_2O_5 nutrient content level is in the *P. ostreatus* PO2 formulation treatment which is 6.42% and the highest K_2O nutrient content level is also in the *P. Ostreatus* PO2 formulation treatment which is 7.65% Inoculate with the molding fungi showed nutrient content capable of being released by microbes. Organic trash added to fungi *Trichoderma* sp, the NPK nutrient content is higher than composting without using *Trichoderma* sp. (Mardhiansyah and Widyastuti, 2007). The straw compost content that given *Trichoderma* sp. and local microorganisms (Mole) yielded lower C/N ratio and higher N, P, K, Ca and Mg nutrients (Suyanto and Tutik, 2015). Sharma *et al*., (2012) also emphasized that *T. harzianum* appeared to be more effective in the biodegradation of these organics compared with *viridae*.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Research and Higher Education of Indonesia for the financial support through RAPID research funding 2016.

REFERENCES

Aiman, A., Pengujian laju dekomposisi dan mineralisasi hara slude sebagai pupuk organik alternatif. Agusta 3(1): 1-6 (1999).

Altomare, C., W.A. Norvell, T. Bjorkman and G. E. Harman, Solubilization of phosphates and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65: 2926-2933 (1999).

Asngad, A. and Suparti, Development model for making organic fertilizer with inolucation (a case study on Rubbish at TPA Mojosongo, Surakarta). Jurnal Penelitian Sains & Teknologi. 6(2): 101-113 (2005).

Astiko, W., The suitability of packaging materials, temperature and storage duration for commercial inoculum of *Mycorrhiza* fungus on vertisol of Lombok. Crop Agro. 1(2): 144-149 (2008).

Ayodele, S. M. and E. O. Akpaja, Yield evaluation of Lentinus squarosulus (Mont) Sing on selected sawdust of economic tree species supplemented with 20% oil palm fruit fibers. Asian J Plant Sci. 6: 1098–1102 (2007).

Baloch, A., M.A. Abroo, G.H. Jatoi, N.A. Abro, S. Dad, M. rafique, R. Ali, S. Memon and N. Mangi, In-vitro evaluation of different fungicides and the bio-control agent *Trichoderma harzianum* against *Botryodiplodia theobromae*, the cause of guava decline. Pak. J. Biotechnol. 14 (3) 491-496 (2017).

Brinton, W.F. and M.D. Droffner, Test Kits for determining the chemical stability of a compost sample. U.S. patent 5320807 (1994).

Budihardjo, M.A., Studi potensi pengomposan sampah kota sebagai salah satu alternative pengelolaan sampah di TPA dengan menggunakan aktivator EM4 (Effective Microorganism). Presipitasi. 1: 25-31 (2006).

Cappuccino, James G and Sherman Natalie, Manual Laboratorium Biologi. Jakarta: EGC. ISBN:979-044-417-1 (2013).

Dirjen Cipta Karya, Spesifikasi kompos dari sampah organik domestik. SNI: 19-7030-2004 (2004).

Eriksson, K.E.L., R.A. Blanchette and P. Ander, Microbial and enzymatic degradation of wood and wood components. Springer-Verlag Heildeberg, New York, USA (1989).

Gade, R.M., S.R. Wardhe, S. Armarker, U.R. Sangale and P.K. Sharma, Shelf life of *Trichoderma* spp. In different carrier materials. J. Mycol. Pl. Pathol. 38(3): 606-607 (2008).

Hanson, L.E. and C.R. Howel, Elicitors of plant defense responses from biocontrol strains of *Trichoderma virens*. Phytopathology 94: 171 - 176 (2004).

Harman, G.E., Overview of mechanisms and use of *Trichoderma* spp. Phytopathology 96: 190 - 194 (2006).

Jayaraj J, Radhakrishnan NV, Kannan R, Sakthivel K, Suganya D, Venkatesan S, Velazhahan R., Development of new formulations of *Bacillus subtilis* for management of tomato damping-off caused by *Pythium aphanidermatum*. Biocontrol Sci Technol. 15: 55-65 (2005).

Jayaraj, J., N.V. Radhakrishnan and R. Velazhahan, Development of formulations of *Trichoderma harzianum* strain M1 for control of damping-off of tomato caused by *Pythium aphanidermatum*. Arch Phytopathology Plant Protect. 39(1): 1-8 (2006).

Jayaraj, J., T. Parthasarthy and N.V. Radhakrishnan, Characterization of a *Pseudomonas fluorescens* strain from tomato rhizosphere and its use for integrated management of tomato damping-off. Biocontrol. 52(5): 683-702 (2007).

Khare, K. B., J. M. Mutuku, O. S. Achwania, and D.O. Otaye, Production of two oyster mushrooms, Pleurotus sajorcaju and P. florida on supplemented and un-supplemented substrates. Bots. J. Agric. Appl Sci, 6: 4–11 (2010).

Lopez, JLCS, Production of lovastin by Aspergillus terreus: effects of the C:N ratio and the principal nutrients on growth and metabolite production. Elsevier Inc, AS. 33: 270-277 (2003).

Mardiansyah, M., and S.M. Widyastuti, Potensi *Trichoderma* spp. pada pegomposan sampah organik sebagai media tumbuh dalam mendukung daya hidup semai Tusam (Pinus merkusii. Et de Vries). SAGU. 6(1): 29-33 (2007).

Mey, D. Uji efektivitas mikroorganisme terhadap laju dekomposisi limbah jambu mete sebagai pupuk organic di Sulawesi Tenggara. AGRIPLUS 23(2): 85-91 (2013).

Papavizas, G.C., Trichoderma and Gliocladium biology, Ecology and Potential for Biocontrol. Annu Rev Phytopathol. 23: 23-54 (1986).

Rana M. and M.U. Dahot, Optimization of culture conditions to produce secondary metabolites by *Pleurotus ostreatus*. Pak. J. Biotechnol. 14(2): 251-256 (2017).

Sankar, P. and R. Jeyarajan, Biological control of sesamum root rot by seed treatment with *Trichoderma* spp. and *Bacillus subtilis*. Indian J. Mycol. Plant Pathol. 26: 147-53 (1996).

Selby, K., The cellulase of *Trichoderma viride*. Biochem J. 104: 716-724 (1988).

Sharma, B.L., S.P. Singh and M.L. Sharma, Biodegradation of crop residues by Trichoderma species vis-à vis nutrient quality of the prepared compost. Sugar Tech. 14: 174-180 (2012).

Soesanto, L., Pengantar pengendalian hayati penyakit tanaman suplemen ke gulma dan nematoda. Rajawali-Press, Jakarta (2008).

Sriwati, R.T., C. Bukhhari and A. Sanjani, *Tricoderma virens* isolated from Cacao plantation in Aceh as biodekomposer Cacao pod husk. Natural. 13(1): 6-14 (2013).

Suyanto, A. and A.P.I. Tutik, Efektifitas *Trichoderma* sp. Mikroorganisme lokal (MOL) sebagai decomposer dalam meningkatkan kualitas pupuk organik alami dari beberapa limbah tanaman pertanian. Jurnal Agrosains 12(2): 1-7 (2015).

Wigati, D., The effect of packaging and storage on insect attack and physical properties of crumble broiler starter. Thesis. Bogor Agricultural University, Indonesia (2009).

he_Effect_of_Organic_Medium_Use_in_Formulation_of_Trchode.

ORIGINALITY REPORT

%11	%8	%5	%6
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	pjbt.org Internet Source	%3
2	academicjournals.org Internet Source	%2
3	Submitted to Universitas Negeri Semarang Student Paper	%1
4	Submitted to Universitas Brawijaya Student Paper	%1
5	Submitted to Surabaya University Student Paper	%1
6	Jayaraj, J., N.V. Radhakrishnan, R. Kannan, K. Sakthivel, D. Suganya, S. Venkatesan, and R. Velazhahan. "Development of new formulations of Bacillus subtilis for management of tomato damping-off caused by Pythium aphanidermatum", Biocontrol Science and Technology, 2005. Publication	<%1
7	www.scialert.net	

	Internet Source	<%1
8	euroessays.org Internet Source	<%1
9	Submitted to Higher Education Commission Pakistan Student Paper	<%1
10	Brij Lal Sharma, Sujeet Pratap Singh, Mukut Lal Sharma. "Bio-degradation of Crop Residues by Trichoderma Species vis-à vis Nutrient Quality of the Prepared Compost", Sugar Tech, 2012 Publication	<%1
11	www.chemijournal.com Internet Source	<%1
12	Submitted to University of California, Los Angeles Student Paper	<%1
13	Francesco Vinale, Krishnapillai Sivasithamparam, Emilio L. Ghisalberti, Roberta Marra, Sheridan L. Woo, Matteo Lorito. "Trichoderma–plant–pathogen interactions", Soil Biology and Biochemistry, 2008 Publication	<%1
14	P. Narayanasamy. "Biological Management of Diseases of Crops", Springer Nature, 2013 Publication	<%1

15	theaasm.org Internet Source	<%1
16	epdf.tips Internet Source	<%1
17	Submitted to East Tennessee State University Student Paper	<%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	< 5 WORDS
EXCLUDE BIBLIOGRAPHY	ON		