Trichoderma harzianum Enriched Vertical Column Enhances Black Pepper Growth

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Abstract— *The method of pepper cultivation with vertical columns is more efficient and effective because it can be planted with more pepper cuttings compared to the conventional method with only one plant per stake so that the productivity of the plant is higher. The application of Trichoderma harzianum in the vertical column can increase pepper growth. The results of this study indicate that there is an increase in plant height, number of leaves, number of nodes, and number of lateral fruiting branches by applying Trichoderma 1x10^9/ml in the vertical column. Statistically, the interaction of column media and the addition of Trichoderma shows a significant difference in plant height. Trichoderma can increase nutrient uptake and act as an antagonist for plant pathogens. This microbe is able to dissolve phosphates and certain types of soil minerals. Consequently, the strain effectively increases the growth of pepper. It is important to maintain the suitability of the Trichoderma environment around the pepper so that the microbes continue to survive and provide benefits for plant growth in the long term.*

Keywords— Organic material, Black pepper, Vertical column, Trichoderma harzianum.

I. INTRODUCTION

Black Pepper is a medicinal plant and spices with high value in foreign countries which plays an important role in the economy of Indonesia as it has been a leading export commodity in the plantation sector. Indonesia is also known as one of the largest pepper producers and exporters in the world and is incorporated in the International Pepper Community (IPC) along with other pepper exporting countries such as India, Malaysia, Brazil, Sri Lanka and Vietnam [1]. However, Indonesia's position as a pepper exporting country is currently weakening, occupying only the third position after it was recognized as the world's largest pepper exporter for a few decades. This is caused by a dramatic decrease in the productivity and quality of national pepper and in intensive pepper development from other IPC members [2]. Thus, improving cultivation technology to increase productivity seems to be the viable option to restore the glory of pepper in Indonesia. The most basic improvement in cultivation technology is the provision of quality seeds and adequate parent plantations.

The propagation technique of black pepper using the vertical column method is one of the alternative technologies because it is more efficient and faster [3]. This method is considered efficient and economical because it does not require large tracts of land and conventional stakes that are relatively expensive. Each column in the vertical column method can be used up to ten planting materials depending on the size of the column. Another advantage of using the vertical column method is the support stake which also serves as a medium for growing the vine pepper can be substituted with organic material so that the root can also help the absorption of nutrients from the degradation of the organic material.

This method does not require conventional stakes like those commonly used by pepper growers, but uses vertical columns made of 4 cm wire mesh covered with plastic and then formed cylindrical with a height of 2 m. The column section is then filled with organic material such as coconut coir, oil palm empty fruit bunches (OPEFB), coconut charcoal, or other types of organic material that has been sterilized beforehand. Moisture is maintained in the range of 75-80% at 25-28ºC. This method is very efficient because in one column, several plant cuttings can be planted.

The use of organic material on column provides enough pores for oxygen and biological agents to develop. Biological agents that can be applied to organic column media are *Trichoderma harzianum* because they can act as antagonists of plant diseases and also play a role in increasing growth through auxin production.

The waste of organic material in West Kalimantan is relatively abundant resulting from the processing of oil palm or deep coconut. Therefore, in this study the media from organic material was combined with *T. harzianum* in order to decompose properly. In addition, it is also important to further investigate the optimum dosage of this type of organic material if it is used as a medium in the vertical column method for breeding pepper plants. The application of the vertical column method will also increase the productivity of pepper plants and efficiency in land use.

II. MATERIALS AND METHODS

Pepper cultivation activities were carried out at the Experimental Field, while the analysis of parameters, fungi preparation, and observations were conducted in the Plantation Plant Science Laboratory of Agricultural Technology Department.

2.1 Equipment and Material

The equipment used in this study were shakers, petri dishes, test tubes, laminar airflow, autoclaves, incubators, stirring hot plates, spectrophotometers, analytical scale, pH meters, plant cutter, and wires. The materials used were black pepper cuttings, Podzolic soil, oil palm empty fruit bunches (OPEFB), coconut coir, coconut charcoal, manure, and sand. Materials used in laboratories such as PDA (Potato Dextrose Agar) media, ethanol, and chemicals for analysis

2.2 Methods

The stages in this research were the preparation of planting media, preparation of *Trichoderma harzianum* suspension, column preparation, planting, growth observation, analysis of soil chemical and biological properties. The details of the research stages are as follows:

2.2.1 Planting Media Preparation

Planting media preparation was carried out two weeks before planting. The soil used for the main roots was podzolic soil. Soil was mashed and sieved before it was used so that plant roots can develop properly. Media used in columns such as coconut coir, oil palm empty fruit bunches (OPEFB), coconut charcoal, and rocks / coral. All types of media were sterilized using autoclave.

2.2.2 Preparation of *Trichoderma harzianum* suspension

Trichoderma harzianum Isolate is a collection of Plantation Plant Laboratory, Pontianak State Polytechnic. Fungi were grown aseptically on Potato Dextrose Agar (PDA) media. *T. harzianum* isolates on PDA media aged 1 week after incubation were made a suspension with a concentration of 1x107/ml and $1x10^9$/ml. Referring to the research [4] conidia suspense used for Trichoderma liquid formulation was $1x10^3$-$1x10^9$ conidia/ml. Trichoderma culture that has been grown on PDA media aged 7 day is put into an erlenmeyer containing 50 ml of sterile distilled water. Subsequently, it was homogenized with a vortex mixer for several minutes. Then the conidia suspension was taken with a volumetric pipette and the number of conidia was calculated using a haemositometer so that the Trichoderma suspension density of 1 x 107 cell/ml was obtained. The *T. harzianum* inoculum was then propagated in the liquid media of Potato Dextrose Broth. The liquid suspension of *T. harzianum* was applied to the black pepper column media by being sprayed. The treatment dose given to the column was 500 ml.

2.2.3 Column Preparation

The column was made vertically as a climbed stake for black pepper with a height of 2 m. The column used a wire filled with several types of media enriched with *T. harzianum*. The pipe containing cement was placed in the middle of the column as a buffer so that the column stands upright and strong. Some media used to fill the column are coconut coir, oil palm empty fruit bunches and rock or coral. Black pepper column has a diameter of 30 cm and height of 2 m. In each column, 4 plants were planted around the column.

2.2.4 Planting

Black Peppers were planted in podzolic soil growing media. The pepper seedlings used was 8 months old and was obtained from seed breeding. Planting media used in the form of a mixture of sand, manure, and topsoil with a ratio of 1: 1: 2. Planting pepper seedlings of 4 plants in each column by surrounding the column. Plant maintenance included watering and controlling plant-disturbing organisms. Weed control was done manually.

2.2.5 Soil Analysis

Analysis on the column media was carried out at the beginning of planting and the end of observation. Soil chemistry observed consisted of total N, P_2O_5, and K. Soil samples were dried and sieved on a 2 mm sieve and continue to measure soil moisture and pH. Soil samples that pass a 0.5 mm sieve are used for analysis of water content, pH, total N, P, and K.

III. RESULTS AND DISCUSSION

3.1 Soil Analysis

Analysis of the planting and vertical column media to determine the nutrient content in each treatment.

TABLE 1
THE CONTENT OF NITROGEN, PHOSPHORUS, AND POTASSIUM ON BLACK PEPPER PLANTING MEDIA

Treatments	Total N (%)	P_2O_5 (ppm)	K (ppm)
M0	0.62	109.08	428.63
M1	0.31	97.08	559.37
M2	0.52	99.86	513.6
M3	1.35	100.76	509.01

M0: Planting Media with rocky vertical column, M1: Planting Media with coco charcoal vertical column, M2: Planting Media with coco coir vertical column, M3: Planting Media with OPEFB vertical column

Table 1. shows the results of the soil analysis in the planting media in each treatment. Soil test on the planting media aims to determine whether the column media affects the chemical composition of the planting media. From the results of the soil analysis, the NPK content in each treatment was relatively equal. This means that the column media does not have a large influence on the planting media and black pepper growth can be observed properly because it is mainly influenced by the vertical column media.

TABLE 2
THE CONTENT OF N, P, K ON BLACK PEPPER VERTICAL COLUMN MEDIA

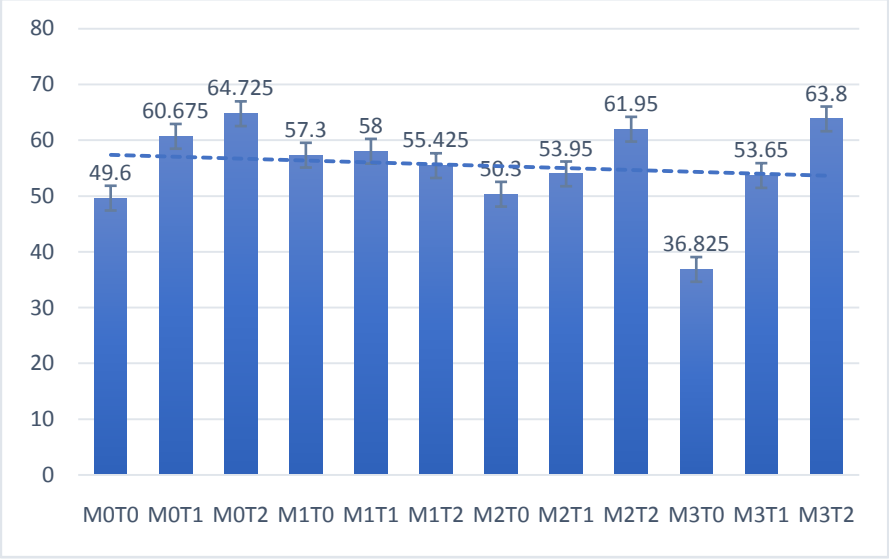
Treatments	Total N (%)	P_2O_5 (%)	K (%)
M1	0.61	0.08	0.4
M2	0.97	0.04	0.05
M3	0.97	0.05	0.03

M1: coco charcoal vertical column, M2: coco coir vertical column, M3: OPEFB vertical column

Media analysis on the column was carried out on the media of coconut charcoal, coconut coir, and oil palm empty fruit bunches (OPEFB). Coconut coir media (M2) and OPEFB (M3) had the same total N value and were higher than other media, while P_2O_5 (%) and K values were lower than coconut charcoal column media. Column media analysis was to determine the chemical characteristics of each treatment in each column.

3.2 Black pepper Growth

3.2.1 Plant Height



M0T0: rocky vertical column without *T. harzianum*, M0T1: rocky vertical column with *T. harzianum* 10^7/*ml*, M0T2: rocky vertical column with *T. harzianum* 10^9/*ml*, M1T0: coco charcoal vertical column *without T. harzianum*, M1T1: coco charcoal vertical column with *T. harzianum* 10^7/*ml*, M1T2: coco charcoal vertical column with *T. harzianum* 10^9/*ml*, *M2T0:* coco coir vertical column *without T. harzianum*, M2T1: coco coir vertical column with *T. harzianum* 10^7/*ml*, M2T2: coco coir vertical column with *T. harzianum* 10^9/*ml*, M3T0: OPEFB vertical column without *T. harzianum*, M3T1: OPEFB vertical column with *T. harzianum* 10^7/*ml*, M3T2: OPEFB vertical column with *T. harzianum* 10^9/*ml*.

FIGURE 1. The Effect of various Media and *T. Harzianum* in Vertical Column on Black Pepper Height

Trichoderma can promote growth of *P. nigrum* [5] and also it has the ability to colonize the roots and form a symbiotic relationship with the plant. Hence, they can be used as a biofertilizer [6]. Besides, Trichoderma can help plants survive in adverse environmental conditions by increasing root growth and promoting photosynthesis [7].

Application of *T. harzianum* on the column media can increase the height of the black pepper. Statistically, *Trichoderma* has a significant influence on the growth of pepper height. Fig. 1 shows the M0T2 treatment was the best high growth rate of 64.7 cm followed by the M3T2 treatment of 63.8 cm. The results of Tukey's HSD Test show that *T. harzianum* treatment concentration of $1x10^9$/ml (T2) was significantly different. Addition of *T. harzianum* on column media can increase height growth. It happens because *T. harzianum* can increase nutrient uptake which results in the addition of root length and stem length, as well as increasing plant chlorophyll.

Trichoderma spp. are major plant growth-promoting fungi that broadly exist in the natural environment and efficient in transformation of soil nutrients. These strains have the abilities to grow and do reproduction quickly. In addition, the plant rhizosphere soil environment can be modified by these microorganism [5].

3.2.2 The Number of Leaves

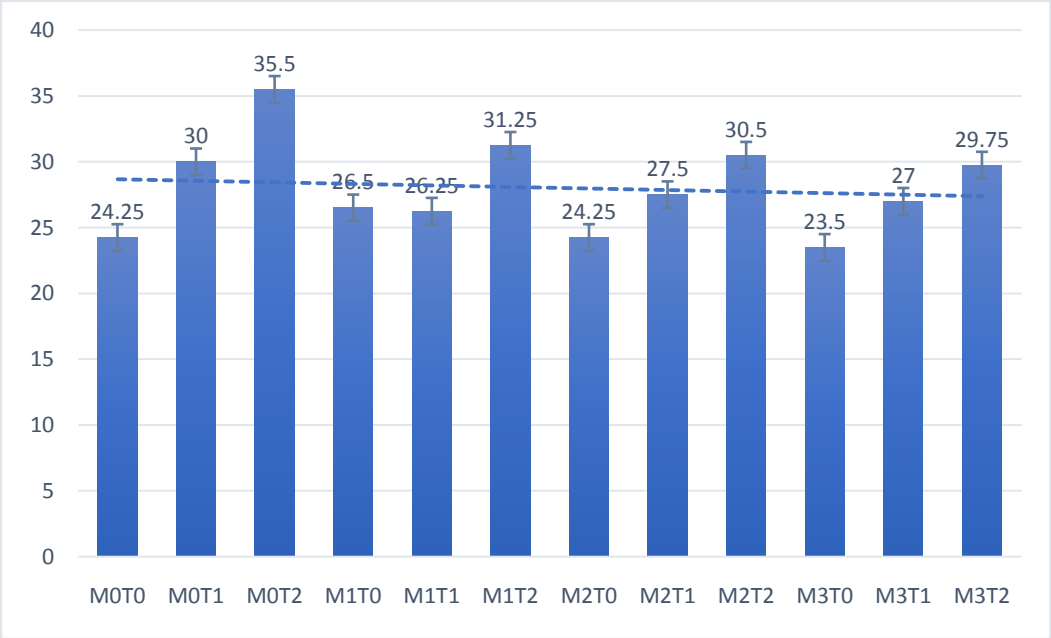


FIGURE 2. The Effect of various Media and *T. Harzianum* in Vertical Column on Leaves Number

T. harzianum essentially affects within the specific abundance of advantageous bacteria and fungi, and consequent growth of black pepper [8]. Trichoderma significantly influences the number of leaves of chili [9]. Based on Fig. 2, in the observation parameters of the number of leaves, M0T2 (rocky vertical column with T. harzianum 10^9/ml) and M1T2 (coco charcoal vertical column with T. harzianum 10^9/ml) treatments were the best with an average number of leaves of 35.5 and 31.25, respectively. Statistical analysis shows that the interaction between column media and *Trichoderma* concentration did not indicate significant effect on the number of leaves, but the concentration of *Trichoderma* significantly affected plant growth and number of leaves. A post hoc test was conducted to measure specific differences between pairs of means and it shows that T2 treatment had a significant difference (Table 3). *T. harzianum* has cellulase and xylanase enzymes so that this fungus acts as an organic material decomposer. Decomposed organic matter makes nutrients available to plants and is useful for supporting plant growth. The ability of *T. harzianum* to dissolve phosphate and certain minerals in the soil is an important key for plant growth. It is also able to excrete certain types of enzymes and can inhibit the pepper pathogens.

3.2.3 The Number of Nodes and Lateral fruiting branches

The use of coir pith compost (CPC) in potting medium under humid conditions can increase the growth of lateral rooting of pepper [10]. According to [11] application of compost lead to significant increase in soil organic matter content, and this will improve long term soil fertility. Fig. 3 shows the average number of nodes in the M0T2 and M2T2 treatments was 14.75, while the average number of fruiting branches was 6.5, in the M2T2 treatment.

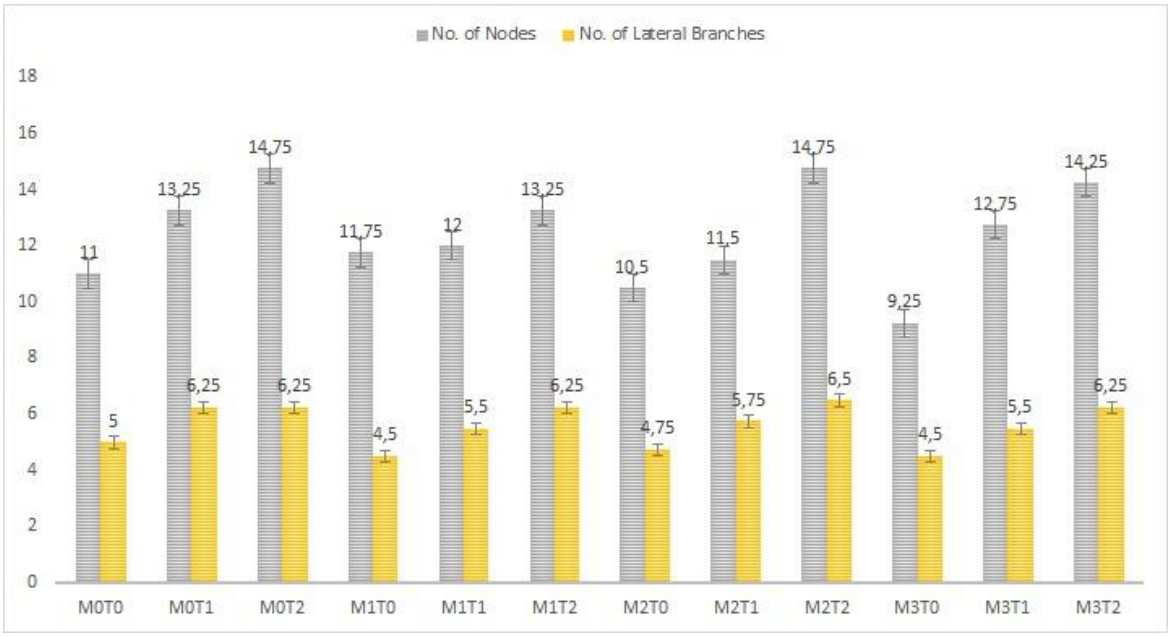


FIGURE 3. The Number of nodes and Lateral Fruiting Branches

In this study, *Based on Tukey's HSD* (*honestly significant difference*) *test* (Table 3), there were statistically significant differences in the parameters from T2 to T0 ($p<0.05$). The increase of the number of nodes and lateral branches is actually influenced by the concentration of *T. harzianum* compared to the type of column media. This shows the ability and contribution of *Trichoderma* in pepper growth. *T. harzianum* provide a significant influence on the number of nodes and lateral branches of pepper plants. The effectiveness of *Trichoderma* is related to various factors, both the abiotic environment such as nutrient concentration, pH, water content, temperature, soil treatment, and the use of fertilizers or pesticides as well as biotic factors such as microbial interactions, fungus species, host plants, and competition between fungi. On research by [12], the application of *Trichoderma* 100 grams/plant is able to influence the growth of pepper plants especially the length of the shoot, the number of shoots and the number of leave. These microorganisms can survive in the soil and the interaction will continue if the abundance of microbes compatible physiologically and inoculum density is quite high [13]. Application of Trichoderma promoted salicyclic acid formation in the root. Regular application of Trichoderma is important in maintaining the population steady, and reducing foot rot disease incidence in black pepper [14].

TABLE 3
TUKEY PAIRWISE COMPARISON

Treatments	**Tukey Pairwise**			
	Heights	Leaves	Nodes	Fruiting Branches
Control	a	a	a	a
T. harzianum 10^7/ml	b	a	b	b
T. harzianum 10^9/ml	b	b	c	b

IV. CONCLUSION

Some media such as oil palm empty fruit bunches (OPEFB), coconut coir, coconut charcoal, and rock can be used as a vertical column media for black pepper. The application of *T. harzianum* suspension has a significant influence on pepper growth. This microbe involves several mechanisms to influence plant growth. Its ability to survive in the soil under suitable environmental conditions and the availability of organic material makes this fungus effective as a biofertilizer especially black pepper. This study shows the positive influence of these microbes on plant height, number of leaves, lateral fruiting branches, and the number of nodes.

The suggestion from this research is to be more selective in choosing the type of column media that will be used by considering the cost and potential of carrying certain types of diseases. The column media must also be sterilized first in order to eliminate the pests and diseases.

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REFERENCES

[1] B. Martin, S. Made, and I. Wiwik, "Pengaruh Media Pembibitan pada Pertumbuhan Setek Lada (Piper nigrum L.)," Jurnal Agro Industri Perkebunan, 3(2), pp. 94–107, 2015.

[2] K. Riski, R. Arifah, and A. A. Sjarif, "Pengaruh Berbagai Konsentrasi IBA dan Urin Sapi Terhadap Pertumbuhan Setek Tanaman Lada (Piper nigrum L.)," Jurnal Agronida, 2(2), pp. 53–61, 2016.

[3] M. Anandaraj, "Status of plantation crops sector in India. Journal of Plantation Crops," 43(1), pp.1-8, 2015.

[4] R. D. Navaneetha, "Liquid Formulation of Trichoderma Species for Management of Gray Mold in Castor (Ricinus communis L.) and Alternaria Leaf Blight in Sunflower (Helianthus annuus L.)," Journal of Biofertilizers & Biopesticides, 06. 10.4172/2155-6202.1000149, 2015.

[5] H. Saiyaremu, X. Deng, S. Xiaoshuang and S. Ruiqing, "Effects of Two Trichoderma Strains on Plant Growth, Rhizosphere Soil Nutrients, and Fungal Community of Pinus sylvestris var. mongolica Annual Seedlings," Forests 2019, doi:10.3390/f10090758, 2019.

[6] C.R. Divya, and M.S.Sharada, "Plant Growth Promoting And Root Colonization Capability Of Trichoderma longibrachiatum(DT1) INPiper nigrumL," IJRAR, Volume 5, Issue 4, 2018.

[7] S.S. Geoffrey, Article in Applied Microbiology and Biotechnology, 2016.

[8] U. Palaniyandi, M. Anandaraj, V. Srivastav, and B. Sailas, "Trichoderma harzianum MTCC 5179 impacts the population and functional dynamics of microbial community in the rhizosphere of black pepper (Piper nigrum L.)," Braz J Microbiol.; 49(3): 463–470, Jul-Sep 2018.

[9] K. Ashish, P. Akhiles, S.N. Singh and R.K. Tiwari, "Effect of Trichoderma spp. in Plant Growth Promotion inChilli," Int.J.Curr. Microbiol. App.Sci 8(3): pp.1574-1581, 2019.

[10] K. A. Ramya Mol, K. N. Harsha, K. A. Saju, and K. Pradip Kumar, "Evaluation of potting mixtures and humidity conditions for rooting and establishment of plagiotropic branches of black pepper (Piper nigrum L.)," Annals of Plant Sciences 6.5 (2017) pp. 1622-1624, 2017.

[11] K, Aousass, L. Kenny, Y. Hashim, A. Ouchen, H. Hajji and M. Bakki, "Soil fertility and pepper yield in response to different sources of nutrients in arid conditions of Southern Morocco," Journal of Environmental and Agricultural Sciences. 15: , pp. 43-50, 2018.

[12] N. Syam, A. Boceng, N. Jufri, and Suharman, "Effect of tricoderma and organic fertilizers on vegetatif growth of black Pepper (Piper nigrum L.) under field condition," IOP Conference Series: Earth and Environmental Science, https://doi.org/10.1088/1755-1315/260/1/012174, 2019.

[13] T. S. Kevin Muyang, H. A. Osumanu, Y. K. Choy, and M. Z. Jarroop Augustine, "Introducing Natural Farming in Black Pepper (Piper nigrum L.) Cultivation," International Journal of Agronomy Volume 2018, Article ID 9312537, pp. 6 , 2018.

[14] W. Dono, M. Dyah and T. Octivia, "Pretreatment Effect Of Black Pepper Seedlings With Pseudomonas, Trichoderma And Mycorrhiza On Foot Rot Disease Incidence," Bul. Littro, Volume 27, Nomor 1, 2016.