International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.13 No.01, pp 222-226, 2020

Exploration and Identification *Trichoderma* spp. as a Biological Control Agents to Plant Pathogens and Starter Making Biological Fertilizers

Henny V.G. Makal*[1], Max M. Ratulangi*[2], Denny S. Sualang*

***Plant Protection Study Program, Faculty of Agriculture, Sam Ratulangi University, Manado, North Sulawesi, Indonesia**

Abstract : The objectives of this study are: (1) to inventory *Trichoderma* spp. in North Minahasa District, South Minahasa District, and Tomohon City-Minahasa District, (2) inventory of *Trichoderma* spp. in the rhizosphere of cultivated and fallow gardens, and (3) calculate the population density of *Trichoderma* spp. all soil samples. The scope of this study is the biological control of plant pathogens, induce plant resistance, and biological fertilizer production. *Trichoderma* isolation spp. has been carried out by dilution method and cultured on PDA + antibiotics. Population density of *Trichoderma* spp. calculated using the plate calculation method. Identification of this species function based on the color and patterns of sporulation in the colony; hyphae and clamydospores; conidiophores; and phialides and phialospores. *Trichoderma* species found in North Minahasa District were *T. harzianum, T. koningii*, and *T. viride*; in South Minahasa District, *T. koningii* and *T. viride*; and in Tomohon City-Minahasa District, *T. koningii* and *T. viride*. In fallow gardens were *T. harzianum, T. koningii*, and *T. viride*, and in cultivated gardens were *T. koningii* and *T. viride*. Population densities of *Trichoderma* sp. in South Minahasa District, North Minahasa District, and Tomohon City-Minahasa District, respectively 1,363.64, 466.67, and 26.67 CFU / g soil.
Keywords: Decomposer, Starter, *Trichoderma harzianum, Trichoderma koningii, Trichoderma viride.*

Introduction

Pesticides and organic compounds have been widely used to control plant pathogens in several countries. This can lead to some unfavorable problems such as the emergence of pest resistance to synthetic pesticides, environmental pollution, and others. Therefore it is very wise if in pest control using natural enemies / biological agents.

DOI= http://dx.doi.org/10.20902/IJCTR.2019.130127

Some *Trichoderma* species have the ability to control the population of soil-borne pathogens such as *T. koningii, T. harzianum, T. lignorum, T. virens, T. hamatum, T. pseudokoningii, T. viride, T. auroviride, T. T. longibrachiatum,* and *T. asperellum*. The mechanisms of action of these fungi in controlling plant pathogens is as follows: (1) competition (microorganisms are more efficient using space and nutrients), (2) antibiosis (producing one or more toxic chemical compounds), (3) parasitism (using targets as food sources or reproductive sites), and (4) inducing plant resistance. *Trichoderma* spp. can also be used as a starter in making fertilizer because of its ability to decompose organic material very quickly[1].

The efficacy of biological control depends on the population density of antagonistic microorganisms per gram of soil sample. Population density of *Trichoderma* spp. can be measured by several methods, one of them with the plate count method[2]. Population density *T. hamatum* 0.6 x 104 CFU / g soil and *T. harzianum* 1.3 x 104 CFU / g soil were able to significantly reduce *Fusarium solani* population[3,4].

This study aims to: (1) inventory *Trichoderma* spp. in North Minahasa District, South Minahasa District, and Tomohon City - Minahasa District, (2) inventory *Trichoderma* spp. in the rhizosphere of cultivated and fallow gardens, and (3) calculate the population density of *Trichoderma* spp. all soil samples.

2. Material and Methods

Exploration of *Trichoderma* spp. has been carried out in the area of North Minahasa, South Minahasa and the City of Tomohon. Three villages were selected per region as a soil sampling location. Soils sampling were carried out on the rhizosphere of estate crops, crops, papayas, vegetables, forests, fallow gardens, and cultivated land. *Trichoderma* isolation spp. has been carried out by dilution method and cultured on PDA + antibiotics. Population density of *Trichoderma* spp. calculated using the plate calculation method[5,2] . Identification of this fungus species based on the color and pattern of sporulation in the colony; hyphae and clamydospores; conidiophores; and phialides and phialospores[6,7,8].

3. Results and Discussion

3.1. *Trichoderma* spp. in North Minahasa District, South Minahasa District, Tomohon City-Minahasa District, and in Fallow and Cultivated Gardens

Trichoderma species found in North Minahasa District are *T. Harzianum* (Figure 1), *T. koningii* (Figure 2), and *T. viride* (Figure 3)); in South Minahasa District, *T. koningii* and *T. viride*; and in Tomohon City - Minahasa District, *T. koningii and T. viride*. In fallow gardens, *T. harzianum, T. koningii*, and *T. viride* were found, and in cultivated gardens, *T. koningii* and *T. viride* were found.

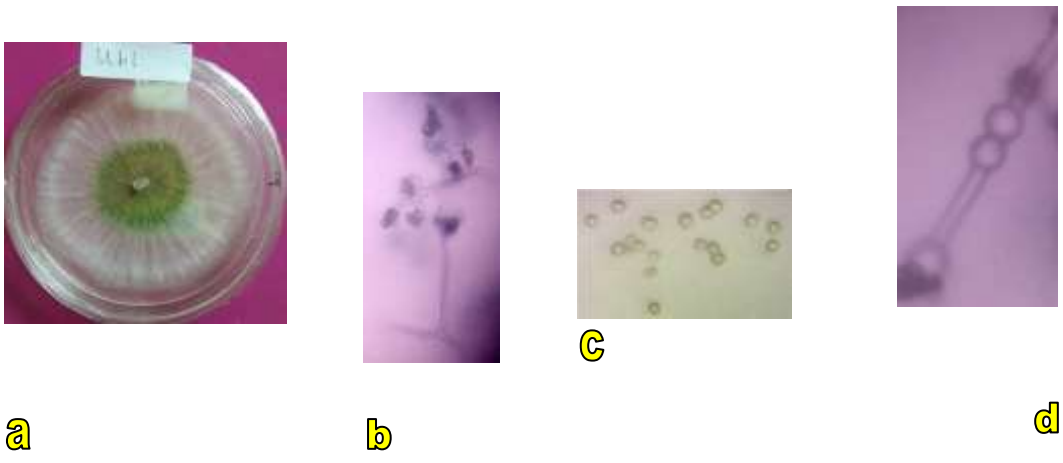

Figure 1. *T. harzianum* morphology: a. Colony, b. Generative, c. Phialospores, d. clamidospores

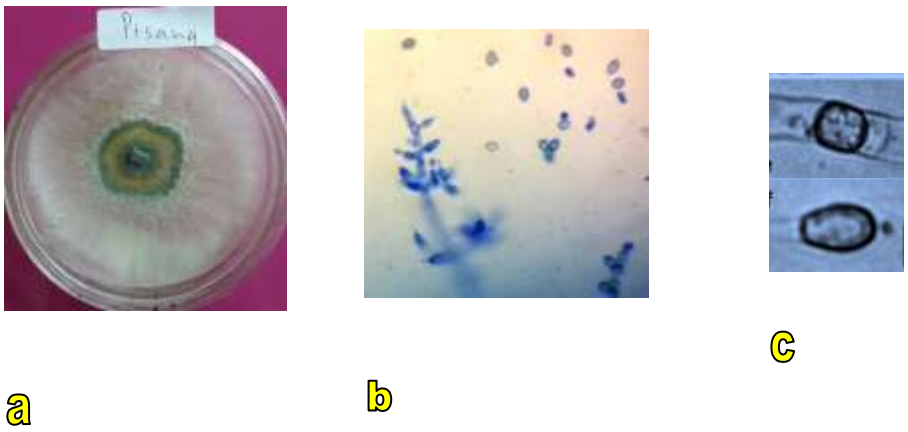

Figure 2. *T. koningii* morphology: a. Colony, b. Generative, c. Clamidospores

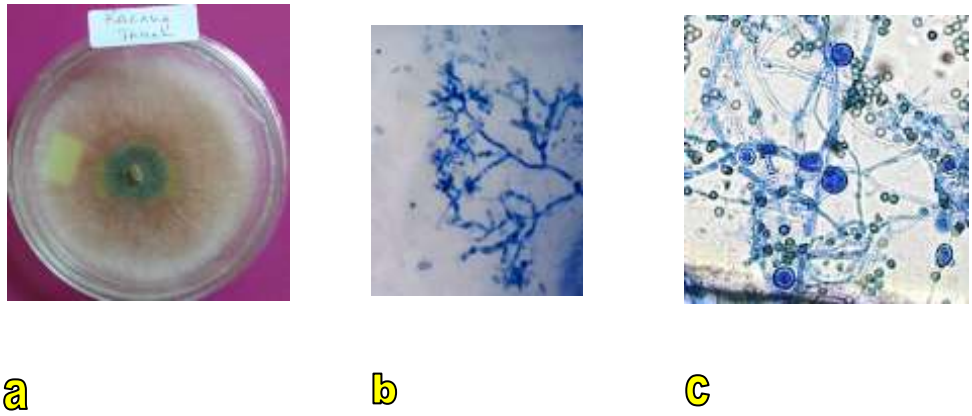

Figure 3. *T. viride* morphology: a. Colony, b. Generative, c. Clamidospores

1. *T. harzianum*

Colony. At 21^{o} C in the light banks, the *T. harzianum* colony grew rapidly, and 72 hours after the subculture had covered the entire surface of the media (9 cm diameter petri dish), concentric sporulation patterns alternating yellowish green and white intermittent, and hyphal growth patterns such as radial patterns (Figure 1a).

Hyphae and clamidospores. Hyphae have septa, branched, smooth-walled, colorless, and have a diameter of 1.5 - 12 µ. Chlamydospores grow intercalary or sometimes in the terminal hyphae, most globose in shape, smooth-walled, colorless, and 6 - 12 µ in diameter (Figure 1d).

Conidiophores. Multi-branched conidiophores and dendroids (look like trees) (Figure 1b) form like rather loose stacks that arise from zones such as rings. Conidiophor branches have a diameter of around 4-5 µ, and produce side branches that appear one-on-one, but most appear in the form of groups of two or three, and each of these branches especially those in the lower part of the branches appear smaller side. The position of the branch with the exit of the branch almost forms a right angle.

Phialides and Phialospores. Lateral phialid grow on verticils to a total of five, and these fidids terminate the ends of the conidiophores, however, they can also grow individually and irregularly along the sides of the smaller branches. The phialides are cone-sized, narrower at the bottom compared to the middle, then immediately narrow towards the neck so that they are conical and sharp, measuring 5-7 x 3 - 3.5 µ. The phialides at the end of the conidiophoric branch is usually longer and thinner, and measures 18 x 2.5 µ. Phialospores are produced one by one and successively and accumulate at the ends of each phialid to form a

globose structure (Figure 1b). The phialospores are subglobose or short obovoid, often with a truncate base, smooth-walled, pale green when observed under a microscope (Figure 1c), but appear darker when in the form of phialospore masses, and measure 2.8 - 3.2 x 2.5 - 2.8 µ.

2. *T. koningii*

Colony. Growth on PDA + antibiotic media takes place rapidly at 21^{o}C in light banks, and at 72 hours after subculture the entire surface of the media has been covered; concentric sporulation patterns alternating greens, yellowish green and white (Figure 2a).

Hyphae and clamidospores. Hyphae hyaline, very branched, septa, smooth wall, and 2 - 10 µm in diameter. Chlamydospores are located intercalary or rarely in terminals and are typically formed (Figure 2c), mostly in hyphae beneath the surface of the media. This resistant structure is globose, ellipsoid or sometimes shaped like a barrel, slippery-walled and up to 12 µ in diameter.

Conidiophores. Multi-branched conidiophores, compact or rather rare in heap zones such as rings. The main branches of conidiophores are about 4 µ in diameter, removing several side branches in groups of two or three. The length of the side branches increases prominently so that a conical or pyramid-shaped branching chart appears.

Phialide and Phialospores. This structure is nine-pin in size (5-) 7.5 - 12 x 2.5 - 3.5 µ (Figure 2b), but the phialide at the ends of the conidiophore branches can reach 30 µ. The phialospores are mostly elliptical-subsilindrical, sometimes oblong or almost angled. The size of these spores is 3 - 4.8 x 1.9 - 2.8 µ, smooth-walled, pale green to green when viewed singly under a microscope, but if in the form of mass they appear darker (Figure 2b).

3. *T. viride*

Colony. On PDA + AB media in light banks and at 21^{o}C, the colony grew rapidly, covering the entire surface of the media four days after subculture. Early colony growth, smooth surface, runny white and rarely mycelium, then become hairy because of the formation of aerial hyphae which is slightly loose, and slightly whitish. Mature colonies, conidial areas are characterized by dark green or dark bluish green. Characteristic odor of coconut released from older cultures (Figure 3a).

Hyphae and Clamidospores. Mycelium consists of hyaline hyphae, smooth walled, septate, many branches, diameter 1.5 - 2 µ. Hyphae often form chlamydospores that appear intercalally or sometimes in terminals (Figure 3c). Chlamydospores are mostly round or almost very round, rarely ellipsoid, hyaline, smooth-walled, more than 14 µ in diameter, and normally present on the lower surface of the media (Figure 3c). Conidiophores are many branches so they look like compact or rather loose stacks. The main branches of conidiophores are 4 µm in diameter, producing single or group side branches (2 or 3). Usually the side branches

3.2. Population Density of *Trichoderma* spp in North Minahasa District, South Minahasa District, Tomohon City-Minahasa District

Population density of *Trichoderma* spp. in North Minahasa District, South Minahasa District, Tomohon City-Minahasa District can be seen in Table 1. The highest density of *Trichoderma* spp population was found in the rhizosphere land in South Minahasa, followed by North Minahasa and Tomohon City-Minahasa District.

Table 1. Population density of *Trichoderma* spp. in North Minahasa District, South Minahasa District, Tomohon City, Minahasa District

Sampling Regions	Population Density of *Trichoderma* spp. (CFU/g soil)
South Minahasa	1.363,64
North Minahasa	466,67
Tomohon City - Minahasa	26,67

Population density of *Trichoderma* spp. in the soil depends on the size of the roots, plant growth phase and plant species. According to[10] that the population density of *Trichoderma* spp. increases with increasing root length, then the pre-harvest phase most effectively increases CFU production, and also Rabbits are the species of plants that have the highest rhizosphere density of *Trichoderma* spp.

In general, *Trichoderma* spp. found in the forest rhizosphere, grasslands, beach swamps, rubbish, and rotting wood. Propagul *Trichoderma* spp. the highest is found in forest soils (3% of total propagul forest samples), then followed by pasture soils and agricultural land[9].

Another factor influencing the population density of *Trichoderma* spp. is soil moisture, the content of organic matter, phosphorus, and potassium. According to[10] that the soil moisture is high and even runny, the density of this mushroom population is higher than that of dry soil, the more organic material the higher the population density of *Trichoderma* spp., And the availability of elements P and K influences the density of this mushroom population.

Information from Table 1 shows that the population density of *Trichoderma* spp. the highest is in South Minahasa District, followed by North Minahasa District and Tomohon City-Minahasa District. Population density of *Trichoderma* spp. in South Minahasa because rhizosphere soil samples generally have higher soil moisture and organic matter compared to other sampling areas. This phenomenon is similar to that reported by[9].

4. Conclusions

Trichoderma species found in North Minahasa District were *T. harzianum*, *T.koningii*, and *T. viride*, in South Minahasa District, *T. koningii* and *T. viride*; and in Tomohon City-Minahasa District, *T. koningii* and *T. viride*. In the fallow garden, *T. harzianum*, *T. koningii*, and *T. viride* were found, and in the cultivated garden, *T. koningii* and *T. viride* were found. Population densities of *Trichoderma* spp. in South Minahasa District, North Minahasa District, and Tomohon-Minahasa District, respectively 1,363.64, 466.67, and 26.67 CFU/g soil.

References

1. Verma M, Brar SK, Tyagi RD, Surampalli RY, and Valero JR. Antagonistic Fungi, *Trichoderma* spp.: Panoply of Biological Control. Biochemical Engineering Journal 37: 1-20. 2007.
2. Kiraly Z, Klemen Z, Solymosy F, Voros J. Methods in Plant Pathology. Elsevier Scientific Publishing Company. Amsterdam. 1984.
3. Sariah M, Choo, Zakaria CW, Norihan MS. Quantification and Characterisation of *Trichoderma* spp. from Different Ecosystems. Mycopathologia 159: 113-117. 2005.
4. Haggag WM, Amin W. Efficiency of *Trichoderma* Species in Control of *Fusarium*-Rot, Root Knot and Reniform Nematode Diseases Complex on Sunflower. Pakistan Journal of Biological Sciences 4 (6) : 679-683. 2001.
5. Christensen M. The Dilution Plate Technique for Isolation of Soil Microfungi. http://carroll.cc.edu/~ jclausz/msamanual/dillq.html. 2006.
6. Anonymous. *Trichoderma* Identification. Unistates Department ofAgriculture: Agricultural Research Service. http://nt.ars.grin.gov/taxadescriptions/ keys/FrameKey.cfm?gen-Tric. 2007.
7. Harman GE. *Trichoderma* spp., Including *T. harzianum, T. viride, T.koningii, T.hamatum* and other spp. http://www.nysaes.comell.edu/ent/biocontrol/ pathogen/trichodenna.hatm. 2002.
8. Rivai MA. A Revision of the Genus *Trichoderma*. Mycological Paper 116: 1 - 56. 1969.
9. Rahman A, Begum MF, Rahman M, Bari MA, Ilias GNM, Alam MF. Isolation and Identification of *Trichoderma* Species from Different Habitats and their Use for Bioconversion of Solid Waste. Turk J Biol 35:183-194. doi:10.3906/biy-0905-8. 2011.
10. Pandya JR, Sabalpara AN, Chawda SK. *Trichoderma*: a Particular Weapon for Biological Control of Phytopathogens. Journal of Agricultural Technology 7(5): 1187-1191. 2011.

*****