Diversity of microbes in organic and non-organic vegetable ecosystem

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Abstract. This study aims to determine microorganisms in organically managed land. The study was conducted in the organic land and non-organic farmer's land in Makassar and continued at the Disease Sciences laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Universitas Hasanuddin. The stages of the study consisted of soil sampling and 10 grams were taken to be diluted into 90 ml of sterile water, then a 10-3 dilution was carried out. The results of the dilution are taken 1ml each and then poured into the PDA media. The fungus that grows is purified on PDA media while bacteria on NA media, then identified. The results showed that in organic vegetable fields more fungi isolates were found (6 isolates): Gliocladium sp, Rhizoctonia sp, Aspergillus sp and two other isolates were unknown and gram-positive bacteria were found while non-organic was found five fungus isolates, Scopulariopsis sp., Verticillium sp., Fusarium sp., Gliocladium sp. Percentage of inhibition of fungi from organic vegetable fields ranged from 33.3 to 100% while non-organic lands 16.7 to 66.7%.

1. Introduction
Organic farming is a production management system that can improve soil health and the quality of soil ecosystems and crop production. In its implementation, organic farming emphasizes the use of renewable and natural inputs and avoids the use of synthetic inputs and genetic engineering products. The adoption of the organic farming approach continues to increase along with the increasingly clear negative impacts of the agricultural development approach with high external inputs (HEIA). Various types of pesticides and fertilizers accumulate in soil and water which adversely affect the entire ecosystem [1]. Conventional agriculture in addition to causing negative impacts from the use of synthetic pesticides, it turns out the provision of inputs in the form of inorganic fertilizers also causes many problems as impacts on soil properties and microbial community compared to the use of organic fertilizers [2].

Organic farming has been shown to increase environmental biodiversity in terms of animals, insects and butterflies when compared to conventional farming [3,4]. Several studies also suggested that microbial abundance [5] and diversity [6] were higher in soils under organic management than in soils under conventional practice. The increased microbial diversity could be related to reduced tillage [7], cover cropping [8] and organic fertilizer incorporation [9] by improving soil organic carbon as the energy source of heterotrophic microbiota [10]. Furthermore, high microbial diversity has been linked to high functional diversity in soil [7], which may cause the important ecological processes mentioned
above. In this context, we investigated the varieties of microbe antagonistic in organic-non organic vegetable cultivation.

2. Materials and methods

2.1. Samplings
The study was conducted in the organic garden at the Universitas Hasanuddin, Faculty of Agriculture (Exfarm), ASYTA organic plantation and non-organic farmer's plantation in Makassar and continued at the laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Universitas Hasanuddin. Soil samples were taken from organic and non-organic plantations, namely vegetable soil on leek and water spinach plantations. Soil sampling was conducted by taking the soil around the plant which is about 30-50 cm with a depth of 10-30 cm from the soil surface. This soil was taken as many as 5 samples from 5 sample points using a 500 gr soil drill and then put into plastic that has been labeled. The soil obtained was then tested in a laboratory.

2.2. Isolation and purification of the samples
Soil samples obtained in weighing as much as 10 grams, put in an erlenmeyer tube containing 90 ml aquadest, then made dilution with $10^{-3}$ suspense. As much as 1 ml of the dilution was poured on sterile PDA (potato dextrose agar) media then spread evenly and incubated for 2-3 days at room temperature.

2.3. Isolation and purification
Growing fungus colonies were transferred into the media by using an oasis needle to regrow it on the PDA media. Subsequently, the colonies were re-isolate to the PDA media to obtain pure culture. Pure culture will later become stock for the identification process. Fungi that grow on PDAs were identified based on morphological characteristics namely spore shape, colony color, mycelium, and fungal identification based on identification using *imperctfungi* [11].

2.4. In vitro antagonist test
Stages of testing using the method of double culture (dual culture) [11], by taking each pure culture characterization results that are classified as test antagonists and pathogenic fungi using cork borer, then inoculated into petri dishes containing PDA media face to face. The placement scheme was shown in figure 1.

![Figure 1. Double Culture Test. A: Pieces of antagonistic fungal test colonies; F: Pieces of pathogenic fungal colonies; R1: Radius of pathogenic colony away from the antagonist fungal test colony; R2: Radius of pathogenic colony approaching the antagonist fungi colony of the test.](image)

2.5. Observed variables
All petri dishes were incubated at room temperature. The observed variables were:

2.5.1 Percentage of inhibition (%). Percentage of inhibition calculated on the 8th day after inoculation with the formula:
Inhibition (%) = \( \frac{R_1 - R_2 \times 100}{R_1} \)

2.5.2 The mechanism of antagonism. Antagonism mechanism was identified according by:

2.5.2.1. Space, nutrition and oxygen competition. Competition between test fungi and pathogenic fungi in the competition for space, nutrition and oxygen was observed by seeing the type of fungi that more quickly filled the petri dish.

2.5.2.2. Antibiosis. Observation of antibiosis is done by measuring the width of the empty zone (barrier) and seeing the presence or absence of change color on the medium due to antibiotic compounds produced by test fungi.

2.5.2.3. Lysis and parasitism. Observation of the mechanism of lysis and parasitism is done by observing the hyphal of the test antagonist fungus that grows on the pathogenic fungus by taking and growing the antagonist fungus hyphae and pathogen using an ose needle, then placed on top of the object glass to be observed microscopically.

3. Results and discussion

3.1. Fungal diversity
Based on the results of research on the microbial diversity in organic and non-organic soil soils, various kinds of fungi were found (table 1). The number of fungus colonies found in organic soil were higher then fungus colonies observed in the non-organic soil.

| Land            | Isolate | Type of Fungi         | Number of Colonies (Colony/ gr) |
|-----------------|---------|-----------------------|---------------------------------|
| Organic         | A       | Gliocladium sp.       | 11 \times 10^3                  |
|                 | B       | Rhizoctonia sp.       | 1 \times 10^3                   |
|                 | C       | x                     | 1 \times 10^3                   |
|                 | D       | Aspergillus sp.       | 3 \times 10^3                   |
|                 | E       | x                     | 5 \times 10^3                   |
|                 | F       | Gliocladium           | 7 \times 10^3                   |
| Non-Organic     | A       | Scopulariopsis sp.    | 6 \times 10^3                   |
|                 | B       | Fusarium sp.          | 3 \times 10^3                   |
|                 | C       | x                     | 5 \times 10^3                   |
|                 | D       | Gliocladium           | 4 \times 10^3                   |
|                 | E       | Verticillium sp.      | 5 \times 10^3                   |

In the soil of organic vegetable plantation, six fungus isolates were found. Four fungi were identified as *Aspergillus* sp., *Gliocladium* sp., *Rhizoctonia* sp., and two others were not yet known. In the soil of non-organic vegetable fields, five isolates of fungus were found. The fungi identified were *Fusarium* sp., *Gliocladium* sp., *Scopulariopsis* sp., *Verticillium* sp., while one isolate was not known. The results show that the fungus isolates were higher in organic vegetable fields compared to non-organic vegetable fields. This is thought to be due to the influence of soil structure, soil fertility, soil moisture and nutrient availability. Healthy soil is not only fertile and contains a lot of material that supports plant health, but is also able to provide a suitable environment for soil microbes, so that plants can be protected from soil pathogens. A number of soil microbes could promote crop growth by producing antibiotics and plant hormones and increase nutrient availability (e.g., excrete phosphatase)
and, as a consequence, benefit plant quality and productivity [13]. Furthermore, high microbial diversity has been linked to high functional diversity in soil [14], which may cause the important ecological processes mentioned above.

Soil biochemical and ecological characteristics in organic fields also improved. Furthermore, organically managed soils have a much higher water holding capacity than conventionally managed soils, resulting in much larger yields compared to conventional farming, under conditions of water scarcity. Because of its higher ability to store carbon in the soil, organic agriculture could represent a means to improve CO₂ reduction if adopted on a large scale. Next, the impact on biodiversity is highlighted: organic farming systems generally harbour a larger floral and faunal biodiversity than conventional systems, although when properly managed also the latter can improve biodiversity. Importantly, the landscape surrounding farmed land also appears to have the potential to enhance biodiversity in agricultural areas [3].

3.2. Fungal antagonism level

Each fungus obtained has a role as an antagonist or cause of the disease. *Aspergillus* sp. is one type of microorganisms including fungi and eukaryotic microorganisms. Fungi *Aspergillus* sp. which is visually isolated appears dark green with white to yellow edges and microscopically fungi *Aspergillus* sp. is easily recognized and distinguished from other fungi that has a conidiophore that is upright, made a hand, not branched and the ends are bulging or enlarged, conidia are round to semi round (table 2). This is consistent with the conidiophor of *Aspergillus* sp. formed freely, bulging edges. Conidia are sequential, numerous and have a round head [15]. *Aspergillus* sp. which was found to be antagonistic to *Fusarium* sp. (table 2 and figure 2A.)

| Land          | Isolate | Genus      | Testing            | Percentage of inhibition (%) |
|---------------|---------|------------|--------------------|-----------------------------|
| Organic       | A       | *Gliocladium* sp. | √ | 100                      |
|               | B       | *Rhizoctonia* sp. | √ | 100                      |
|               | C       | x          | √ | 66.67                    |
|               | D       | *Aspergillus* sp. | √ | 100                      |
|               | E       | y          | √ | 33.33                    |
|               | F       | *Gliocladium* | √ | 100                      |
| Non-Organic   | A       | z          | √ | 16.67                    |
|               | B       | *Scopulariopsis* sp. | √ | 66.67                    |
|               | C       | *Fusarium* sp. | √ | 50                       |
|               | D       | *Gliocladium* sp. | √ | 50                       |
|               | E       | *Verticillium* sp. | √ | -                        |
Figure 2. Antagonistic test of isolates found on organic soil soils with *Fusarium* sp.

Due to the fungus *Aspergillus* sp. has the ability to inhibit the growth of pathogenic fungi because it produces hydrolytic enzymes such as lipases, proteases, cellulases, pectinases [16]. Fungi *Aspergillus* sp. also produces extracellular enzymes including chitinase, α-amylase, β-amylase, glucoamylase, catalase, lactase, invertase. Inhibitory mechanism of fungus *Aspergillus* sp. namely by producing the enzyme chitinase and β-1,3 glucanase (Laminarinase) which have the ability to break down the cell wall components of pathogenic fungi [18]. These fungi exist that live as saprophytes or parasites. Fungi that live parasites can cause disease in humans, animals and plants.

4. Conclusion
Six isolates found in organic field identified as *Gliocladium* sp., *Rhizoctonia* sp., *Aspergillus* sp., and two other isolates were unknown. Five fungus isolates were found in non-organic fields identified as *Scopulariopsis* sp., *Verticillium* sp., *Fusarium* sp., *Gliocladium* sp., and one isolate whose genus was unknown In addition, gram-positive bacteria also found in both field either organic or non-organic vegetable fields. Percentage of inhibition of fungi from organic vegetable fields ranged from 33.3 to 100% while in non-organic lands was 6.7 to 66.7%.

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