

IDENTIFICATION OF SOIL FUNGI ISOLATED FROM ALFALFA (*Medicago sativa* L) TO FIND SPECIFIC FUNGI WHICH IMPROVED THE GROWTH OF ALFALFA

T. Yudiarti, Sumarsono and D.W. Widjayanto
Faculty of Animal Agriculture, Diponegoro University,
Tembalang Campus, Semarang 50275 - Indonesia
Corresponding E-mail: tyudiarti@yahoo.co.id

Received November 20, 2009; Accepted July 12, 2010

ABSTRACT

Objective of the study was to identify all kinds of fungi which can life in the alfalfa plantation in Baturaden Purwokerto-Central Java. Fungi used in this study was 38 isolates. All fungi have been taken from the isolation of soil and root of diseased plant. Macroscopic and microscopic methods were used for identification. Potato Dextrose Agar (PDA) medium was used to grow the fungi. All fungi were identified using book identification of fungi. The results showed that from 38 isolates, six species was determined and one was unidentified. Those species identified were *Cunninghamella sp*, *Trichoderma sp*, *Verticillium sp*, *Eupenicillium sp*, *Pythium sp*, *Aspergillus sp*.

Keywords: alfalfa (*Medicago sativa* L), identification, soil fungi

INTRODUCTION

Alfalfa (*Medicago sativa* L) is one of important forage crops which is belong to legume. The aim of legume growing is to produce fodder rich in protein, alfalfa contains about 15% protein in hay with a dry matter content of 85% (Finck, 1982). Alfalfa requires enough water but it tolerates for dry season although the yield are reduced. A warm climate is preferred. A soil reaction should be approximately neutral. In growing alfalfa there was a problem, diseases attacked. One of the disease caused by microorganism is fungi. Fungi may attack alfalfa (*Medicago sativa* L) on seed or plant (Jones, 1987). If one plant in the plantation have been attacked by the disease, it may spread easily and widely to another plant mediated by helping soil water and root contact (Cook and Baker, 1983; Jones, 1987; Dhingra and Sinclair, 1985).

Many diseases on alfalfa can be caused by fungi and one of this is root rot. This disease is one main problem that found in alfalfa plantation in Baturaden, Purwokerto, Central Java, Indonesia. One of the alternative ways to solve the problem is by knowing the kind of fungi that cause the disease and also fungi lives in the soil and then searching specific fungi that can antagonist to the causing alfalfa disease.

The objective of this study was to identify the kinds of fungi which life in the alfalfa soil plantation in Baturaden Purwokerto.

MATERIALS AND METHODS

The experiment was done in Laboratory of Microbiology of Faculty of Animal Agriculture, Diponegoro University, Semarang, Indonesia. The isolates of fungi was taken from the isolation of soil and root of diseased plant from alfalfa plantation in Baturaden Purwokerto-Central Java. The number of isolate was 38 isolates. Macroscopic and microscopic methods were used in this identification. Potato Dextrose Agar (PDA) was used as a medium for growing and observing all characters of them. All data observed were checked to the book of identification key. For identify of *Pythium* species used identification key of Plaatsma and Nierink (1981) and Robertson (1979), and for other fungi used Alexopoulos and Mims (1979), Ganjar *et al.* (1999), Dhingra and Sinclair (1985) also Gam *et al.* (1987).

Preparing Potato Dextrose Agar Medium

Potato Dextrose Agar medium was prepared by washing about 200 g of potato using distilled water, then was sliced into small pieces, emerged into 1 liter of distilled water and boiled till the texture of potato crumbled. Potato solution then was taken and filtered. Filtrate were put into erlenmeyer and fill up with distilled water till 1 liter of volume was reached. Then, 20 g of sucrose and 17 g agar was added into the solution stirred homogenously. The homogenous solution was sterilized in the autoclave at 121°C. The 250 mg

chloramphenicol antibiotic was added to the solution when temperature reached 40°C. After that, the 10 ml solution was put on Petri dish and allowed to be cool (Dingra and Sinclair, 1985).

Identification of Fungi Isolates

Number of fungi identified in this study was 38 isolates. To identify them, they were grown on PDA which added chloramphenicol antibiotic to protect bacterial growth. All plates were placed at 25°C and examined at regular intervals for observing morphology colony and microscopic preparation like producing of hyphae, sporangia, oogonia and reproduction structures. Identification of all isolates were used the key books from Alexopoulos and Mims (1979), Plaat Niterink (1981), Robertson (1979), Ganjar *et al.*, (1999), Dhingra and Sinclair (1985) and Gam *et al.* (1987).

RESULTS

Identification of fungi isolated from the soil alfalfa plantation showed that most of them have almost the similar characters and that all isolates were belong to six species and one was unidentified. Those species were *Aspergillus sp*, *Cunninghamella sp*, *Eupenicillium sp*, *Pythium sp*, *Trichoderma sp* and *Vertilicium sp*. This finding was according to the key book from Alexopoulos and Mims (1979), Robertson (1979), Plaat Niterink (1981), Gam *et al* (1987), Dhingra and Sinclair (1985), and Ganjar *et al.* (1999).

The species that was obtained: 1) *Aspergillus sp* (Figure 1) was conidiophores consisting of a so-called "foot-cell" and an branches stipe mostly without septa, which terminates in a vesicle. The conidia may be aggregated in columns or diverge in a radiating manner. Some of them can produce large, thick-walled, hyaline cell; 2) *Eupenicillium sp* (Figure 2) produce structure with multi-cellular that called ascomata, The ripening of ascomata take a long time. Asci are produced either in chains or singly from crosiers; 3) *Cunninghamella sp* (Figure 3) produce sporangiola. Their colony grows fastly, initially white colour turn to dark grey. Sporangiphores have branches, verticillate or solitary; 4) *Pythium sp* (Figure 4) produce sporangia, zoopores. Sporangia are terminal or intercalary. Most species have smooth-walled oogonia, and are homothallic. Some species are heterothallic. Heterothallic species only develop oogonia in mating of two opposite strains. Some species have ornamented oogonia; 5) *Trichoderma*

sp (Figure 5) produce spora, conidia. They fast grow and easily sporulate green colonies have been listed in almost every soil fungal analysis. Conidiophores are irregular verticillate and bear cluster of flask-shape phialides. Vegetative colonies can be recognized by fast and thin growth, wide hyphae and characteristic smell, somewhat reminiscent of camphor. They also produce large hyaline chlamydospores; 6) *Vertilicium sp* (Figure 6) produce colony with white to pale yellow. Reverse colorless, yellow or ochraceous. Phialides solitary or in whorls arising from conidiophores or from slightly differentiated prostrate aerial hyphae. Some species absent in chlamydospores.

DISCUSSION

In this study PDA medium for observing and growing of all fungi was chosen. This because the medium was suitable for all microorganism including fungi. According to Dhingra and Sinclair (1985), Ganjar *et al.* (1999), medium is belong to one of common medium means that the medium is suitable for growing fungi and also bacteria, while to protect for other microorganism except fungi which grows in the medium then was added with chloramphenicol antibiotic. Antibiotic like chloramphenicol is not preferable for bacteria growing but appropriate for fungi. As mention by Murwani (2008) that the function of chloramphenicol antibiotic in the body of bacteria is to protect protein synthesis. Therefore if it occur then it will affect to the life of the bacteria.

The findings showed that in the soil of alfalfa plantation there were many kinds of fungi can grow. This findings was agree with the findings of Chen *et al.* (2008) that the soil planted which legumes has been found fungal community, Yudiarti (2007) that the population of soil born microorganism including fungi generally range from 250 to 3,000 propagul per gram soil.

Some of the species which were found in this study and have been identified were *Aspergillus sp*, *Pythium sp*, *Trichoderma sp* and *Vertilicium sp*. As mention by Alexopoulos and Mims (1979) that all species were belong to the common species of soil fungi. In addition, those species were also found by Eapen *et al.* (2005) and Chavarriaga *et al.* (2007). The other species e.g. *Cunninghamella sp*, and *Eupenicillium sp* are also species which belong to soil born fungi and they are ubiquitous in soil (Alexopoulos and Mims, 1979).

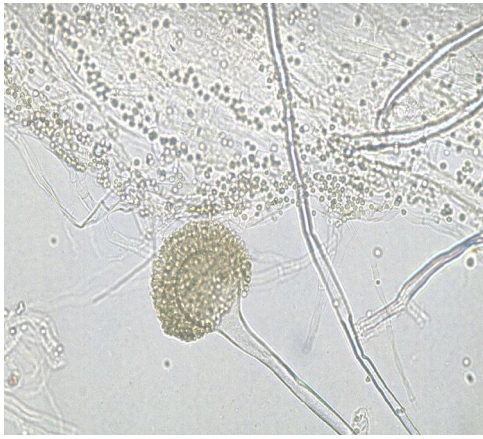


Figure 1. *Aspergillus sp*

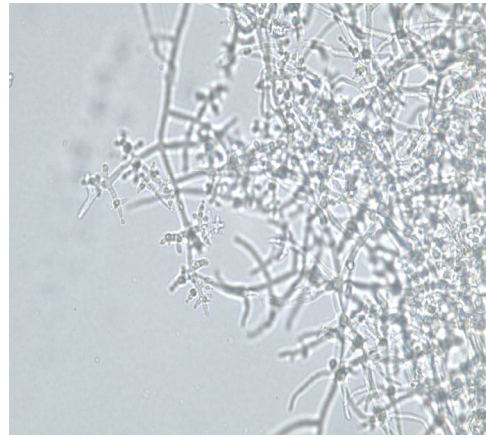


Figure 2. *Eupenicillium sp*

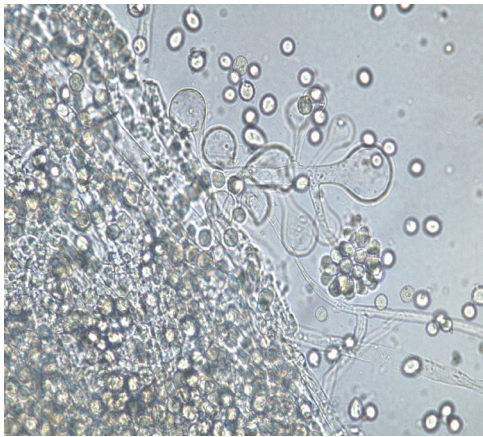


Figure 3. *Cunninghamella sp*

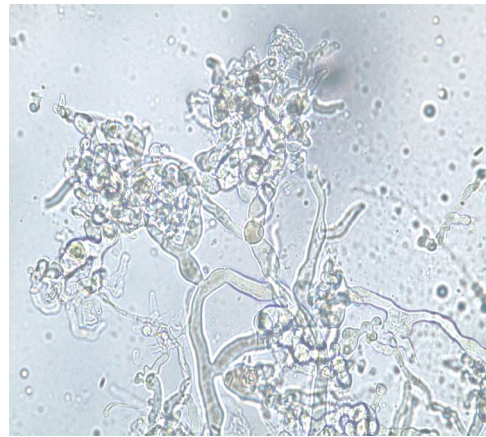


Figure 4. *Pythium sp*



Figure 5. *Trichoderma sp*

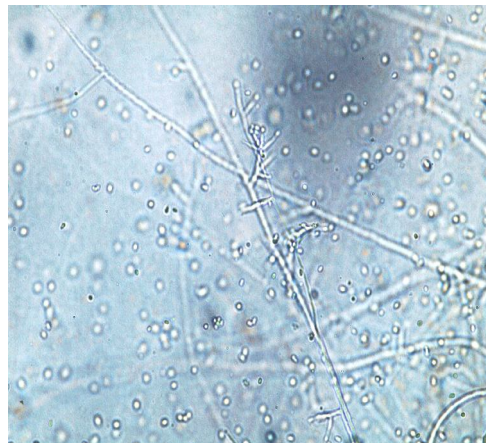


Figure 6. *Verticillium sp*

One of the findings fungi found in study was not unidentified. This was pointed to the character of some fungi was not clear. The reason was it might be the fungi was belong to specific fungi that needs the using a selective media. According

to Baruch and Stack (1990), the specific fungi needs a selective medium for their growing and for appearing of all their character. In this study used PDA medium which was not belong to selective medium and that may be the problem

why the unidentified fungi difficult to grow.

CONCLUSION

In conclusion, it was obtained six species and one unidentified from the identification of soil fungi isolated from alfalfa plantation in Baturaden, Purwokerto-Central Java. The six species were *Aspergillus sp*, *Cunninghamella sp*, *Eupenicillium sp*, *Pythium sp*, *Trichoderma sp* and *Verticillium sp*

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