Exploration of pathogenic and non-pathogenic fungi on alfalfa (*Medicago sativa L*)[1]

[*]Turrini Yudiarti,* Sumarsono,† and Didik Wisnu Widjajanto†

*Laboratory of Microbiology Faculty of Animal Agriculture, Diponegoro University, Semarang, Indonesia; and †Laboratory of Forage Crop ScienceFaculty of Animal Agriculture, Diponegoro University, Semarang, Indonesia

ABSTRACT: The aimed of the experiment was to explore Pathogenic and Non-Pathogenic fungi isolated from Alfalfa (*M. sativa* L) plantation in the tropic area. Soil and root of diseased plant samples were collected from the Alfalfa plantation in Baturaden, Banyumas Municipal, Indonesia (7°18'57.10"S; 109°14'1.29"E). Its location is about 650-1000 m above the sea level. The rainfall average is about 4,000 mm annually with the daily temperature is ranging between 15-25^{o}C. Samples were taken on the 23rd of July 2009. Fungi were isolated from both soil and root samples. Soil were crushed into small pieces and put on the Potato Dextrose Agar (PDA) media on plate directly. The root of diseased plant samples was rinsed in water, cut into small pieces (± 2 cm) and put on the same plate of media of soil sample. All plates were incubated in the room temperature for about 24-48 hours. Each fungus that grew on the plate then was separated by planting on a new media and incubated in the room temperature for about 24-48 hours. Purification has been done for each plate contained only one fungus. Six species: *Aspergillus* sp., *Cuninghammela* sp., *Eupenicillium* sp., *Pythium* sp., *Trichoderma* sp., and *Vertilicium* sp. were identified during the experiment. All of the identified isolates then were tested pathogenically. Experiment resulted that one species (*Pythium* sp.) was identified as a pathogenic fungus and others were non-pathogenic fungi.

Key words: isolation, identification, pathogenicity test, fungi, alfalfa (*M. sativa L*)

INTRODUCTION

Alfalfa (*Medicago sativa* L) is one of the important forage crops that have both high on protein and fiber content. At the first defoliation its protein and fiber content were 21.8 and 26.4%, respectively (Widyati *et al*., 2007). Under 4 weeks interval of defoliation, its protein and fiber content reached 22.0 dan 24.9%, respectively (Vetrianto *et al.*, 2007), meanwhile the dry matter and organic matter digestibility, respectively, were 64.9 dan 70.0% (Surahmanto *et al.*, 2007).

Fungi, unfortunately, may attack alfalfa either through seeds or mature plant (Jones, 1987). The disease caused by fungi may spread widely from one plant to another (Cook and Baker, 1983; Dhingra and Sinclair, 1985; Jones, 1987). Many problems in connection with fungi have been found at alfalfa plantation including at the experimental site of Baturaden Alfalfa's plantation, Banyumas Municipal, Central Java Province, Indonesia. The main problem was suspected due to the high rainfall that may give favourable condition for the growth of microorganisms, mainly fungi.

Root rot was one of diseases suspected on most alfalfa at Baturaden Alfalfa's plantation. To prove the suspection and to find the best alternative in tackling the problem, therefore, experiment on exploration of either pathogenic or non-pathogenic of fungi that may cause disease on alfalfa has been carried out. One of the alternative ways to solve the problem is to understand what kinds of fungi that live at alfalfa's plantation. Isolation of fungi that live in the soil and in the part of plant that attacked by the fungi should be done prior other works. Then, it should be followed by identification of fungi

[1] Authors thanks to the Directorate General for Higher Education for funding through multi years scheme of research fiscal year 2009, with the contract no.: 23.Rnc-I/HB 23/C1/FP/DIKTI/2009. Thanks were also extended to the Head of Microbiology laboratory and Forage Crop Science laboratory Faculty of Animal Agriculture, Diponegoro University for facilitating place and equipments during experiments. Thanks to Yohana Liani for assistance throughout the experiment.

that have been isolated and finally the pathogenicity test will be performed on the isolates (Yudiarti 1990).

MATERIALS AND METHODS

Sample was taken from alfalfa plantation that located at Baturaden, Banyumas Municipal, Central Java Province (CJP), Indonesia. It is located about 400 km South West of Semarang city, the Capital city of CJP. Its location is about 650-1000 m above the sea level. The rainfall average is about 4,000 mm annually with the daily temperature is ranging between 15-25^{o}C. Samples were taken place on the 23rd of July 2009. These were collected from root of alfalfa diseased plant and soil from the around of the root.

Preparing Potato Dextrose Agar Medium

About 200 g of potato was washed using distillated water, sliced into small pieces, emerged into 1 L of distillated water and boiled till the texture of potato crumbled. Potato solution then was taken and filtered using casa. Filtrate then were put into erlenmeyer and fill up with distillated water till 1 L of volume was reached. After that 20 g of sucrose and 17 g of agar were added. It then was shacked homogenously. The homogenous solution then was put into autoclave and sterilized at 121^{o}C 1 atmosphere. About 250 mg of chloramphenicol antibiotic was added into the solution after sterilization when temperature of solution reached 40^{o}C. Solution then was shacked homogenously. About 10 ml of solution then was put into dish and allowed to be compacted.

Isolation of Fungi from the Samples

Fungi were isolated from both soil and root samples using non selective methods modified by Warcup (Dhingra and Sinclair, 1985). Soil were crushed into small pieces, and then these were put into Potato Dextrse Agar (PDA) media on plate directly. The root of diseased plant samples was rinsed in water then also cut into small pieces about 2 cm and put on the same media on plate. All plates were incubated in the room temperature for about 24 – 48 hours. Each colony of fungi that grew on the plate were separated by planting on a new media and incubated in the room temperature for about 24 – 48 hours. The purification must always be done until each plate only contained one fungus.

Identification of Isolates

Thirty eight isolates of fungi were isolated from soil of alfalfa plantation. They then were grown on PDA with added-chloramphenicol antibiotic to protect bacterial growth. All plates were placed at 25°C and examined at regular intervals for observing of morphological colony and microscopic preparation like producting of hypha, sporangia, oogonia and reproduction structures. All isolates were identified using the key of Alexopoulos and Mims (1979); Plaat Niterink (1981); Robertson (1979); Ganjar *et al*. (1999); Dhingra and Sinclair (1985), and Gam *et al.* (1987).

Pathogenicity Test

Identified fungi were tested on pathogenicity on alfalfa plant on soil medium in pot. Soil medium was taken from the experimental sites of Forage Crop Sciences Laboratory, Faculty of Animal Agriculture, Diponegoro University. It is a latosol soil with the pH about 5.5, low in soil elements with field capacity and wilting point, respectively, were 39 and 21%. Soil samples were sterilized using autoclave at about 121^{o}C 1 Atmosphere for about 30 minutes. About 500 g of soil then was put into a small pot (Ø = 10 cm). Soil in the pot then was watered using distillated water till reached the field capacity. Five seeds of alfalfa then were planted into the pot then was allowed for germination. In the same time soil was inoculated with the identified fungi. Each fungi for each treatment and has three replicates. Seven days after planting, the plants were evaluated.

RESULTS AND DISCUSSION

The result showed that thirty eight isolates were isolated from soil and root of alfalfa diseased-plant samples. All isolates belong to species of *Aspergillus sp, Cuninghammela sp, Eupenicillium sp, Trichoderma sp, Vertilicium sp, Pythium sp,* and one species could not be unidentified. From the pathogenicity test of seven isolates to alfalfa were determined that six isolates belong to non-pathogenic fungi: *Aspergillus sp, Cuninghammela sp, Eupenicillium sp, Trichoderma sp, Vertilicium sp* and one was unidentify species. One species that was one *Pythium sp* was belong to pthogenic fungi.

Added-chloramphenicol antibiotic of PDA medium was used throughout the experiment as it is belong to one of the common media for growing fungi (Dhingra and Sinclair, 1985; Ganjar *et al*., 1999). Therefore, all fungi that were isolated from soil samples can grow at PDA medium easily. Addition of chloramphenicol antibiotic into PDA medium made it does not suitable for growing bacteria. Chloramphenicol antibiotic is belong to one antibiotic that has a function to protect bacterial growth (Murwani, 2008). Using non selective methods modified by Warcup (Dhingra and Sinclair, 1985) were possible to put all particels of soil into the media directly. Therefore, all soil particles can be utilized properly and all fungi that live in the soil can be isolated.

Thirty isolate of fungi were obtained throughout the experiment. This finding indicated that many kinds of fungi were existed at alfalfa plantation. It was matched with previous findings (Yudiarti, 2007) that the population of soil born microbe including fungi generally range from 250 to 3,000 propagules/g soil. It was also in connection with the findings of Alexopoulos and Mims (1979); Robertson (1979); Plaat Niterink (1981); Gam *et al*. (1987); Dhingra and Sinclair (1985);Yudiarti (1990); Ganjar *et al*. (1999). They found that kinds or the species of the findings fungi was also same species that live and born in soil. One of the findings fungi was not unidentified. This is may be due to the fact that fungi need a selective media for their growth. So, when the identification used the common media some fungi may not grow as profusely as in selective media.

On the basis of the pathogenicity test, six isolates belong to non pathogenic fungi were obtained. Those were *Aspergillus sp, Cuninghammela sp, Eupenicillium sp, Trichoderma sp, Vertilicium sp.* and one unidentify species. Microbe is belong to non pathogen if they do not have any potentially causing disease on plant (Jones, 1987; Yudiarti, 1990). One species that was *Pythium sp*. tend to be pathogenic fungi as it has potentially to causing disease on plant Jones, 1987; Yudiarti, 1990). It was shown that plant inoculated with *Pythium sp* isolate showed a similar symptom to that was found by Yudiarti (1990). The similar findings was also reported by Cook and Baker (1983); Jones (1987); Dhingra and Sinclair (1985); Yudiarti (2007); Gam *et al.* (1987). It was reported that *Pythium sp* is one of fungi that belong to the important patogen to many plant including alfalfa.

CONCLUSIONS

Experiment resulted that thirty isolates were obtained and six of them was identified as *Aspergillus sp, Cuninghammela sp, Eupenicillium sp, Pythium sp, Trichoderma sp, Vertilicium sp and* one was unidentify species. On the basis of the pathogenicity test it was found that six isolates were found as non-pathogenic fungi: *Aspergillus sp, Cuninghammela sp, Eupenicillium sp, Trichoderma sp, Vertilicium sp* and one was unidentify species. One species that was *Pythium sp* belong to pthogenic fungi.

LITERATURE CITED

Cook, R.J. and K.F. Baker. 1983. The Nature and Practise of Biological Control of Plant Pathogens. The Ame. Phytophatological Soc. Minnesota.

Dhingra, O.D. and J.B. Sinclair. 1985. Basic Plant Pathology Methods. CRC Press, Inc. Florida.

Gam, W., H.A. Van der Aa, A.J. Van der Paats Niterink, R.A. Samson and J.A. Stalpers. 1987. CBS Course of Mycology. Central Bureau Voor Schmimmelcultures. Baarn.

Ganjar, I., R. A. Samson, K. van den Tweel-Vermeulen, A. Oetari dan I. Santoso. 1999. Pengenalan Kapang Tropik Umum. Yayasan Obor Indonesia. Jakarta.

Jones, G. 1987. Plant Pathology. Principles and Practise. Open Univ. Press. England.

Murwani, R. 2008. Aditif Pakan. Aditif Alami Pengganti Antibiotik. Unnes Press, Semarang.

Pelczar, Jr. Chan. 1986. Dasar-dasar Mikrobiologi. Pradnya Paramita, Jakarta.

Samson, R.A., E.S. Hoekstra, Connie A.N. van Oorschot. 1984. Introduction to Food-Borne Fungi. Central Bureau Voor Schmimmelcultures. Baarn.

Yudiarti, T. 1990. The use of *Pythium periplocum* Isolated from Indonesian Soil as An Antagonist for Biological Control of *Pythium* Damping off. The Royal Veterinary and Agric. Univ., Denmark (Master Thesis)

Yudiarti, T. 2007. Ilmu Penyakit Tumbuhan. Graha Ilmu. Yogyakarta.