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Effects of Tannin to Control Leaf Blight Disease on *Toona sureni* Merr. Caused by Two Isolates of *Rhizoctonia* sp.

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ABSTRACT
This study investigated the efficacy of tannin in order to control leaf blight disease on *Toona sureni* caused by *Rhizoctonia* sp. by measuring disease intensity and percentage of leaf damages. Two *Rhizoctonia* sp. (R1 and R2) isolates were induced on the *T. sureni* leaves. Tannin solution with concentration of 0.5, 1, 2 and 3% and control (water) were sprayed on the leaves surface every two days. The percentage of disease sign on the leaves was measured every two days for one month. Results revealed that all tannin solution treatments reduced intensity and leaf death percentage. No poisonous impacts on the seedling were observed. The highest tannin concentrations which reduce disease activity were 3% for both isolates. It reduced the disease by stopping the fungal enzymatic activity of cellulolytic and pectinolytic. The highest increase of disease intensity and the percentage of leaf death was found in the control treatment. It can be concluded that tannin could be used to control leaf blight disease on *Toona sureni* caused by *Rhizoctonia* sp.

Key words: Tannin, *T. sureni*, *Rhizoctonia* sp., Isolates, enzymatic activity

INTRODUCTION

Toona sureni Merr. belongs to Meliaceae family and recognized as Suren. Naturally, *T. sureni* exist almost in all areas of Java and Bali, Indonesia. They spread also in West Sumatra and Ambon (Heyne, 1987). In the world, they spread naturally also in South-East Asia and South Asia countries such as in Srilanka, India, Myanmar, Malaysia, Laos, Pakistan, Nepal and Philippines. The tree species also present in some parts of East Australia and Hawaii (Edmonds, 1993). *Toona sureni* represents multipurpose plant, possesses high quality of wood and high social and economic potential and durability. It grows fast with a life cycle between 25-30 years and easy to process for wood industry (Heyne, 1987). The woods are used for raw materials of furniture, handy craft, decorative board, wooden floor, oil barrel, cigar box, pencil, some castanets (Edmonds, 1993). It also provides other benefit, i.e., used as a traditional medicine such as to heal dysentery, fever and stomach disease. With all of these benefits, this type of wood is suggested to plant as industrial forest plantations.

The efficacy program of industrial forest plantations depends on some factors; among them is the availability of healthy seed. But there are some resistances especially the plant disease problem. One of the diseases is leaf blight. Effect of the pathogen infection to the leaf may cause destruction of physiological function and lead to death. Soft attack can cause wood degradation or obstruction of growth (Achmad, 1991). Agrios (1997) has described that pathogen attack the hosts involving some mechanism such as mechanic pressure, chemical weapon which is in the form of enzyme or toxin. Agrios (1997) has reviewed the involvement of pectinase, cellulase and proteinase enzymes in course of infection of leaf disease. Then the symptom that happens localized wound at host leaf causing death and damage of cell. Porter *et al*. (1961) indicated that the active inhibitor of pectinase found in *scuppernong* grape leaves is tannin. Other work has shown that tannins are effective inhibitors for pectinase.

Grossmann (1958) reported that pectinolytic enzymes from *Fusarium oxysporum* were inhibited by added tannins. Bell *et al.* (1962) initiated to isolate the pectinase and cellulose inhibitor from seven species of plants. Special attention was given to *Lespedesa cuneata*, because of its potential as the inhibitor of both enzymes due to its high tannin content.

This research aimed to identify the pathogen species that infected the leaves of *Toona sureni* Merr. (leaf blight disease), to characterize the infection mechanism of the pathogen and to observe the use of tannin as a control agent on leaf blight disease.

MATERIALS AND METHODS

Fungal isolates: Fungal pathogen was collected at two different periods and named as R1 and R2 for the first and the second, respectively. They were collected through isolation from the infected *Toona sureni* Merr. leaf host. The host was originated from Pokpolandak Perum Perhutani nurseries in Cianjur, West Java, Indonesia. The isolates were cultured in Potato Dextrose Agar (PDA) media. The identification of isolates was conducted by using Ogoshi and Sneh key book.

Enzyme activity assays of the fungal isolates: Enzyme activity was assayed by Filter Paper-ase (FP-ASE) and polygalacturonase activity test. Glucose and galacturonic monohydrate acid were used as standard to determinate cellulase-C1 and polygalacturonase activities, respectively. Other test to detect pectinase production was conducted by culturing the fungi on pectin media (Atlas and Parks, 1993) and incubated for 10 days. Pectinase production was marked by changing the media color from chocolate to red.

Effect of tannin: Tannin was used as a substance to control the leaf blight disease due to the fungal infection. The seed of *Toona sureni* Merr. was divided into three treatment groups, i.e., R1, R2 and without isolate (control). Each group was sprayed by 5 tannin levels, i.e., 0, 0.5, 1, 2 and 3% in water. The experimental steps were consisted of: (1) Fungal cultivation, (2) Enumeration of hyphae density 5 days after cultivation, counted with Haemacytometer method, (3) Inoculation of the fungal species to healthy plant leaf by smearing using paintbrush in each leaf and sprayed with tannin solution at various levels, conducted every 2 days until one month and (4) Disease symptoms observation during the time of earliest leaf blight formed and number of infected leaves. Calculation of disease intensity was according to the method of Unterstenhofer (1976).

Data analyses: A factorial treatment design was employed in which the fungal isolates and tannin levels were the factors. Allocation of the treatments to each experimental unit was based on a completely randomized design by considering the effect of repeated measurement. Data obtained was analyzed by using analysis of variance (ANOVA) and were continued with Duncan multiple range tests and least square curve fitting method (Mattjik and Sumertajaya, 2002).

RESULTS AND DISCUSSION

Fungal isolates: Result of macroscopic and microscopic observation to both isolates shows that both are in the form of colony consist of thread-like growth called hypha (plural, hyphae); large masses of hyphae are referred to as mycelium (Fig. 1). At R1 isolate an oval-solid irregular shape lump was seen, light to dark brown, not differentiated into rind and medulla. While, in R2 isolate their existence has not been seen.

Microscopically, both isolates have characteristics among others appears as vertical branch at 90^{o} angles hyphae, critical branch at base, possessing partition (septa) (Fig. 2). There are no conidia or spore and clamp connection. Others found also monilloid cell with diameter : long, 1:1 comparison. Both isolates have hyphae diameter <15 µm and cell length <250 µm.

According to Sneh *et al*. (1991), based on the result of macroscopic and microscopic observation to both isolates it shows that they are recognized as *Rhizoctonia solani*. Hereinafter they also explain that *R. solani* do not have to produce sclerotium. Sclerotium (if present) is an oval-solid irregular shape lump, light to dark brown, not differentiated into rind and medulla. R1 isolate has sclerotium whereas R2 isolate do not.

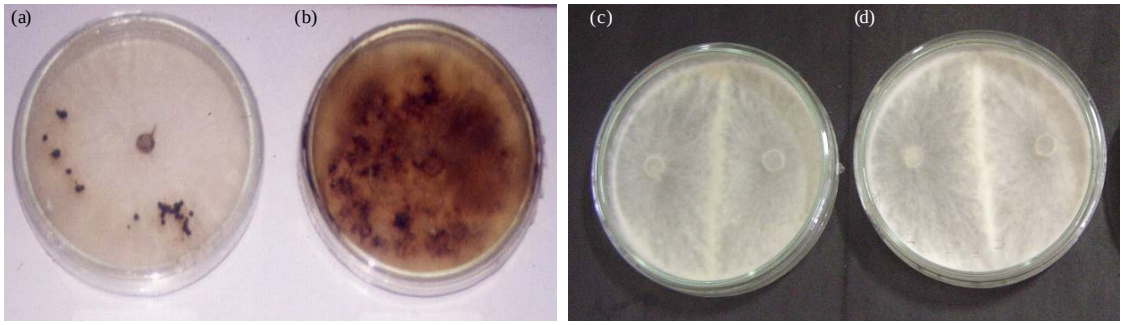

Fig. 1(a-d): Growth of two isolates *Rhizoctonia solani* at potato dextrose agar media, (a) R1 Isolate of first weeks, (b) R1 Isolate of third weeks and (c, d) R2 Isolate of first weeks

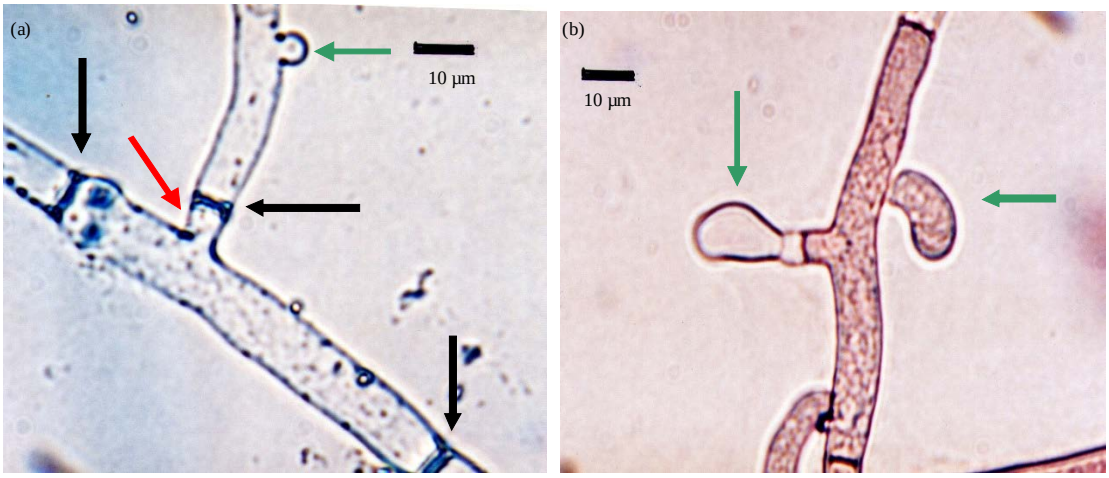

Fig. 2(a-b): Pathogen hyphae under microscope with 1000x magnification. Green dart show cell monilloid, red dart show critically branch at base and the black dart show cell wall

Microscopically, both isolates have *R. solani* characteristic. The characteristics are appears as vertical branch at 90° angles hyphae, critically branch at base, possessing partition (septa). There are no conidia or spore and clamp connection. Others found also monilloid cell with diameter: long, 1:1 comparison. Both isolates have hyphae diameter <15 µm and cell length <250 µm.

Ceresini (1999) reviewed that *R. solani* primarily attacks below ground plant parts such as the seeds, hypocotyls and roots, but is also capable of infecting above ground plant parts (e.g., pods, fruits, leaves and stems). The most common symptom of *Rhizoctonia* disease is referred to as "damping-off" characterized by non germination of severely infected seed whereas, infected seedlings can be killed either before or after they emerge from the soil. Infected seedlings not killed by the fungus often have cankers, which are reddish-brown lesions on stems and roots. In addition to attacking below ground plant parts, the fungus will occasionally infect fruit and leaf tissue located near or on the soil surface. This type of disease often occurs because the mycelium and/or sclerotia of the fungus are close to or splashed on the plant tissue.

Enzyme activity assays: Results of examination indicate that the fungal infecting seed leaf shows cellulase and pectinase activity existence commonly by detecting of glucose and galacturonic acid (Fig. 3). They are tested with filter paper-ase (FP-ase) and Bernfeld method. Increasing level of ammonium sulfate increases both cellulase and pectinase activities in pellet whereas, on the contrary, it decreases the enzyme activities in supernatant. The optimum level of ammonium sulfate with regard to cellulase and pectinase activities is 80%.

Enzyme represents a pathogen weapon to infect the host. Hydrolytic enzyme of cellulose and pectin is known to have important role in pathogenesis. Figure 3 explaining that *R. solani* possesses a number of enzymes which can be used to penetrate leaf cell wall. The biggest component from cell wall

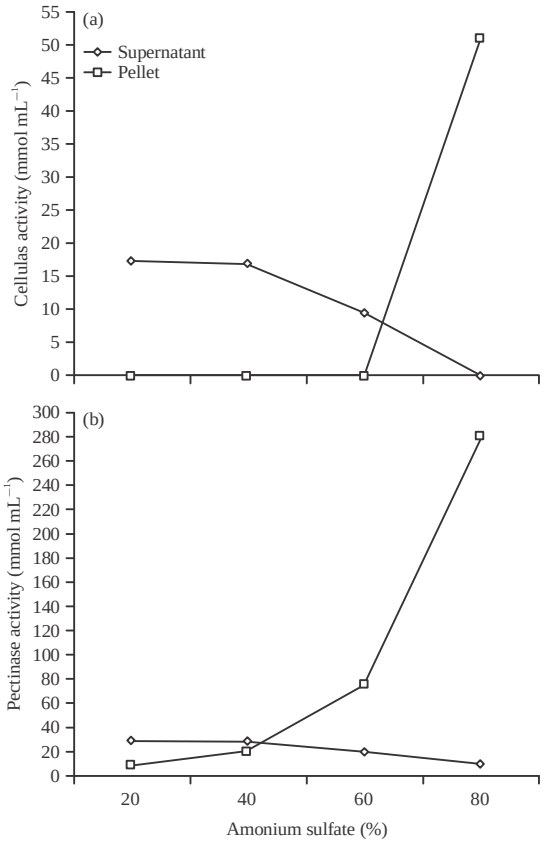

Fig. 3(a-b): Activity of (a) Cellulase and (b) Pectinase at various concentration of ammonium sulphate

is cellulose and pectin. So that to penetrate it is needed an enzyme which can degrade cellulose and pectin; that is cellulase and pectinase.

Effect of tannin: The effects of tannin to diseases intensity of leave blight are shown in Fig. 4-6. Based on statistical

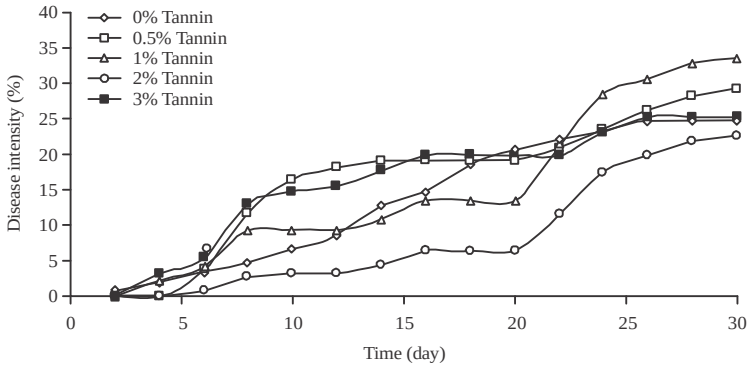

Fig. 4: Disease intensity curve at unsmeared isolates group treatment in various tannin level

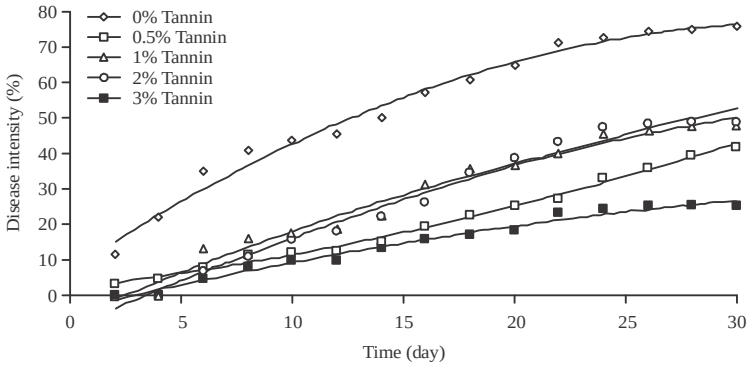

Fig. 5: Disease intensity curve at smeared R1 isolates group treatment in various tannin level

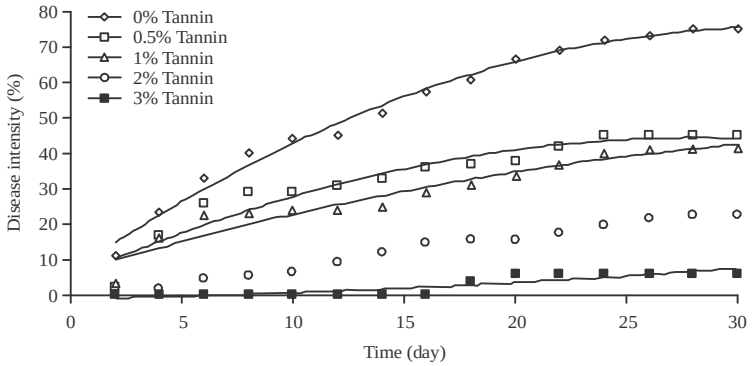

Fig. 6: Disease intensity curve at smeared R2 isolates group treatment in various tannin level

analyses, all of the treatments are significantly different. From Duncan Multiple Range Test result, it is found out that most ferocious isolate attack is by R1 isolate, followed later then by R2 isolate. The group of treatment which does not get smeared with disease solution remains to generate disease, only its distance is far enough from R2 isolate. This is the reason the curve of intensity of attack of disease to unsmeared with disease solution group of treatment (Fig. 5) is not too regular. Although treatment of tannin is given, it remains to generate disease because this disease has been formed before given tannin treatment.

Here in after from Duncan Multiple Range Test, it is known also that treatment of tannin 3% is the test which obstructs disease the most, so that its intensity is very low both in treatment of R1 isolate and also treatment of R2 isolate. One assistive equipment *R. solani* in infecting its host is celulase and pectinase enzyme. Hence, if the enzyme is obstructed or do not work, the ability of *R. solani* in infection will far decrease. This matter is supported by previous study that the material which successfully insulate enzyme resistor of cellulase and pectinase of vine leaf and after researched it is learned to be tannin. Bell *et al*. (1960, 1962) and Bell and

Etchells (1958) supporting function of tannin as material of enzyme inhibitor of cellulase and pectinase by insulating tannin from other crop and test its ability in obstructing the enzyme.

CONCLUSION

It was revealed in the present experiment that the cause of leaf bright disease on *Toona sureni* Merr. is a fungal species namely *Rhizoctonia solani*. The mechanism of attack of the fungal species is by secreting a number of enzymes particularly cellulase and pectinase. Tannin is a substance that can inhibit the enzyme activities released by *R. solani* and thus is potential to be used as biocontrol agent against the fungal species.

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