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EFFECT OF MANCOZEB 80% CONCENTRATIONS ON THE GROWTH OF *CENOCOCCUM GEOPHILUM* FR. UNDER *IN VITRO* CONDITION

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ABSTRACT

Fungicides, such as Mancozeb 80% are used in nurseries to prevent the plant root against pathogenic fungi. These fungicides may have negative impacts on beneficial organisms such as ectomycorrhizal fungi. *Cenococcum geophilum* is an important ectomycorrhizal fungus associated with some forest trees species.

An *in vitro* experiment was conducted in laboratory condition. *Cenococcum geophilum* was cultured on solid Modified Melin Nokrans' (MMN) medium containing Mancozeb 80 % at different concentrations (0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 μM). A completely randomized design was used with 8 replicates Petri dishes. Mancozeb 80 % decreased the growth of mycelia of *C. geophilum*. The radial growth of mycelia was not inhibited by Mancozeb 80 % at 0 to 400 μM concentrations. Fungi-static effect of Mancozeb 80 % was found at 500 to 600 μM concentrations, meanwhile fungi-toxic effect of Mancozeb 80 % was obtained at concentration more than 700 μM. A lethal level of Mancozeb 80% to the growth of *C. geophilum* was not found.

Key word : fungicide/Mancozeb 80 % */Cenococcum geophilum*/fungi-static, fungi-toxic

INTRODUCTION

Background

Seedlings production is one of the important processes for the success of forestry programs. Good quality seedlings must be provided in sufficient number and in appropriate time prior to the planting activities. Seedling quality is affected by genetic and environmental factors. The presence of mycorrhizal fungi on the root system of seedlings is one environmental factor that has been proven to increase plant growth.

Mycorrhizal research in Indonesia has been promoted by the Government, because of its potential to increase the quality of seedlings via growth acceleration (Marx 1973), to control some root pathogenic microorganisms (Marx 1973), to improve rooting due to hormone production (Gay and Debaud 1987), to increase nutrient and water uptake (Boyle *et al.* 1987), to increase drought resistance (Boyle

et al. 1987), and to increase survival and accelerate xylem formation of plants obtained by tissue culture techniques (Supriyanto 1989, Chang 1993).

The incidence of disease in tropical nurseries can lead to loss of nursery stock. The damping off diseases, caused by *Fusarium* sp., *Rhizoctonia* sp., or *Pythium* sp., are of most concern. These fungi destroy the root system and curtail water and nutrition absorption.

Therefore, fungicides are commonly used in nurseries to protect the plant root against these pathogenic fungi. Concentrations of mancozeb 80% normally used in forest nurseries, such as in *Pinus merkusii* nurseries, are between 1.8 – 2.0 gram/liter. Fungicides may inhibit the growth of the pathogenic fungi or kill them. However, non-target microorganisms, especially the beneficial ones such as mycorrhizal fungi, may also be killed.

Cenococcum geophilum is an important mycorrhizal fungus in the nursery. This fungus belongs to imperfect fungi, and is commonly found in the nursery as mycorrhizal fungi. *Cenococcum geophilum* doesn't produce a fruiting body. Its isolation started from isolated mycorrhizal root tips. In nursery techniques for seedlings production, the nursery man used commonly pesticide and fertilizer intensively. Therefore, it is possible that *C. geophilum* could survive under high concentration of pesticide and fertilizer, high water retention, and less oxygen. In such condition, *C. geophilum* became a promising mycorrhizal fungi to be used for seedlings production of forestry species.

The use of fungicides such as Mancozeb 80% (Dithane M-45) could have a negative effect on the growth of *C. geophilum.* Several studies have pointed out the negative effect of pesticides on the growth and development of mycorrhizal fungi (Marx and Rowan 1981). In pure culture, Mancozeb 80 % has a strong toxic effect on the growth of mycorrhizal fungi including *Pisolithus tinctorius, Laccaria laccata, Rhizopogon luteolous,* and *Corticium bicolor* (Thapar 1987). However, so far no information is available for *C. geophilum.* Aside from the effect of fungicides on the growth of mycorrhizal fungi, the work on herbicides indicates opposite growth responses in *Scleroderma aurantius* and *Pisolithus tinctorius* (Lake *et al*. 1981).

Hence, the objectives of this research were : to study the effect of different concentrations of Mancozeb 80% on the growth of *C. geophilum* and to determine the fungi-toxic level of Mancozeb 80% to *C. geophilum* under *in vitro* conditions.

MATERIALS AND METHODS

Source of culture come from the laboratory of Silviculture SEAMEO-BIOTROP, that was isolated from *Pinus merkusii* seedlings. *Cenococcum geophilum* was propagated on solid Modified Melin Norkrans' medium (Marx 1969). Small pieces of dense mycelia (1 cm in diameter) were cultured on MMN media containing Mancozeb 80 % at 0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and

1000 μM concentrations. Mancozeb 80% was added in the culture media and autoclaved at 120^{o} C, 1.5 bar, 30 minutes. The experiment was carried out in an incubator. The temperature in the incubator was 28^{o} C to 30^{o} C and in dark condition. Radial growth of mycelia was observed every 5 days for two months. Measurement of radial growth of *C. geophilum* mycelia was conducted at 8 different directions. The colour change of mycelia was also observed. Morphological change of mycelia from different fungicide treatments was observed under a light microscope. The experiment was arranged in completely randomized design with 8 replicates Petri dishes. Analysis of variance was done followed by Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

Radial Growth of Mycelia of *Cenococcum geophilum*

The effects of different concentrations of Mancozeb 80 % on the growth of mycelia of *C. geophilum* is shown in Figure 1. At 0, 50, 100, 200, 300 and 400 μM concentrations, the growth of mycelia was always better than the other concentrations ($\geq$ 500 μM). Inhibition of mycelial growth was found clearly at 30 days after treatment. The mycelia of *C. geophilum* grew very well at 5 days after treatment, especially at 0, 50, and 100 μM concentrations. However, in general the increase of fungicide concentration significantly inhibited the growth of mycelia. Finally, the effects of Mancozeb 80 % on the growth of mycelia of *C.geophilum* can be classified into two groups as follows : fungi-static and fungi-toxic effects (Figure 1).

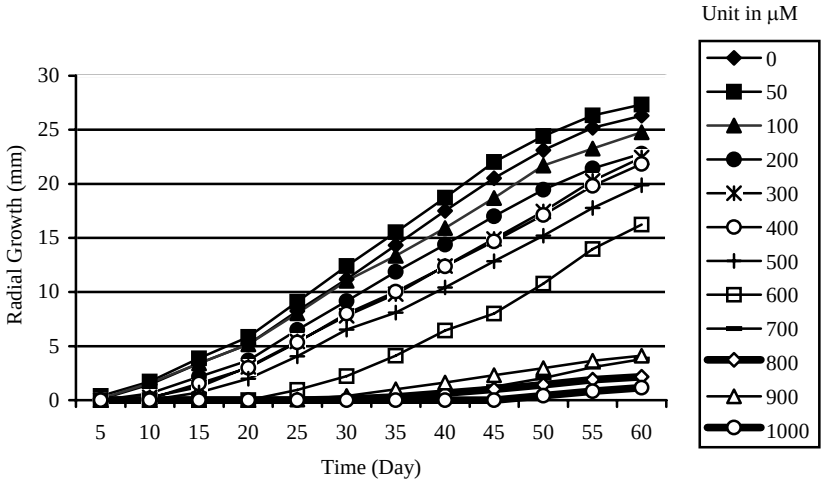

Figure 2 shows the radial growth of two-month-old *C. geophilum* mycelia in different concentrations of Mancozeb 80 %.

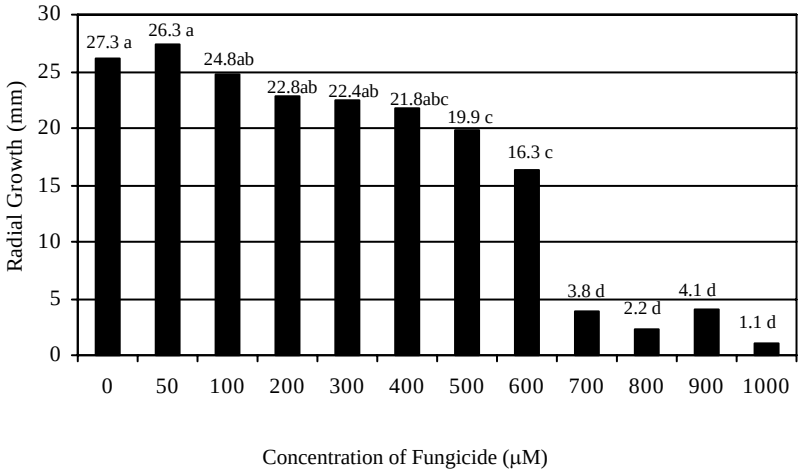

Figure 2. Radial growth of two-month old *Cenococcum geophilum* mycelia in different concentrations of Mancozeb 80 %

The radial growth of *C. geophilum* mycelia in different concentrations of Mancozeb 80 % was divided into three groups as follows:

(1) The radial growth of mycelia at concentration of 0-400 μm (21.8 – 27.3 mm),
(2) The radial growth of mycelia at concentration of 500-600 μm (16.3 -19.9 mm),
(3) The radial growth of mycelia at concentration of 700 – 1000 μm (1.1 - 4.1 mm).

Inhibition effect of Mancozeb 80% to radial growth of *C. geophilum* mycelia in the solid medium of MMN was not significant at 50 to 400 μM concentrations. The fungi-static effect (the concentration in which fungicide starts to inhibit the growth of mycelia) of Mancozeb 80 % was started at 500 μM up to 600 μm concentrations. While, the fungi-toxic effect (the concentration in which fungicide starts to kill the fungi) of Mancozeb 80 % was found at 700 μM concentration. Figure 3 shows that *C. geophilum* mycelia could not grow in solid medium of MMN containing Mancozeb 80% of 700 – 1000 μM. In this case, Mancozeb 80 % disturbed cellular organ of *C. geophilum.* Moreover, the normal process of fungal intracellular digestion did not run well. It will cause the dead of the fungi or disturb the fungal growth (Finholt 1952).

Fungicides will cause the decrease of membrane permeability surrounding fungal hyphae. Three systemic fungicides, Benlate, Aliette, and Ridomil, showed

effects on the percentage of mycorrhizal infection (Jabaji -Hare and Kendrick 1987). Benlate, affected all aspects of fungal development, not only the fraction of the root length infected but also the total root length (Carey *et al.* 1992). Benlate severely reduced the number of intercellular hyphae and arbuscules per section and there was a small but non-significant effect with respect to vesicles (Sukarno and Smith 1993). This condition will give a negative effect on the intracellular digestion process. The decrease in membrane permeability caused inhibition of liquid material absorption through the fungal membrane. Moreover, the fungicide could dis-activate extra-cellular enzymes (Finholt 1952). Enzyme availability is very important for fungal activity in decomposing its nutrient source to the simplest form to be absorbed. The inactivity of this kind of enzyme will inhibit the fungal nutrient absorption that may cause the disturbance on the fungal growth. Finally, this condition will cause the death of the fungi.

The effects of Mancozeb 80 % on the performance and structure of mycelia of *Cenococcum geophilum* is shown in Figure 4.

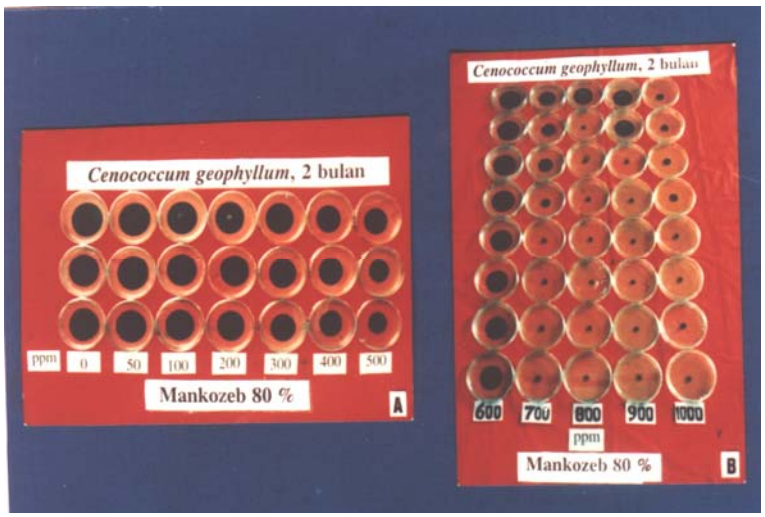

Figure 3. Radial growth of two-month old *Cenococcum geophilum* mycelia in different concentrations of Mancozeb 80 % in solid medium of MMN

In solid medium of MMN containing Mancozeb 80% most of the hyphae became fragmented dark black color thick and branching while in the control

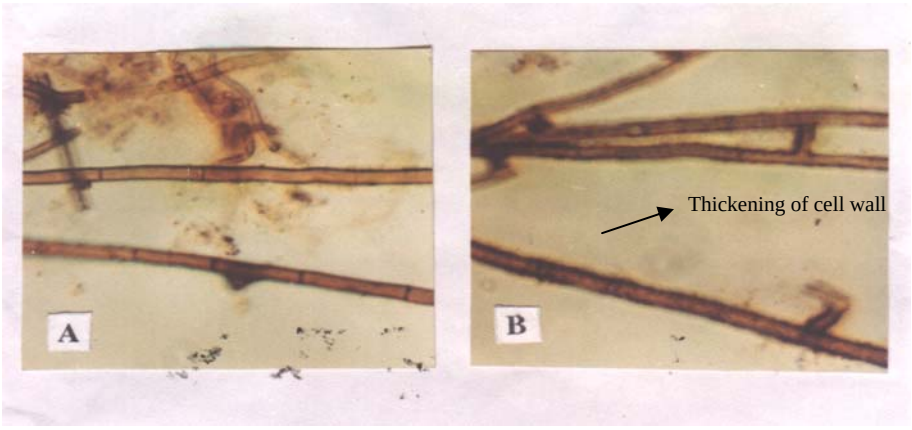

figure 4. Two-month-old *Cenococcum geophilum* mycelia in solid medium of MMN (40 X) without fungicide (A) and with fungicide addition (B)

CONCLUSIONS

An *in vitro* experiment showed that Mancozeb 80 % reduced the growth of *Cenococcum geophilum* mycelia at more than 700 μM concentration as fungi-toxic level. Mancozeb 80 % affected also the mycelial morphology (fragmented, dark black) of *Cenococcum geophilum.*

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