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Effects of Indigenous Fagaceae-Inhabiting Ectomycorrhizal Fungi *Scleroderma* spp., on Growth of *Lithocarpus urceolaris* Seedling in Greenhouse Studies

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ABSTRACT
Ectomycorrhizal fungi, *Scleroderma* spp., is potential to promote the growth of seedlings for forestry plants. This research explored the *Scleroderma* spp., from rhizosphere of Fagaceae in School of Biology forest, investigated the compatibility of *Scleroderma* spp., with *Lithocarpus urceolaris* seedlings and studied its effectiveness. The result showed that there were three species of *Scleroderma*: *Scleroderma sinnamariense*, *Scleroderma columnare* and *Scleroderma citrinum*. *Lithocarpus urceolaris* inoculated with *Scleroderma sinnamariense*, resulted in the highest growth of plants (56.55 cm) compared to *S. columnare*, *S. citrinum* and control. Diameters of seedlings inoculated with the three species of *Scleroderma* did not show significant different but they were significant different from control. The three species of *Scleroderma* had the same growth of colonizations (30%) classified as middle colonizations. There were changes in morphology and anatomy of roots from the infection of three species of *Scleroderma*. Mantle was clearly observed to cover the root surface and the mycelia formed the Hartig net. There was compatibility between *L. urceolaris* and three species of *Scleroderma*. It is suggested that inoculating these *Scleroderma* to *L. urceolaris* is necessary to increase the quality of growth seedling.

Key words: Ectomycorrhizal fungi, *Scleroderma* spp., *Lithocarpus urceolaris*, compatibility

INTRODUCTION

The School of Biology Forest (SBF) of Andalas University is one of the protected lowland forests in West Sumatera as a natural water resource for Padang City. Besides its economic value and environmental service as town lungs, SBF is also used as a research and education areas. SBF has approximately 150 ha width with hilly topography, 10-30% slope, located in 260-465 m above sea level with temperature ranges from 24-27°C and relative humidity 68-90%.

Actual condition of SBF has been disturbed, for instance there are areas of farming, illegal logging and land clearing by fire. As a protected forest, these conditions have disturbed the ecosystem and endangered the existent populations that leads to extinction. However, SBF is an unique area since it is dominated by Fagaceae plants. This circumstances gives great chances for scientists to study about Fageceae especially about its association with ectomycorrhizal fungi in West Sumatera. Fagaceae had high growth rates of diameter and physically hard wood of a stem (Yoneda *et al*., 1997). It has economy

value since it could be used for floor, furniture and construction. Its fruits are usually used for medicinal and food.

High domination of Fagaceae in SBF gives a significant contribution to the structure and function of this lowland forest ecosystem. So, a good quality of Fagaceae seedlings are really needed for the conservation of SBF. Breeding this plant using seeds take a long time period and small percentage of seed germinations. One of the methods to increase the growth of seedlings is by utilizing the ectomycorrhizal technique. Introducing the ectomycorrhizal is a solution to recover the quality of forest which has been already damaged. Ectomycorrhiza used could be obtained from the same species of plants or others.

Ectomycorrhiza is one of mycorrhiza which usually infects the forest plants. The fungi of ectomycorrhiza obtain the source of carbon from their host plants, on other hand, mycelium of the fungi help the plants to absorb water and nutrient from soil. The ectomycorrhizal fungi not only accelerate the shoot growth but also rehabilitate degraded land. According to Bougher *et al*. (1996) ectomycorrhizal fungi have great benefits to ecological function and have role in food chains in rhizospheres. Smits (1992) mentioned that Dipterocarpaceae seedlings from seeds and vegetative multiplication, need the ectomycorrhizal fungi for their growth.

Some of the forest trees such as Pinaceae, Betulaceae, Fagaceae, Dipterocarpaceae and Myrtaceae associated with the ectomycorrhizal fungi (Wilcox, 1983). Most of the fungi forming ectomycorrhiza, are Basidiomycetes such as *Scleroderma* sp., *Laccaria* sp., *Amanita* sp., *Pisolithus tinctorius*, *Boletus* sp., *Telephora* sp., *Russula* sp., *Suillus* sp., (De la Cruz, 1983; Bougher *et al*., 1996).

Scleroderma forms ectomycorrhizal associations with a wide range of woody plants, including members of the Pinaceae, Myrtaceae, Fagaceae, Mimosaceae, Dipterocarpaceae and Cistaceae (Sims *et al*., 1997; Jeffries, 1999). Some beneficial isolates can vigorously compete with other ectomycorrhizal fungi in field (Jeffries, 1999; Martin *et al*., 2003).

No studies have addressed the diversity or distribution of ectomycorrhizal fungi on Garry oak (*Quercus garryana* Hook). In spite of interest in habitat, systematic, tree health, natural regeneration and restoration of Garry oaks, no studies have gone below ground to see which fungal associates are present (Valentine *et al*., 2002).

The SBF as a part of the lowland tropical forests has significant biological resources, therefore the existence of ectomycorrhiza is expected to give contribution for forest trees. The loss of various types of plants as a result of forest destruction will be followed by the loss of ectomycorrhizal fungi associated with plants of the forest. So far there has not been a study to investigate the potential of indigenous ectomycorrhiza in West Sumatera forests.

The existence and diversity of ectomycorrhizal fungi in SBF in colonization of Fagaceae is very important to find indigenous *Scleroderma*. Utilizing indigenous *Scleroderma* is expected to give an effective method with a low cost to promote initial growth of *Lithocarpus urceolaris* (Fagaceae). Objectives of this study were to explore *Scleroderma* spp., from rhizosphere of Fagaceae plant in SBF and study their compatibility to observe their effectiveness to *Lithocarpus urceolaris* seedlings.

MATERIALS AND METHODS

Collection of ectomycorrhiza fungi, *Scleroderma* spp.: Fruit bodies of *Scleroderma* spp., found in rhizosphere of Fagaceae were collected and identified. The fruit bodies found were taken carefully by taking some mycelia in soil attached to roots of host plants by using small shovel. The fruit bodies were cleaned from dirts and then put in brown paper bags separately then placed in plastic bags. Then they were labeled and identified. In laboratory the fruit bodies were collected in formalin (4%) after being washed with water then with sterile water and alcohol. Identification was based on basidiocarp characteristics referred to Sims *et al*. (1995) and Bougher *et al*. (1996).

Preparation of *Scleroderma* spp., inoculum: The inoculum was obtained from the fruit body tissues. The young fruit bodies were chosen to be isolated because their tissue were actively growing. There fore the mycelia grew rapidly. Isolation was done by cutting fruit bodies aseptically then hyphae were taken from center of gleba with inoculation needle and shifted to the Petri dishes filled with MMN medium then incubated. The mycelium suspension was made by blending the mycelia in water by adding one drop Tween 20 for 500 mL water. The ratio between mycelium and water was 1:10 (m/v). Every seedling was inoculated with 1 mL mycelium suspension.

Seed germination: The seeds of *Lithocarpus urceolaris* were collected from SBF. The surfaces were sterilized by sodium hypochlorite solution (NaOCl) and rinsed by sterile water. Germination phase was done in seedling trays which were filled by the mixture of soil and sand with 1:1 volume ratio. This medium was sterilized by autoclave for 30 min at 1.5 atm and 121°C. The medium was not compact, so it had enough porosities to prevent damage to the roots at the time of weaning. The seedlings were watered to keep the humidity.

Preparation of seedling media: Media used for the growth of *Lithocarpus urceolaris* seedlings was a mixture ultisol and sand with ratio 1:1. The medium was sterilized by autoclave for 30 min at 1.5 atm and 121°C. The sterilized medium was put into polybags.

Compatibility test: The prepared medium in the polybag was watered until saturated. The two weeks of *L. urceolaris* seedlings which had uniform growth were selected and moved into the polybags. Then, the roots of seedlings were inoculated by indigenous *Scleroderma* spp. As a control, seedlings were not inoculated. The seedlings were not watered for the first two days after inoculated to prevent inoculum from being leached. After that, watering was done once in two days. This

research used randomized block design. Data were analyzed by ANOVA and Duncan's New Multiple Range Test (DNMRT) with 95% confidence interval.

Ten months after inoculation all seedlings were harvested. Root systems of the seedlings were photographed and root samples were taken for further analysis and examination under a microscope. The seedlings were taken out from the polybags and carefully washed without damaging the roots. The clean roots were observed to find out the progress of mycorrhizal based on the colonization percentage of ectomycorrhiza. Colonization percentage of ectomycorrhiza were observed visually.

Growth parameters observed were height and diameter of seedling measured after 10 months. Height of seedling was measured from the base of seedling to the top. Stem diameter measured was the part at the ground surface using caliper.

Root preparation for microscope observation: To make sure that the roots were infected by mycorrhiza, root histology was observed. Roots specimens were prepared following the method of Sass (1958) which covered the process of fixation, dehydration, parafinas, cutting and staining. Ectomycorrhizal roots of *L. urceolaris* were soaked in Formalin Alcohol Acetic Acid (FAA) for 24 h and sliced using a rotary microtome for 5-10 µm thick, then stained with tryphan blue. The existence of mantle and Hartig net indicated that the seedling had been infected by ectomycorrhiza *Scleroderma* spp.

Morphology of ectomycorrhiza from *L. urceolaris*: The characteristics of root morphology like branch patterns and colors of sheath were also observed using loop. Roots with mycorrhiza symbiont were marked by the existence of mantle or hyphae covering the roots.

RESULTS

Exploration of *Scleroderma* spp.: The result showed that the isolates found in rhizosphere of Fagaceae in School of Biology Forest, Andalas University showed variation in the shape, size, color and the stipe of their basidiocarp, also the shape and color of their spores. Morphologically, the result showed that there were three species of *Scleroderma* found in rhizosphere of Fagaceae in School of Biology Forest, Andalas University, i.e., *S. citrinum*, *S. columnare* and *S. Sinnamariense*.

According to Hawksworth *et al*. (1995) cited in Bougher *et al*. (1996) *Scleroderma* ectomycorrhizal fungi could be classified into:

Division: Basidiomycotina
Class: Holobasidiomycetes
Sub-class: Gasteromycetes
Order: Sclerodermatales
Family: Sclerodermataceae
Genus: Scleroderma
Species: *Scleroderma citrinum*, *Scleroderma columnare* and *Scleroderma sinnamariense*

Microscopically external hyphae surrounding the ectomycorrhiza were septate and formed clamp connection which is one of characteristics of fungi in Basidiomycetes (Fig. 1).

Growth of *L. urceolaris* seedling: Effects of *Scleroderma* spp., inoculation on *L. urceolaris* seedlings were presented in Table 1 and Fig. 2.

Inoculation of three species of *Scleroderma* on *L. urceolaris* showed significant effect on seedling height and

Table 1: Effects of *Scleroderma* spp., inoculation on growth of *Lithocarpus urceolaris* seedlings

Treatments	Seedling high (cm)	Seedling diameter (mm)	Colonization (%)	Mantle (µ)	Hartig net (µ)
S. sinnamariense	56.55[a]	3.92[a]	30	20	15
S. columnare	40.25[b]	3.92[a]	30	7-8	15
S. citrinum	39.45[c]	3.91[a]	30	5-10	20
Control	22.33[d]	2.39[b]	0	0	0

Numbers in the column followed by the same lower case letter are not significantly different according to DNMRT at 5% significance level

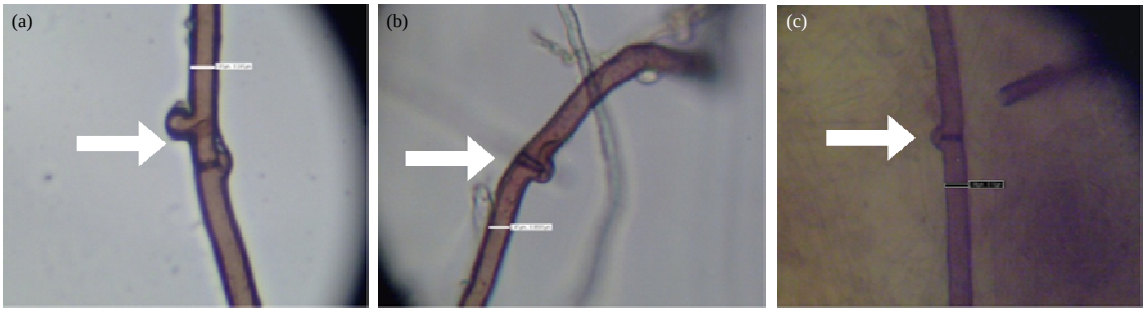

Fig. 1(a-c): Hyphae *Scleroderma* spp., with clamp connection (white arrow), (a) *Scleroderma citrinum*, (b) *Scleroderma columnare* and (c) *Scleroderma sinnamariense*

Fig. 2: *Lithocarpus urceolaris* seedlings inoculated with three species of *Scleroderma*, a: *Scleroderma citrinum*, b-c: *Scleroderma columnare*, d: *Scleroderma sinnamariense* and e: Control

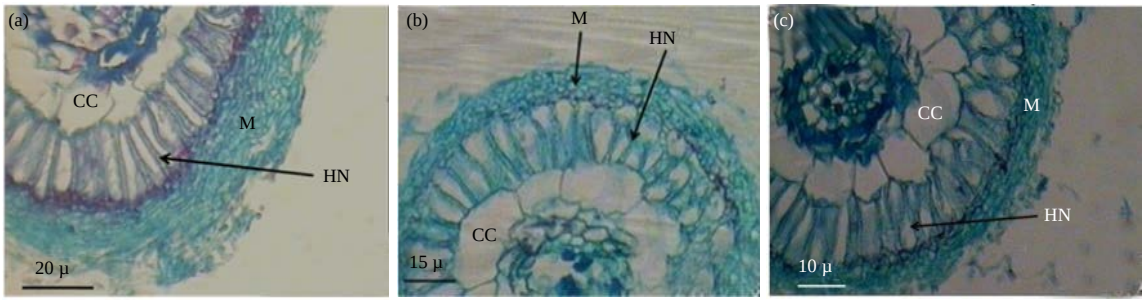

Fig. 3(a-c): Cross section of *L. urceolaris* roots infected by *Scleroderma* spp. (a) *Scleroderma sinnamariense*, (b) *Scleroderma columnare* and (c) *Scleroderma citrinum*. M: Mantle, HN: Hartig net, CC: Cortex cell

diameter. Height and diameter of seedlings inoculated with three species of *Scleroderma* were higher compared to those of control. Among the treatments, seedling height showed significant effect but not in diameter. The highest seedlings were obtained from inoculation with *S. sinnamariense* (56.55 cm) followed by *S. columnare* (40.25 cm) and *S. citrinum* (39.45 cm). Figure 2 showed the seedlings of *L. urceolaris* grew much better than control.

Percentage of ectomycorrhizal colonization: Inoculation of *Scleroderma* spp., on *L. urceolaris* affected the colonization percentage of ectomycorrhiza. The control seedlings were clear from the ectomycorrhizal since they were not inoculated. Based on the morphology analysis, the ectomycorrhiza development occurred on roots of *L. urceolaris* seedlings inoculated with *Scleroderma* spp. Three species of *Scleroderma* in this research showed the same colonization growth (30%).

Mantle and Hartig net thickness: At cross section of roots, mantle and the Hartig net were not observed on seedlings of *L. urceolaris* uninoculated with *Scleroderma*. On the other hand, the inoculated seedlings by *S. sinnamariense*, *S. columnare* and *S. citrinum* showed a complete structure of mantle and Hartig net. The mantle was clearly shown to cover the surface of the roots and the mycelia of *Scleroderma* formed the weaving Hartig net (Fig. 3). The three types of *Scleroderma* formed a single layer mantle. The thickness of the mantle was 5-20 µ and the one of the Hartig net was 15-20 µ (Table 1).

Morphology of ectomycorrhiza on *L. urceolaris* seedlings: Ten months after inoculation, *L. urceolaris* seedlings were well colonized by *S. sinnamariense*, *S. columnare* and *S. citrinum*. Macroscopically, ectomycorrhiza formed on the roots of *L. urceolaris* seedlings associating with three species of indigenous *Scleroderma* in SBF, had the same morphological characters resulting in monopodial branches. Most of the surface sheaths were white.

DISCUSSION

The higher height and diameter of seedlings indicated that there was association between *L. urceolaris* with *Scleroderma* spp. The positive effect of the association was also shown on *Scleroderma*. This indicated that association gave benefits for both seedlings and ectomycorrhizal *Scleroderma*.

All three species of *Scleroderma* found had the same colonization growth (30%), classified into medium colonization. Marx *et al*. (1991) divided the percentage of colonization into four groups, (1) 75-100% (very good/high), (2) 50-74% (good), (3) 24-49% (medium) and (4) 1-24% (bad/low). Infection of *Scleroderma* ectomycorrhiza caused the change in root structures. In infected root there was found hyphae covering roots of *L. urceolaris* seedlings. Mantels were formed to cover roots and Hartig net was formed among cortex cells. This depended on the compatibility between host plants and ectomycorrhizal fungi penetrating roots, age of root seedling inoculated and environment that support growth of fungi mycelia. Every species of plant should have compatibility with a fungi to form mycorrhiza. The compatibility is necessary to form symbiosis between fungi and plants. Every species of ectomycorrhizal fungi has a very high compatibility with its host plant. It is marked by higher percentage of ectomycorrhizal colonization, high growth rate and formation of mantle and Hartig net. According to Wolf and Wolf (1947), anatomically and morphologically roots infected by mycorrhiza are generally marked by formation of mantle covering roots and formation of Hartig net consisting of hypha penetrating among cortex tissue. If both structures can be observed in root colonized, it means that ectomycorrhizal fungi inoculated compatible with species of seedling inoculated. Peterson and Farquhar (1994) said that roots colonized by ectomycorrhizal fungi mostly undergo changes in morphology and anatomy. Infected roots usually undergo alteration of their diameter, length and branches. According to Zak (1971, 1973), different host plant species showed different branch forms and sheath color of ectomycorrhiza.

There was compatibility between *L. urceolaris* seedling with indigenous *Scleroderma* in SBF. Therefore, colonization level and compatibility of three species of *Scleroderma* on *L. urceolaris* would influence seedling growth. Host plant species and different level of association are important in relation to introduction of exotic trees species.

In ectomycorrhiza, sometimes it took longer time for fungi to infect host plants. Lu *et al*. (1998) reported that colonization percentage of ectomycorrhiza *Scleroderma* on *Eucalyptus urophylla* was very low after nine months inoculations. The success of root colonization depends on spore germination, mycelia growth in soil and susceptibility of roots against mycorrhizal infection (Bowen, 1994; Reddy and Natarajan, 1997). Physiological and ecological difference between the same and different species of ectomycorrhiza influence its success in colonizing roots (Bonfante *et al*., 1998). Selection of compatible ectomycorrhiza species is very important for the success of developing inoculation program (Dell *et al*., 1994). Generally, the existence of association of ectomycorrhiza in plant roots could increase the plant growth. The increase in plant growth means the *L. urceolaris* seedling can be moved to field earlier so that it could decrease the cost of seedling rearing.

Scleroderma is one of groups of ectomycorrhizal fungi growing in Indonesia forests. Hyphae *Scleroderma* has a major role in absorbing nutrients and water for host plants. According to Chen *et al*. (2006), *Scleroderma* inoculation could increase seedling growth of Pinus and Eucalyptus up to 105% after colonization.

Inoculation has a role to form mycorrhizal colony because spores is a reproductive organ of ectomycorrhizal fungi that in turn would develop to form hypha in suitable environment. On the following stage hypha will grow to cover roots in infection area to form colony and finally hypha will penetrate roots (Alexopulos and Mims, 1979). Inoculation of indigenous ectomycorrhizal *Scleroderma* in SBF on *L. urceolaris* seedling was meant to study the compatibility between the two.

Shemakhanova in Julich (1988) indicated that infecting fungi inoculums to seedlings on one of Fagaceae like oak (*Quercus* sp.) could increase leaves size up to 70% or two fold bigger than control. The existence of mycorrhiza indicated that there was mutualism functional interaction between certain plants with one or more mycobion strain and vice versa in space and time.

Successful formation of ectomycorrhiza showed the compatibility between *S. sinnamariense*, *S. columnare* and *S. citrinum* with *L. urceolaris*. This means that *L. urceolaris* could become the host plant for *S. sinnamariense*, *S. columnare* and *S. citrinum.* Most *Scleroderma* sp., have a wide range of host plants (Jeffries, 1999) but they need a specific host for producing fruiting bodies. For example, *S. columnare* needs *Pinus merkusii* and *S. dictyosporum* needs dipterocarps to produce fruiting bodies. During the field observation, fruiting bodies have never been found. Potential ectomycorrhizal association often indicated by the formation of fruiting bodies. According to Massicotte *et al*. (1994), for *Scleroderma* the percentage of ectomycorrhizal colonization on the secondary host was lower than that in the primary host. The low colonization on the secondary host has also been found for other ectomycorrhiza fungi.

CONCLUSION

There were three species of *Scleroderma* identified associated with *L. urceolaris* in School of Biology Forests at Andalas University: *S. sinnamariense*, *S. columnare* and *S. citrinum*. Symbiosys of the three species could increase the growth of *L. urceolaris* seedlings. There was changes in morphology and anatomy of roots infected by the three species of *Scleroderma*. There was also compatibility between *L. urceolaris* with the three species of *Scleroderma*. It is suggested to inoculate *L. urceolaris* seedlings with the three species of *Scleroderma* to increase seedling growth.

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