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Effect Indigenous Ectomycorhizal Fungi *Scleroderma* spp. Isolated from Botanical Garden of Andalas University on Roots of *Lithocarpus urceolaris* (Jack) Merr Seedlings under Different Density Shading Net

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ABSTRACT

Root anatomy of Lithocarpus urceolaris seedlings inoculated with Scleroderma spp. at different density of shading net was studied in greenhouse and in laboratory. L. urceolaris seedlings were inoculated with S. sinnamariense, S. columnare and S. citrinum at different of shading net (55 %, 65 % and 75 %) where the fungi were isolated from indigenous Fagaceae grown at Botanical Garden of Andalas University. The roots were studied for anatomical structures of mantle and Hartig net, percentage of ectomycorrhizal colonization and morphology of roots infected. The results showed that the roots of L. urceolaris could associate with all ectomycorrhizal fungi tested. The roots inoculated with S. columnare and S. citrinum under 65 % shades while S. sinnamariense in all condition shading density (55%, 65% and 75%) could give the best results with 60% colonization and catagorized as "good" colonization. L. urceolaris seedlings without shade, the colonization by the three ectomycorhizal of Scleroderma spp was around 30 %, and catagorized as "medium". In uninoculated seedling, no formation of ectomycorrhiza was observed. The structure of root anatomy of seedlings inoculated with S. sinnamariense, S. columnare and S. citrinum showed that the seedling inoculated with S. columnare using 65% of shade, some of its mantles formed of three layers with the thickness of mantle 300 µm. Two layers of mantles were found under 55 % shade with inoculant S. sinnamariense and S. columnare with mantle thickness 200 µm and 75% shade, with inoculant S. sinnamariense mantle thickness was 150 µ and some formed one layer of mantle (single layer). Ectomycorrhiza formed had the same morpholocical characters, i.e. producing monopodial branches and the color of surface of seedling roots colonized by mycorrhiza was white because there was mysellia which covered roots.

Keyword : *Scleroderma* spp, *Lithocarpus urceolaris*, colonization, mantle, Hartig net.

INTRODUCTION

Mycorrhiza is a mutualistic association structure between fungi (mykes) and root (rhiza) of plants. Symbiosis between mycorrhiza with its host could be classified into three groups based on growth structure and mechanism of infection on host root system, i.e. Ectomycorrhiza, Mycorrhiza Arbuskula Fungi (MAF) and Ectendomycorrhiza. Ectomycorrhiza fungi can only infect woody plants and its presence is needed very much for the survival of forest

trees. According to Brundrett *et al.* (1996), ectomycorrhiza fungi has more perfect sporocarp and roots are surrounded by mantle formed and Hartig net(1).

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 (1; 2). *Scleroderma* forms ectomycorrhizal associations with a wide range of woody plants, including members of the Pinaceae, Myrtaceae, Fagaceae, Mimosaceae, Dipterocarpaceae and Cistaceae (3; 4). Some beneficial isolates can vigorously compete with other ectomycorrhizal fungi in field (3; 5). *Scleroderma columnare* is one species of fungi that form ectomycorrhiza that can associate with conifer and woody plants (6). Naturally, *Scleroderma sinnamariense* could associate with *Gnetum gnemon* (3).

Contribution of ectoycorrhizal fungi in their association with plants among other things increasing the absorption of nutrition (7; 8) and water (9), increasing resistance to drought (10), and disease (11), and as bioindicator of forest soil productivity (12). On the other hand, ectomycorrhizal fungi obtain carbon from host plants. According to Alexander and Selosse (2009), studies about mycorrhiza in tropical forests are still limited(13). *Lithocarpus urceolaris* is one of dominant Fagaceae tropical forest in low land areas in West Sumatera. Fagaceae has a high diameter growth rate and physically has a hard wood (14) and economically important (15). Alamsjah *et al.*, (2015) stated that the seeds of *L. urceolaris* takes a long time to germinate. One of ways to speed up the growth of seedlings after germination is inoculating them with ectomycorrhizal fungi. Alamsjah *et al.* (2015) selected ectomycorrhizal fungi indigenous at Botanical Garden of Andalas University (BG-AU), and resulted in three best species in increasing the growth of *L. urceolaris* seedlings, i.e. *Scleroderma sinnamariense, S. columnare* and *S. Citrinum* (16).

One of environmental factors which very much affects the success of association of ectomycorrhizal fungi with their hosts is light intensity. So, it is very important to study further about the effect of light intensity. This research objective was to study the response of *L. urceolaris* seedlings inoculated with ectomycorrhizal fungi, *S.sinnamariense, S. columnare* and *S. citrinum* indigenous to BG-AU at different density of shades on root anatomical structure i.e. the presence of mantle and Hartig net, percentage of ectomycorrhizal colonization and morphology of roots infected.

MATERIALS AND METHODS

Tools and Material

Materials used in this study are the seeds of plants Fagaceae (Lithocarpus urceolaris), container sprouts, ultisol soil, sand, inoculant Scleroderma sinnamariense, columnare S. and S. citrinum, polybags, media Modified Melin Norkrans (MMN), antibiotics, alcohol, distilled water sterile, spritus, HgCl2, tissue paper, filter paper, label paper, cotton, Alcian blue, FAA, safranin, xylol, paraffin, shade/paranet.

The tools used are shears, scissors, cameras, stationery, rulers, calipers, loup, tweezers, needle ose/needle inoculation, Petri cup, test tubes, beakers, beaker, stir bar, Erlenmeyer, lights spritus, pipette , bottles films, autoclave, glass slide, cover glass, cork borer, hand sprayer, a dissecting microscope, a photo-microscope, microtome, oven.

Isolate Culture

Isolate used were *Scleroderma sinnamariense, S. columnare* and *S. citrinum* explored from Botanical Garden of Andalas University (BG-AU). Isolation and isolate culture of *S.sinnamariense, S. columnare* and *S. citrinum* were done using media Modified Melin Norkrans (MMN) that had been given antibiotic. Mycelia produced on media was used as materials of trials. All the process was mycelia propagation was done aseptically.

Media for Seedlings

Media used for growing seedlings of *L. urceolaris* was mixture of ultisol soil with sand with ratio 1:1 (volume). Soil and sand were screened from coral and stone. Then the media were sterilized using autoclave for 30 minutes with 1,5 atm pressure, and temperature 120°C. Sterile media were then put in poly bags.

Effect of shade and inoculation of *Scleroderma* spp. on percentage of ectomycorrhizal colonization and Anatomy of root of *L. urceolaris*

Seeds of *L. urceolaris* that had germinated were selected based on their good growth and then were planted in sterile media provided in poly bags. At the same time inoculum of ectomycorrhiza fungi *S. sinnamariense, S. columnare* and *S. citrinum* were placed near roots of seedlings and for control there was no inoculation of three species of fungi. The seedlings were then placed in diffrent intensity of shades, 55%, 65%, 75% and no shade. During the first two days the seedlings were not watered to avoid washing inoculum.

Seedlings care

Seedlings were taken care by watering and weed control. Watering was done every two days using hand sprayer. Weeds growing around seedlings were pulled out. Observation was done until ten months after inoculation of three species of *Scleroderma*.

Parameters measured

Root morphology of *L. urceolaris* seedlings

After 10 months, seedlings were separated from planting media. Roots were washed in a container contained water and they were kept intact. Morphological characters of ectomycorrhiza (pattern of branches, color of mantle) on roots that had been clean were observed using loop.

Microscopic observation of *Scleroderma* spp.

Isolates of *Scleroderma* spp. which associated with roots of *L. urceolaris* seedlings were observed under microscope to determine the presence of clamp conection on its mycelia which is one of characters of ectomycorrhiza fungi in Class of Basidiomycetes.

Anatomical Structure of roots colonized by ectomycorrhiza

To determine anatomical characters of root colonized by ectomycorrhiza, the parameters measured were structure of mantle, Hartig net and rhizomorph. Roots that had symbiosis was marked by the presence of mantle or hypha of ectomycorrhizal fungi *Scleroderma* spp. which covered roots and the presence of Hartig net. For root anatomical observation the process of making preserved preparate was done using paraffin methode (Sass, 1958) which covered the process of fixation, dehydration, parafinas, cutting and staining.

Percentage of ectomycorrhizal colonization

Roots that had been cleaned were observed. Percentage of roots colonized by mycorrhiza was determined by comparing root mass colonized by mycorrhiza with the total mass of roots.

RESULTS AND DISCUSSION

Effects of Shade and Inoculation of Ectomycorrhizal Fungi on Percentage Ectomycorrhizal colonization

Table 1 showed that ectomycorrhizal fungi, *S. sinnamariense*, *S. columnare* and *S. citrinum* indigenous Botanical Garden of Andalas University (BG-AU) could colonize roots of *L. urceolaris* seedlings. Colonisation of ectomycorrhiza was not formed on seedlings that were not inoculated with three species of *Scleroderma*.

Table 1. Percentage of roots of *L. urceolaris* seedlings colonized by different species of ectomycorrhiza fungi indigenous of BG-AU at different density of shade (ten months after inoculation)

Shade	Percentage of ectomycorrhizal colonization (%)			
	S. sinnamariense	*S. columnare*	*S. citrinum*	No mycorrhiza
No shade	30	30	30	0
55 %	60	50	50	0
65 %	60	60	60	0
75 %	60	50	50	0

Effects of Shade and Inoculation of Ectomycorrhizal Fungi on Mantle thickness and the penetration depth of Hartig Net

Observation on anatomical structure of roots of *L. urceolaris* seedlings inoculated with *S. sinnamariense, S. columnare* and *S. citrinum* showed the formation of fungi mantle out side the layer of epidermal cells and Hartig net in space among cortex cells (Table 2 and Fig. 1: A-F). On roots of *L. urceolaris* seedlings which were not inoculated with the three species of fungi, mantle and Hartig net were not formed.

Table 2. Mantle thickness (M) and penetration depth of Hartig net (HN) at different density of shade and inoculation of ectomycorrhrizal fungi *Scleroderma* spp. indigenous of BG-AU (ten months after inoculation)

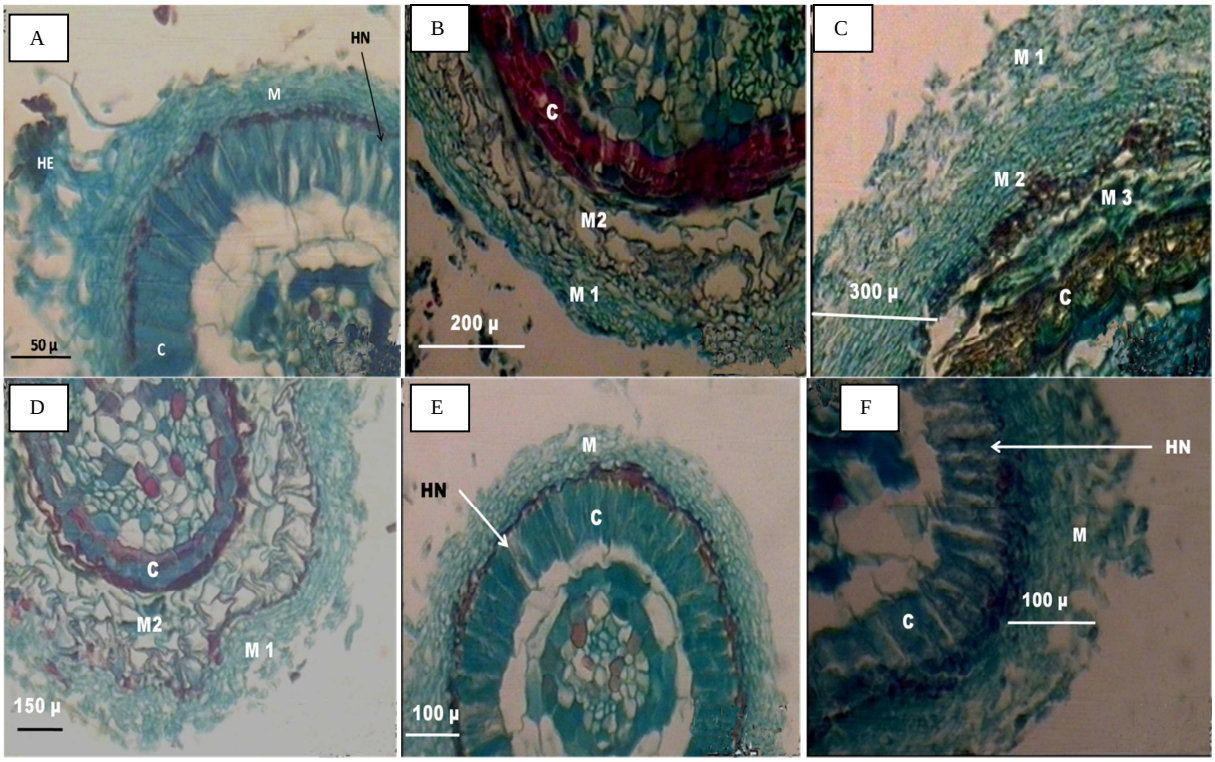
Shade	*S. sinnamariense*		*S. columnare*		*S. citrinum*		No mycorrhiza
	M (µ)	HN (µ)	M (µ)	HN (µ)	M (µ)	HN (µ)	
No shade	80	40	50	25	10	10	0
55 %	200	40	200	50	100	40	0
65 %	150	50	300	-	100	50	0
75 %	150	100	100	50	100	25	0

Root Morphology of *L. urceolaris* seedlings

Macroscopically it was shown that on roots of infected seedlings there was hypha covering roots. Ectomycorrhiza formed had the same morphological characters, producing monopodial branches and the surface of roots colonized with ectomycorrhiza had mostly white color (Fig. 1-G).

Microscopic observation of *Scleroderma* spp.

Microscopic observation on hypha of three species of *Scleroderma* on roots of *L. urceolaris*, showed the presence of clamp connection (Fig. 1-H).



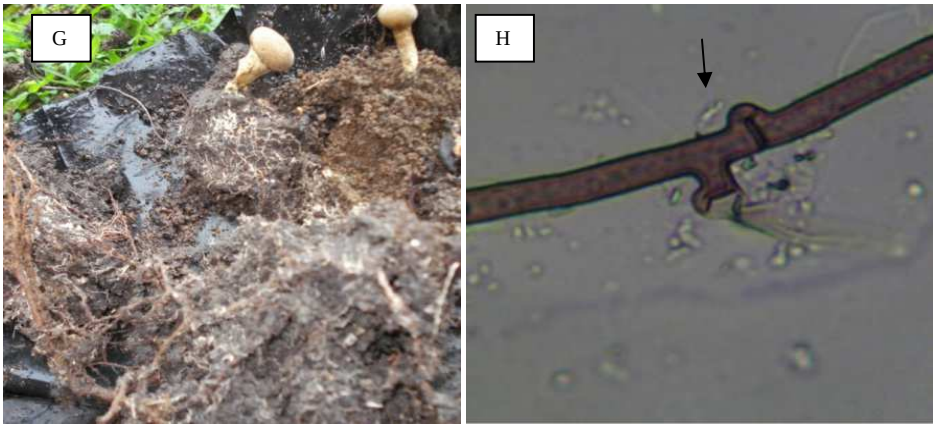


Figure 1. A-F. Cross section of *L urceolaris* roots infected by ectomycorrhiza fungi (ten months after inoculation); G. Infected root; H. Hypha of fungi (arrow shows clamp connection); M = Mantel, HN = Hartig Net, CC = Cell Cortex, HE= External Hypha

Shading-net and the inoculation of *S. sinnamariense*, *S. columnare* and *S. citrinum* isolated from BG-AU to seedling of *L.urceolaris* showed the difference effect on anatomical form and the presence of mantle and Hartig net, while none was found on seedlings in condition without shade and no inoculants. Result showed that at 55%, 65% and 75% shading net and inoculating *L.urceolaris* with *S. sinnamariense*, and 65 % shade with inoculation with *S. columnare* and *S. citrinum* gave the best results with the same percentage of colonization, i.e. 60 % which catagorized as "good" colonization. Giving 65 % shade and inoculating with *S. columnare* and *S. citrinum* showed the same colonization i.e. 50 % which was also catagorized as "good" colonization. Unshaded condition, the three species of fungi produced 30% colonization catagorized as "medium" colonization. The shade of 65% might cause optimum light intensity and temperature compared to other density of shades (55 % and 75 %). The condition of environment would decrease evapotranspiration rate so that it could protect seedlings from drying out and water was available in planting media. This indicated that inoculation of *S. sinnamariense*, *S. columnare* and *S. citrinum* could increase percentage of ectomycorrhizal infection on roots of *L. urceolaris* seedlings. Hight percentage of root colonized by ectomycorrhiza fungi indicated that plants would absorb more nutrition and water (17). Marx *et al*, (1992) classified percentage of colonization into four catagories: 75-100% (very good), 50-74% (good), 24-49% (medium) and 1-24% (bad)(18).

Compatibility of species of ectomycorrhrizal fungi to associate with their hosts would have an effect on percentage of ectomycorrhizal colonization. This case relates to the rate of sporal sprouting of ectomycorrhizal fungi(8). The more quickly the spores sprout the higher the possibility of spores to colonize roots (19). Mycelium is a very dynamic part and functions in forming symbiosis. The rate of growth and development of mycelium on plant roots would determine the percentage of colonisation by fungi (20), because 80% of biomass of ectomycorrhiza fungi is exstra radical mycelium (21). According to Jeffries and Dodd (1991), level of plant dependent on ectomycorrhizal fungi besides determined by the plant its self, it is also determined by the fungi isolate(22).

Structure of roots underwent changes by the infection of ectomycorrhizal fungi, *S. sinnamariense*, *S. columnare* and *S. citrinum*. After inoculation the mantle was formed and mycelium penetration to cortex tissue was deeper, then Hartig net was formed between cortex cells. Analysis of root anatomy showed that the mantle formed on roots of seedlings given shade, some consist of three layers with mantle thickness 300 µ and this was found on seedlings under 65% shade with inoculant *S. columnare* (Table 2, Fig. 1-C). Some consist of two layers, where the layer of young mantle attached to roots and old layer developed outward and this was obtained on seedling under 55% of shade with inoculant *S. sinnamariense* and *S. columnare* (Table 2, Fig. 1-B), mantle thickness is 200 µ while with 75% of shade and inoculant *S. sinnamariense* (Fig. 1-D) the thickness of mantle is 150 µ. Mantle formed looked thick and attached evenly around root. Some also formed single layer (Fig. 1:A,E,F).

Table 2 and Fig 1, shows that mantle thickness formed were ranged from 10 – 300 µ and Hartig's net were ranged from 10 – 100 µ. According to Supriyanto *et al.* (1994), the mantle thickness usually varied from 20 – 100 µ, and mostly found was 30 – 40 µ(23). The estimate dry weight of mantle was 25–40% from the total weight of the whole organ of fungi. The component functions as device for selection and storing. The result also showed that roots of *L.*

urceolaris seedlings inoculated with three species of *Scleroderma* with shade, the thickness of mantle formed ranged from 100 – 300 µ. This indicated that there was a strong symbiosis between *S. sinnamariense*, *S. columnare* and *S. citrinum* indigenous of BG-AU with roots of *L. urceolaris* seedlings. The thicker the mantle formed the more mycelium colonizing roots of *L. urceolaris* seedlings and its penetration was deeper because there was compatibility between ectomycorrhizal fungi and seedlings. This result was supported by higher percentage of ectomycorrhizal colonization compared to the one without inoculation of the three species of *Scleroderma*.

Mantle is an outer structure of ectomycorrhiza that consists of hypha covering surface of roots and as consequency there is no direct contact between roots and rizosphere. Mantle might consist of one or two layers of hypha which function as pseudoparenchyme. Mantle is formed by hypha and rhizomorph. Hartig net is arranged by hypha in a complex labirintik branches system. The structure grows in spaces intercell cortex toward root centre up till endoderm or other tissue near root centre that has been differentiated. Based on the result of research of Supriyanto (1999), the presence of mantle and Hartig tissue on root system explained the compatibility status between plants and ectomycorrhiza fungi(24).

Morphological observation indicated that ectomycorrhiza had developed in roots of *L.urceolaris* inoculated with *S. sinnamariense*, *S. columnare* and *S. citrinum*. In roots infected there was hypha coating roots of seedling. Ectomycorrhiza formed in root of *L. urceolaris* which associated with three species of *Scleroderma* had the same morphological characters, producing monopodial branches and root surface of *L. urceolaris* seedling colonized by mycorrhiza showed mostly white color. Mycelium of ectomycorrhizal fungi coated roots and showed white color.

In Fig.1-G, it was shown that giving 65% shade and inoculation with *S. columnare* caused ectomycorrhiza colony formation on roots of *L. urceolaris* seedling. On further stage, root infection would cause the fruitbody of *S. columnare* to form in the surface of planting media. The fruitbody found in *L. urceolaris* seedling colonized by mycorrhiza, had the same characters with *S. columnare* inoculated to seedling.

The way to determine level of success in forming and developing ectomycorrhiza in a seedling based on the presence or absence of ectomycorrhiza on root. Sometimes, certain fungi have variation in their ability to form ectomycorrhiza. Sufficient light intensity is needed for the root to be able to accumulate carbohydrate for the growth of fungi symbiont. Suhardi (1995) stated that the increase of light intensity could cause room and soil temperature increase, and indirectly could affect development of ectomycorrhiza(25). According to Yasman (1995), too much light was not good because it would cause the soil warmer, while too little light could reduce the number of root with ectomycorrhiza because of less carbohydrate produced from photosynthesis(26). Moore-Landecker (1972) stated that light affected growth rate of fungi, synthesis capasity, and reproductive structure. Shading is an effective way to reduce light intensity and temperature so it could increase humidity(27). The increase of plant photosynthesis rate would increase karbohydrate content so that infection of mycorrhizal fungi would also increase. On early stage of inoculation, generally most of photosynthesis production is used to support the formation of mycorrhizal association.

CONCLUSION

The results demonstrate that L. urceolaris could associate with more than one ectomycorrhizal fungi, i.e. with Scleroderma sinnamariense, S.columnare and S. citrinum. Inoculation of *S. sinnamariense* on roots of *L. urceolaris* seedlings under 55 %, 65 % and 75 % shade and that of *S.columnare* and *S. citrinum* under 65 % shade gave the best result with 60% colonization which categorized as good colonization. Anatomical structures of seedling roots inoculated with *S. sinnamariense*, *S.columnare* and *S. citrinum* showed the presence of mantle which cover roots and Hartig net consisted of hypha between cortex cells. Some mantles formed could consist of three layers with thickness 300 µ and these were obtained from inoculant *S. columnare* under 65% shade, two multy series layers were obtained from inoculants *S. sinnamariense* and *S. columnare* under 55% shade with thickness of mantles 200 µ, and under 75 % shade with inoculant *S. sinnamariense* the thickness of mantle 150 µ. Some only formed single layer.

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