UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF MYCORRHIZA ON NITROGEN AND PHOSPHORUS REQUIREMENT OF NURSERY GROWN EUCALYPTUS

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MASTER OF AGRICULTURAL SCIENCE
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By

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Thesis Submitted in Fullfilment of the Requirements for the Degree of Master of Agricultural Science in the Faculty of Agriculture Universiti Putra Malaysia

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LIST OF ABBREVIATIONS

PT. IIU	Perusahaan Terbatas. Inti Indorayon Utama
Spo	Spora
Inf	Infection
NP	main plot : nitrogen + phosphorus
CI	sub plot : commercially produced + indigenous mycorrhiza

Abstract of thesis presented to the Senate of the Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

THE EFFECT OF MYCORRHIZA ON NITROGEN AND PHOSPHORUS REQUIREMENT OF NURSERY GROWN EUCALYPTUS

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April 1999

Chairperson : Professor Azizah Hashim, PhD.

Faculty : Agriculture

Eucalyptus is a potential forest species which is fast growing and has multi-purpose uses. The forest plantation of PT. Inti Indorayon Utama in Indonesia faces serious problem of seedling survival during transplanting. Furthermore, the budget for fertilizer in the nursery is also very expensive. The objective of this study therefore was to examine the effects of mycorrhiza application on nitrogen and phosphorus requirement of nursery grown eucalyptus.

The study consisted of three components. The survey examined the occurrence of mycorrhizae in several plantation sectors. The effectivity trial evaluated the effectiveness of indigenous versus commercially produced mycorrhiza inoculum. The nursery trial examined the effect of selected mycorrhiza on nitrogen and phosphorus requirement of eucalyptus seedlings.

Results from the survey showed that both ectomycorrhiza and arbuscular mycorrhiza were present in the almost all plantation sectors examined. Increase in

plant age increased AM spore number, root colonization and presence of EM. Plantation management practices and the amount of soil organic matter in the forest were factors that contribute to the decrease in AM spore number under the young eucalyptus stands. The combined effect of spore number and several soil properties(Cu, Zn and pH) as well as the stage of eucalyptus stands affect percentage AM root colonization.

The results from the effectivity study found that indigenous inoculum from Habinsaran was the most effective. However, its effectivity was lower than the commercially produced mycorrhiza.

The results from the nursery study showed that mycorrhiza was able to increase seedling height, while reducing the amount of phosphorus fertilizer applied to the plants. However, mycorrhiza was not able to replace the nitrogen function in seedling growth. Application of combined indigenous with the commercial mycorrhiza inoculum gave the best overall seedlings growth compared to individual mycorrhiza inoculum (indigenous or commercial). The amount of N and P fertilizers applied also influenced the mycorrhiza role in increasing seedling growth.

In conclusion, growth of Eucalyptus seedlings was found to be significantly ($p \leq 0.05$) affected by both mycorrhiza and fertilizer factors.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Pertanian

KESAN PENGGUNAAN MIKORIZA TERHADAP KEPERLUAN NITROGEN DAN PHOSPHAT DI PERSEMAIAN EUCALYPTUS

Oleh

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Eucalyptus merupakan tanaman hutan dengan kadar pertumbuhan yang cepat dan mempunyai pelbagai kegunaan. Di PT. Inti Indorayon Utama Indonesia, pengurusan tanaman ini mengalami masalah dalam daya tahan benih semasa penanaman. Tambahan pula, kos pembiayaan baja di tapak semaian juga sangat tinggi. Kajian ini bertujuan untuk mengkaji kesan penggunaan mikoriza pada keperluan N dan P semasa persemaian *Eucalyptus*.

Kajian ini mengandung 3 bahagian iaitu tinjauan : i. untuk memeriksa kewujudan mycorrhiza di beberapa kawasan penanaman, ii. kajian untuk menilai kadar keberkesanan inokulum mycorrhiza semulajadi berbanding dengan yang dihasilkan secara komersial, dan iii. kajian di tapak semaian bagi menguji kesan mikoriza terpilih ke atas keperluan N dan P semasa penyemaian *Eucalyptus*.

Hasil kajian menunjukkan ektomikoriza dan arbuskular mikoriza terd pada hampir semua sektor penanaman yang dikaji. Peningkatan usia *Eucalyptus* meningkatkan jumlah spora AM, akar yang berjangkit AM dan kewujudan EM. Pengurusan kawasan dan kandungan bahan organik tanah merupakan faktor utama dalam pengurangan jumlah spora AM pada kawasan yang baru di tanam. Kesan dari kombinasi seperti faktor jumlah spora, sifat-sifat tanah (Cu, Zn dan pH) serta umur penanaman mempengaruhi peratusan akar yang dijangkiti AM.

Keputusan kajian keberkesanan menunjukkan bahawa inokulum yang paling effektif berasal dari Habinsaran. Namun tahap keberkesanannya masih rendah berbanding mikoriza yang dihasilkan secara komersial.

Kajian dipersemaian memperlihatkan mikoriza mampu meningkatkan pertumbuhan benih, yang mana jumlah P tersedianya adalah rendah. Namun demikian, mikoriza tidak mampu menggantikan fungsi N dalam pertumbuhan benih. Penggunaan kombinasi inokulum semulajadi dengan komersial memberikan hasil yang terbaik terhadap pertumbuhan benih berbanding penggunaan inokulum mikoriza secara individu (semulajadi atau komersial). Jumlah baja N dan P yang digunakan juga mempengaruhi peranan mikoriza dalam meningkatkan pertumbuhan benih.

Kesimpulan dari kajian yang dijalankan, pertumbuhan benih *Eucalyptus* dipengaruhi nyata ($p \leq 0.05$) oleh faktor mikoriza dan baja.

CHAPTER I

INTRODUCTION

General

Indonesia comprises over 13,000 islands arranged along the equator, covering approximately 143 million hectares of forestland (Smith, 1992). In the tropics, under good management practices some species of eucalyptus have been reported to show very fast growth and produce large quantities of wood. However, eucalyptus plantations are often believed to result in drastic reduction of soil fertility, due to high dry matter production and utilization within a short rotation period (Lapeyrie *et al.*, 1992).

Tropical soils undergo rapid degradation under improper management, thereby reducing the fertility status. Continuous chemical fertilizers are often used to improve the productivity of impoverished soils. Such practice however usually results in a decrease in the natural fertility of the soils, while posing another health hazard to the environment (Azizah, 1997).

Hence utilization of the mycorrhiza fungi and other beneficial microbes offers possible approach in enhancing and maintaining soil fertility.

Mycorrhiza is a fungus that forms symbiotic relationships with plant roots, with the fungal symbiont becoming a major interface or connection between soil and plant. Mycorrhizal infection may change root activity, root growth and exudation. The mycorrhizal external mycellium can extend the root surface area for acquisition of soil mineral nutrients and / or water. Way beyond the zones explored by the plant roots. The mycelium in mycorrhiza communities can spread from plant to form a linked nutrient absorbing network that has access to the soil in which all the roots are growing. The extraradical hyphae function as absorptive structures for uptake of mineral elements and water, extending several centimeters from the roots and absorbing immobile elements adsorbed on soil colloidal particles.

The application of the mycorrhiza inoculum to forest trees seedlings may be necessary in view of the low and ineffective mycorrhiza populations in the field. This is particularly exacerbated by the high phosphorus fixation capacity and low mineral nutrient content of most tropical soils. Forest practices that increase soil compaction and erosion adversely affect mycorrhizal formation and hence seedling establishment. Increasing soil density and decreasing soil organic matter contents can also decrease fungal growth. In addition, decreasing soil structure restricts the movement of oxygen and water into the soil, hence limiting the AM fungal growth.

The genus eucalyptus forms both the arbuscular mycorrhiza (AM) or endomycorrhiza and ectomycorrhiza (EM). The AM fungus colonizes the inner part of the root cortex while the EM fungus is usually confined to the outer cell layer. Some evidence shows that the succession between AM and EM during host

plant aging, could be related to competition for infection sites. Knowledge of such specific relationships between plant-fungus combinations is therefore of fundamental importance for the effective utilization of AM or EM fungi on eucalyptus.

It has been reported that under natural ecosystems, some trees could not survive without the presence of mycorrhiza (Janos, 1983 ; Janos, 1988) . Fungi forming mycorrhiza on tree seedlings in nurseries must be ecologically adapted to the planting sites. Without mycorrhiza the seedlings will hardly survive unless supplemented with fertilizers. Mycorrhiza deficiencies are most prevalent in forest nurseries. This is due to the type of potting media used and the practice of spraying chemicals to control pathogens and weeds. Furthermore, excessive fertilization (Asimi *et al.*, 1980) and the use of fungicides will also reduce or eliminate mycorrhiza (Trouvelot *et al.*, 1992).

Although the mycorrhiza fungal populations may be high in some soils, the indigenous mycorrhiza populations however may not be effective in enhancing the growth of any particular plant. Host plant specificity by EM and AM fungi is one of the problems faced in the selection of effective fungi for field applications.

In view of the above, it is therefore felt that application of the mycorrhiza fungi from selected species is appropriate in the eucalyptus nursery of Inti Indorayon Utama forest plantation company, in Northern Sumatera. To date, only limited studies have been conducted on mycorrhiza Eucalyptus interaction. Such studies in North Sumatera Eucalyptus plantation has never been conducted.

Hypothesis

Inoculation of effective mycorrhiza onto eucalyptus seedlings will reduce fertilizer application while enhancing the growth of these seedlings.

Objectives

The general objective of these studies was to determine the effect of mycorrhiza on N and P nutrition of nursery grown eucalyptus seedlings.

The study which was conducted from January 1997 to June 1998 consisted of three experiment: i.) survey on the occurrence of mycorrhiza on Eucalyptus sp in PT. Inti Indorayon Utama forest plantation, Indonesia, ii.) a pot trial in the glasshouse at Universiti Putra Malaysia, and iii.) a nursery trial at the Research and Development Station of PT. Inti Indorayon Utama in Porsea, Indonesia.

CHAPTER II

LITERATURE REVIEW

The Association between Fungus and Plant Root

Certain soil microorganisms colonize roots of higher plants and form symbiotic relationships. Symbiotic associations formed between fungi and living roots of higher plants are known as mycorrhizas. Frank (1885) as cited by Harley and Smith (1983) introduced the Greek word mycorrhiza to this association, which means "fungus root".

Depending on the physical relationship of the fungus with plant roots and based on the types of fungus-root structures formed, mycorrhizas can be divided into two major types, ectotropic and endotropic mycorrhizas (Safir, 1987).

In ectomycorrhizas, the fungus forms a sheath around all or some of the fine absorbing rootlets. Ectomycorrhiza hyphae penetrate between the root cells and occasionally enter the cells but they never penetrate beyond the cortex.

The intercellular hyphae also do not cause destruction of the host cell. The fungus grows as a mantle around the root, as Hartig net inside the root, and as an external mycelium outside the root. There is however no intracellular penetration.

In contrast, the endomycorrhizas or arbuscular mycorrhiza (AM) is the association of roots with fungi that forms the external hyphal networks in the soil as well as grows internally in the root cortex forming specific fungal structures. A fungal mantle is not present, but the hyphae enter root cortical cells without causing visible damage to the invaded root.

Ectomycorrhiza

Ectomycorrhizae is the fungi that form sheaths around or at all parts of the fine absorbing rootlets. Ectomycorrhizas can be easily recognized without any special staining procedure. The infected roots are usually swollen, branched, mantled by the fungal tissues, and lack root hairs. Hyphae grow from the root surface and around the cells of root epidermis and cortex. The hyphal growth between the root cells, termed as Hartig net, does not damage root cells, and indicates a mutualistic symbiosis (Figure 1) (O'Dell, *et al.*, 1992).

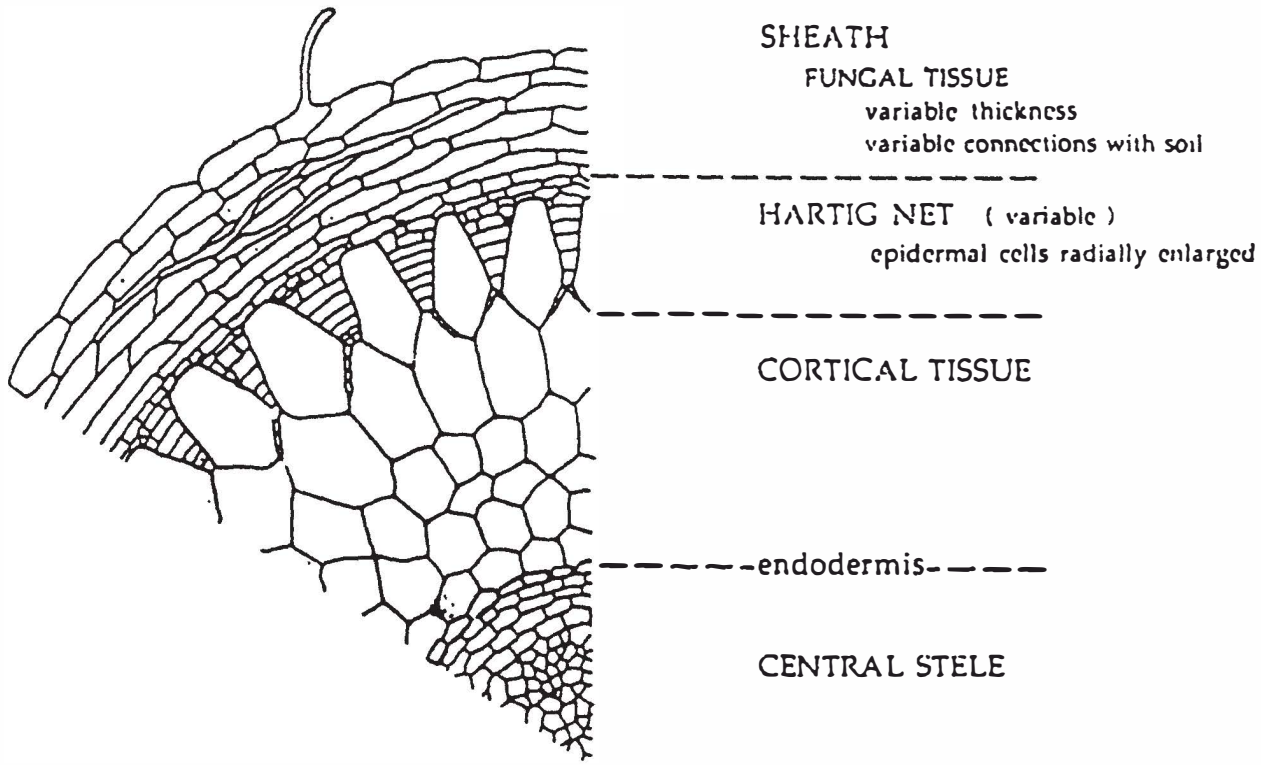

Figure 1: Ectomycorrhiza Relationship with Host (Isaac, 1992)

The Hartig net is assumed to be the distinguishing feature of true mycorrhiza. The Hartig net consist of *lames fungique*, and the formation of the Hartig net did not disrupt the plasmodesmata between the cortical cells of the host, so that symplastic continuity is retained in the cortex (Nylund, 1980).

The tannin layer consists of a layer of cells, which are naturally dark brown in color. The function and origin of the tannin layer has been the center of some controversy. Some researcher suggested that tannins secreted by the host may act as a biological screen that selects only fungal species which can tolerate the compound (Foster and Marks, 1966, 1967).

The cell walls of the mantle hyphae were covered with an amorphous layer, which on mild maceration revealed two layers of microfibrils. The inner one being more organized than the outer as is common in fungi of many groups. The inner mantle was characterised by more closely interwoven hyphae, an increase in the number of cytoplasmic organelles and an increase in the concentration of glycogen granules (Marks and Foster, 1973).

In Eucalyptus, the hyphae of the outer mantle are usually devoid of cytoplasmic contents which are sometimes partially collapsed and transversely stretched, whereas those of the inner mantle are more rounded and richly cytoplasmic.

Ectomycorrhizae infection was often found to decrease the cell volume of host root tissues of Pinus (Hatch and Oak, 1933). However Chilvers and Pryor (1965) have found a slight increase in the volume of mycorrhizal epidermal root cells of Eucalyptus, but this depend on the extent of the increase of radial elongation of the epidermal or outer cortical.

Ectomycorrhizae are more prevalent in the member of *Pinaceae*, *Fagaceae*, *Betulaceae*, and *Salicaceae*, with fewer genera from other families such as Eucalyptus, *Tilia*, and *Arbulus*. The member of *Caesalpiniaceae* and *Dipterocarpaceae* also show EM association. Most genera of woody plants which exhibit ectomycorrhiza, are important forest components of the cool and temperate regions. While in tropical forests, especially the rain forests, the proportion of