CHAPTER 3

SURVEY OF ARBUSCULAR MYCORRHIZAL FUNGI IN TREE CROPS IN AGROFORESTRY SYSTEMS IN LUANGPRABANG PROVINCE, LAOS PDR

3.1 Introduction

Agroforestry is a land - use system in which woody perennials are deliberately used on the same land - management units as agricultural crops and / or animals, in some form of spatial arrangement or temporal sequence.

Many of the tree crop species employed in agroforestry systems are legumes that form symbiotic associations with N_2 - fixing bacteria (Alvarez-Solis and Anzzueto-Martinez 2004). The tree crops may also benefit from symbiosis with arbuscular mycorrhizal fungi (Habte and Turk 1991; Ingleby *et al* 2001). These symbioses enable them to sustain growth in the phosphorus and nitrogen deficient soils that have been degraded through over - cultivation and erosion. Intensification of land - use may lead to insufficient or ineffective population of microsymbionts (Alvarez-Solis and Anzueto- Martinez 2004). In these cases, inoculation with effective rhizobia and AM fungi may be needed for the re- establishment of trees, while long term improvements in soil fertility and growth of the crops will require land management regimes which sustain and promote AM fungi populations (Sieverding 1991).

The distribution of AM fungi community may influence composition of plant communities as various fungal species preferentially associate with different plant species (Pringle and Bever, 2002). The host-specificity in mycorrhizal fungal response might promote the coexistence of AM fungi and the distinct fungal communities are associated with different hosts (Vandenkoonrnhuyse *et al.*, 2002). However, other authors have suggested that plants are typically colonized by a mixture of AM fungal species since host specificity of AM fungi appears to be very low (Smith and Read, 1997). On the other hand the participation of AM fungi in the biodiversity and agroforestry system functioning is now being recognized, particularly due to their effect on plant diversity and productivity (Yimyam,N *et al.*, 2005).

The AM fungi is potentially useful for tree crops in agroforestry systems in Laos and this study set out to examine the abundance of AM fungi in rubber, agarwood teak and paper mulberry in agroforestry systems in northern Laos.

3.2 **Materials and Methods**

Study site and sampling

This field survey was conducted in two seasons consist of dry season (April to May, 2007) and wet season (October, 2008). This work was conducted in two sites including Phonxay district and Nan district (Agroforestry Research Station), in Luangprabang province of Lao PDR Mycorrhizae and nutrients status of four crops tree as teak(*Tectona grandis*), rubber (*Hevea brasiliensis*), agarwood(*Aquilaria crassa*) and paper mulberry(*Broussonatia paprifera*), were determined in each site. Soil, roots and leaf samples were collected from six plants of each species in each site. Each plant was a replication.

- Root and soil sampling

Three holes (10 cm diameter, 25 and 50 cm deep) were excavated form canopy area of 6 plants of each species from each site. Fine roots from 3 holes were collected and combined for determining AM fungi colonization. Soil samples were collected separately in 2 depths (0 - 25 cm and 25 - 50 cm). Soil samples from the same depth from the 3 holes were combined for AM fungi spore counting (Figure3.1).

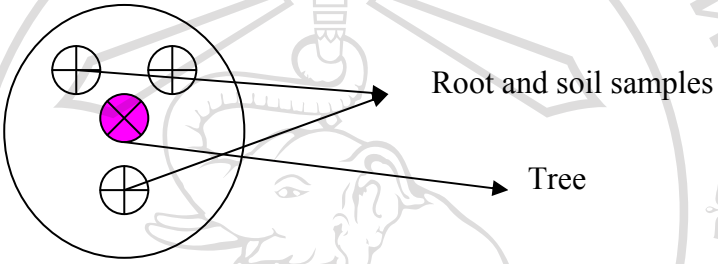

Figure 3.1 Root and soil sample were collected in on plant (replication)

- Leaves sampling

Leaf samples were separately collected from 6 plants of each species in each site.

In each plant seven leaves of youngest fully expended leaf (YFEL) were collected for determination of phosphorus concentration.

Sampling preparation and analysis

The fine root samples were mixed together, washed over a 2 mm under running water and root was cut into pieces of 1-2 cm length, cleared in 10% KOH for four days and rinsed with water on a 90 µm sieve. Cleared roots were stained with 0.05% trypan blue (Brundrett *et al.,* 1996). Thirty pieces of fine roots (≤ 1 mm diameter) was taken at random from each sample and mounted on microscopic slides to assess root colonization (Brundrett *et al.,* 1996).

Soil samples were air dried then 30 g sub-sample from each soil sample was used for spore extraction by wet sieving and sucrose centrifugal method (Brundrett *et al.,* 1996).

AM spores were separated from the soil by wet sieving through 250 µm and 90 µm sieve and processed separately. Each was centrifuged for 5 min at 2000 rpm to remove floating debris. The spores were re-suspended in 50% sucrose with vigorous shaking. The samples were centrifuged for 1 minute at 2000 rpm to separate spores from dense soil compartments. After centrifugation, spores in the supernatant was poured over the finest sieve and washed with water to remove the sucrose before vacuum filtration on filter paper with gridlines. Spore on filter paper were kept in Petri dishes. Spores were counted under stereomicroscope (Brundrett *et al.,* 1996).

Percent colonization was calculated by the formula:

$$\% \text{ colonization} = \frac{\text{Total number of colonized root pieces}}{\text{Total number of root pieces examined}} \times 100$$

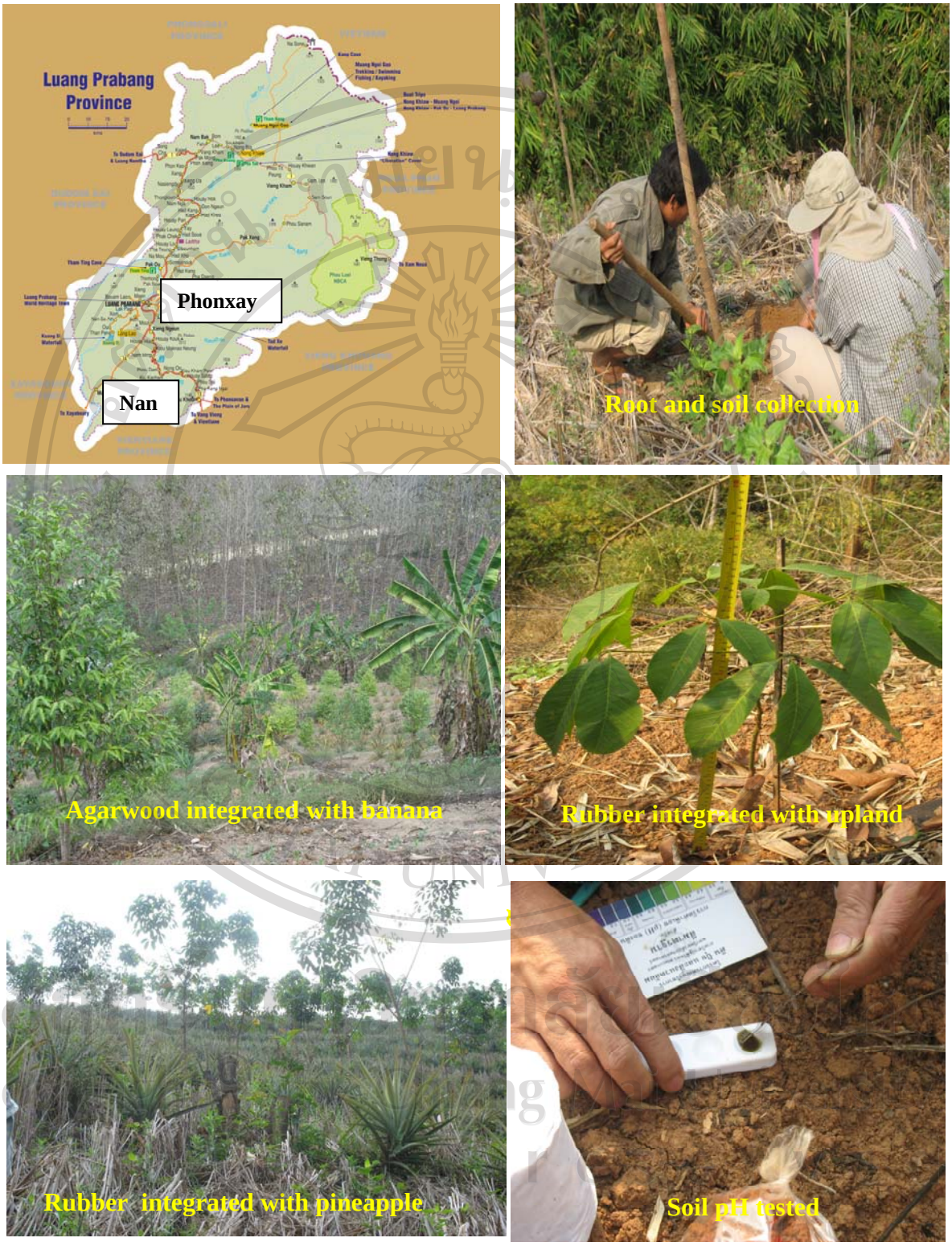

Figure 3.2 Survey the distribution of AM fungi at Phonxay and Nan district.

3.3 Results

Spore and percentage of root colonization of tree crops from dry season

Spores

Most of the AM fungi spores were distributed in shallow level (0- 25 cm) in all trees at both sites. In Phonxay, plant species had no effect on spore density in soil of root zone (Figure 3.3). In the shallow level of 0-25 cm, there were 14±4.8 spores per 100 g soil in root zone of paper mulberry, 24±8.0 spores in rubber, 12.0±6.8 spores in agarwood and 18.0±7.8 spores in teak

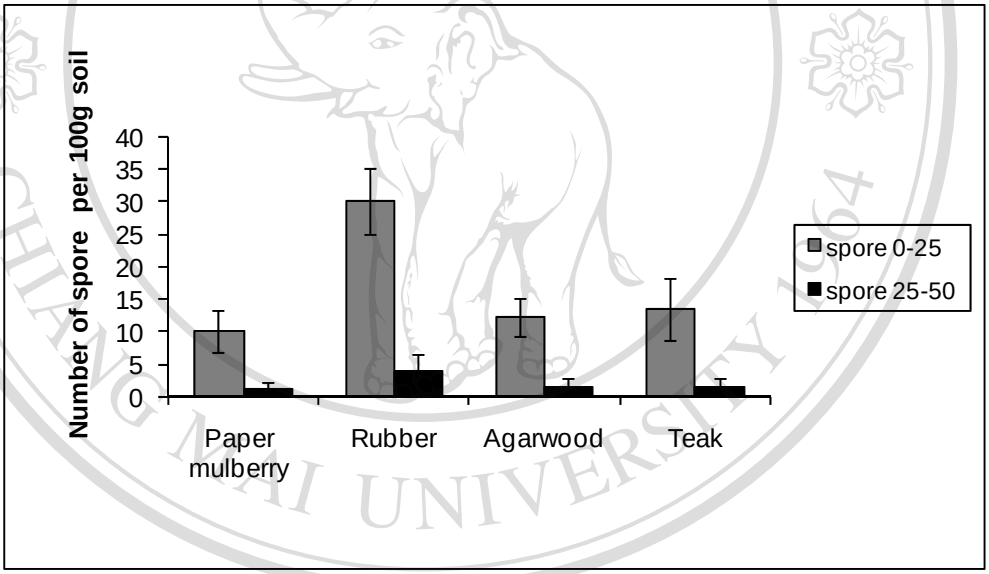

Figure 3.3 Spore density per 100 g of soil (with error bars) in the root zone of 4 tree crops (Paper mulberry; rubber, agarwood and teak) at 2 depths at Phonxay district.

In Nan district, the spore density in root zone soils was highest in rubber at 43± 4.9 spore per 100g. The second was agarwood with 31± 5.6 spore per 100g, followed by teak with 20±8.0 spores per 100 g and paper mulberry with 16±6.2 spore per 100g soil (Figure 3.4).

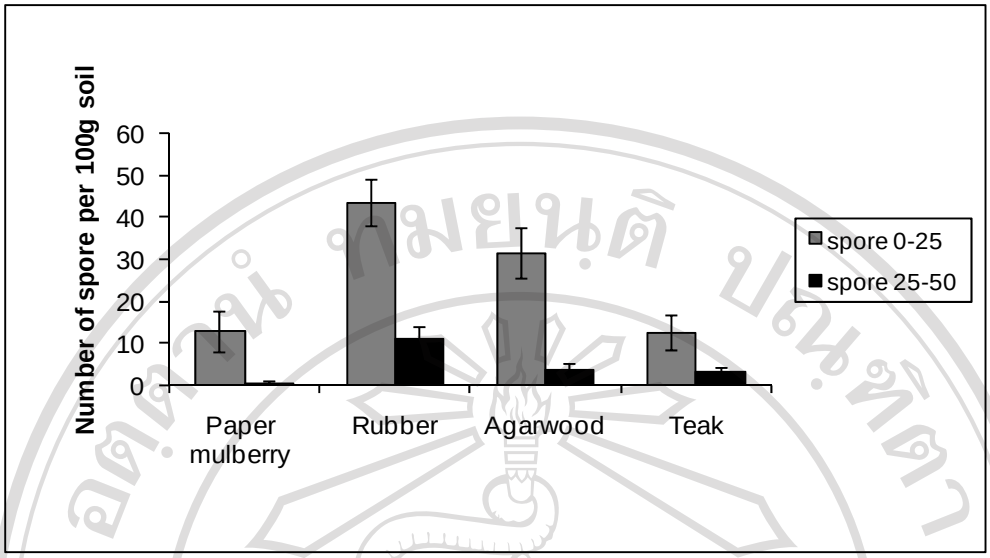

Figure 3.4 Spore density (with error bars) of 4 tree crops (Paper mulberry; rubber, agarwood and teak) at depths at Nan district.

Root colonization of all plants was quite low in both sites, ranging from 1 to 5% In Phonxay, Root colonization in rubber was higher than the other species. While the root colonization of paper mulberry, agarwood and teak were only about 1% (Figure 3.5).

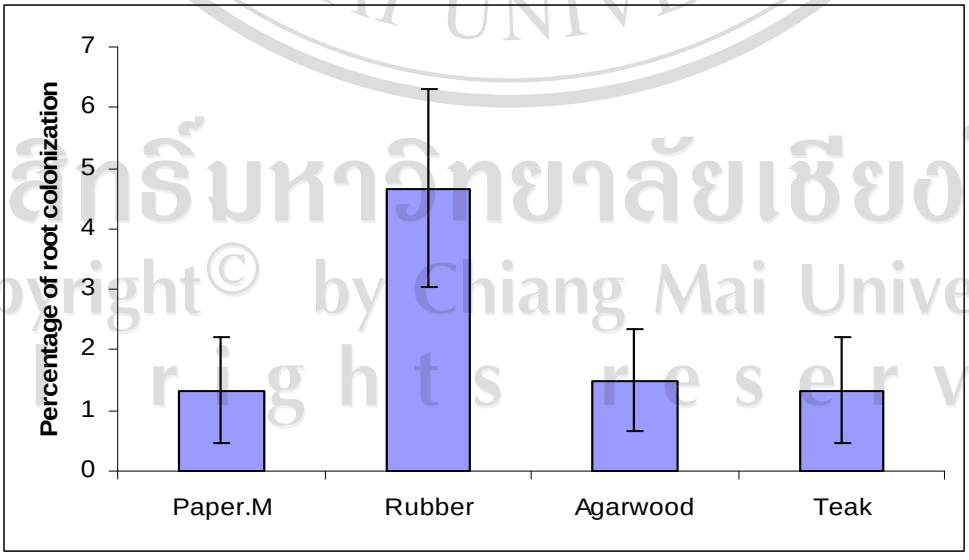

Figure 3.5 Root colonization (with error bars) of 4 tree crops (Paper mulberry; rubber, agarwood and teak) at Phonxay district.

In Nan, root colonization of rubber was highest (43%). It was higher than root colonization in teak and paper mulberry (20 and 16 % respectively).While the root colonization of agarwood was between rubber and teak (43 and 20%). (Figure 3.6).

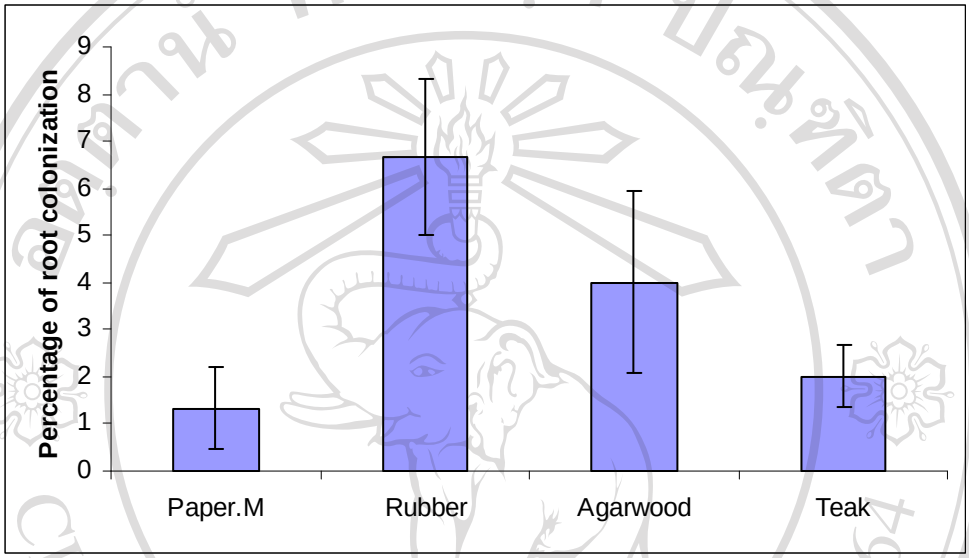

Figure 3.6 Root colonization (with error bars) of 4 tree crops (Paper mulberry; rubber, agarwood and teak) at Nan district.

Spore density and root colonization in rubber and agarwood in rainy season, compared with dry season

There were much higher density of AM fungi spores in the wet season than in the dry season in both agarwood and rubber tree in both Phonxay (Figure 3.7) and Nan (Figure 3.8). In addition, the spore density in the rhizosphere of the rubber was slightly higher in Nan, but AM fungi spore density associated with agarwood did not show any difference between the two sites.

Root colonization by AM fungi of agarwood and rubber were different by season and location.

Root colonization was generally higher in the rainy season than in dry season, except for rubber at Nan where there was no difference between the seasons (Figures 3.9 and 3.10**).**

In Phonxay, root colonization in agarwood and rubber, were at the same level at about 17% in the rainy season and about 5% in the dry season. However at Nan there was a strong interaction between season and tree species. Agarwood had the highest percentage of root colonization in the rainy season at 27% in and lowest at 6% in the dry season. Rubber at Nan on the other had about the same percentage of root colonization in the rainy and dry season, which were less than half that of agarwood in the rainy season and slightly higher than agarwood in the dry season.

Between the two districts, root colonization was equally low in the dry season for both tree species. In the rainy season, however, rubber in Nan had significantly lower root colonization than in Phonxay.

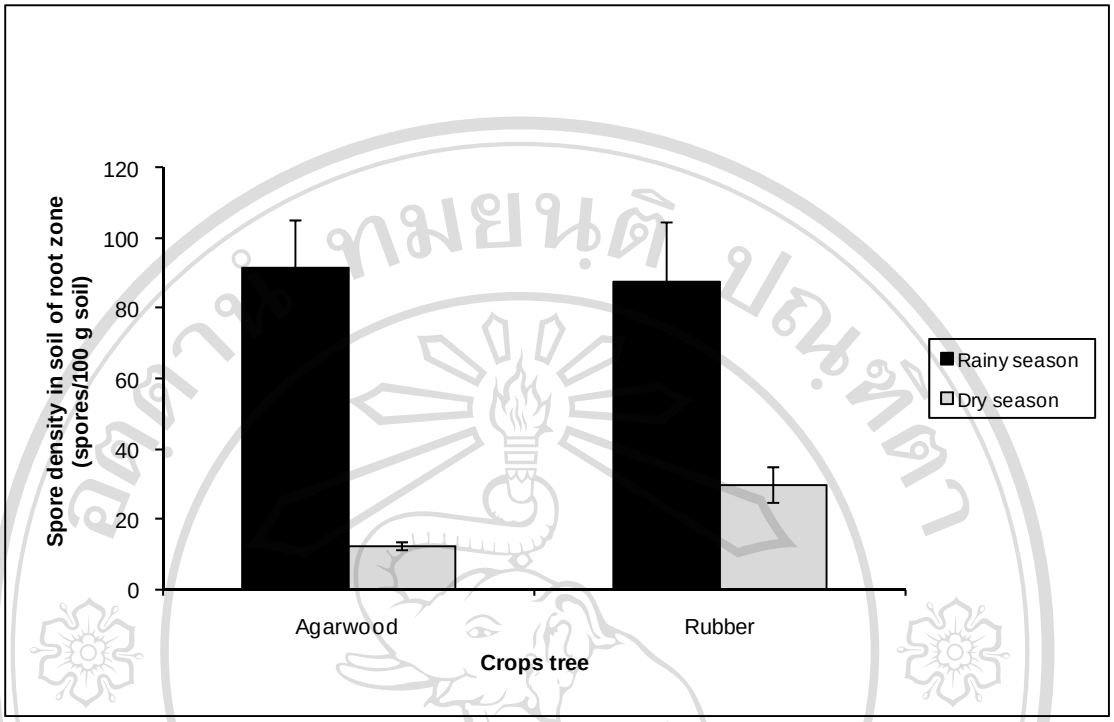

Figure 3.7 Spore density (per 100 g soil, with error bars) of 2 tree crops (agarwood and rubber) in 2 seasons (dry and rainy season) in Phonxay district.

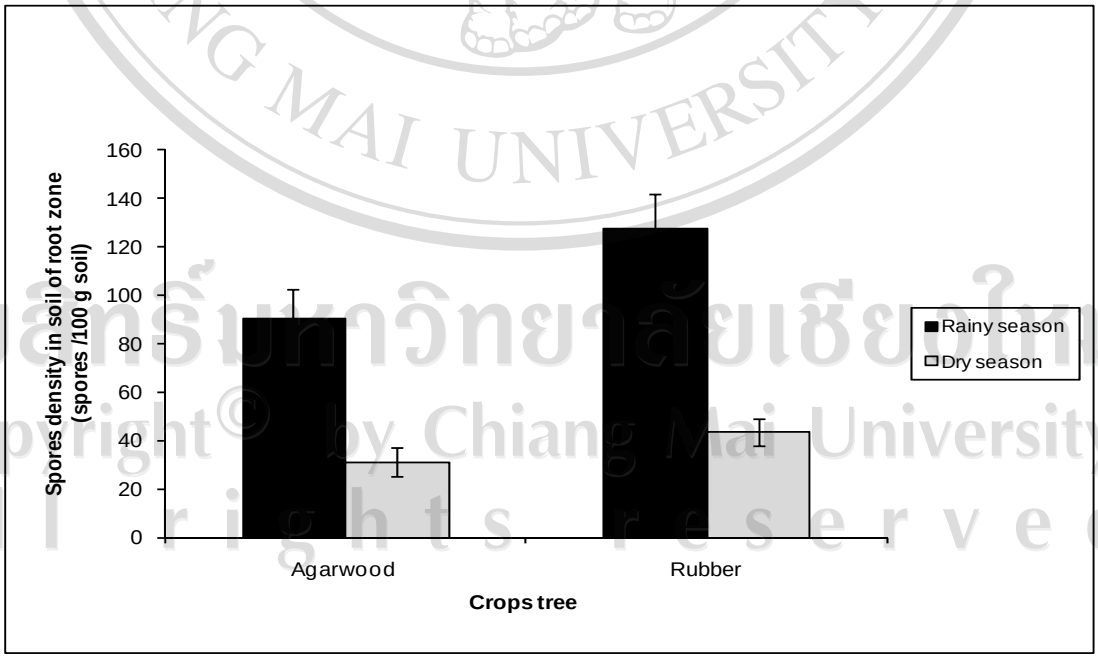

Figure 3.8 Spore density (per 100 g soil, with error bars) in 2 tree crops(agarwood and rubber) in 2 seasons (dry and rainy season) in Nan district.

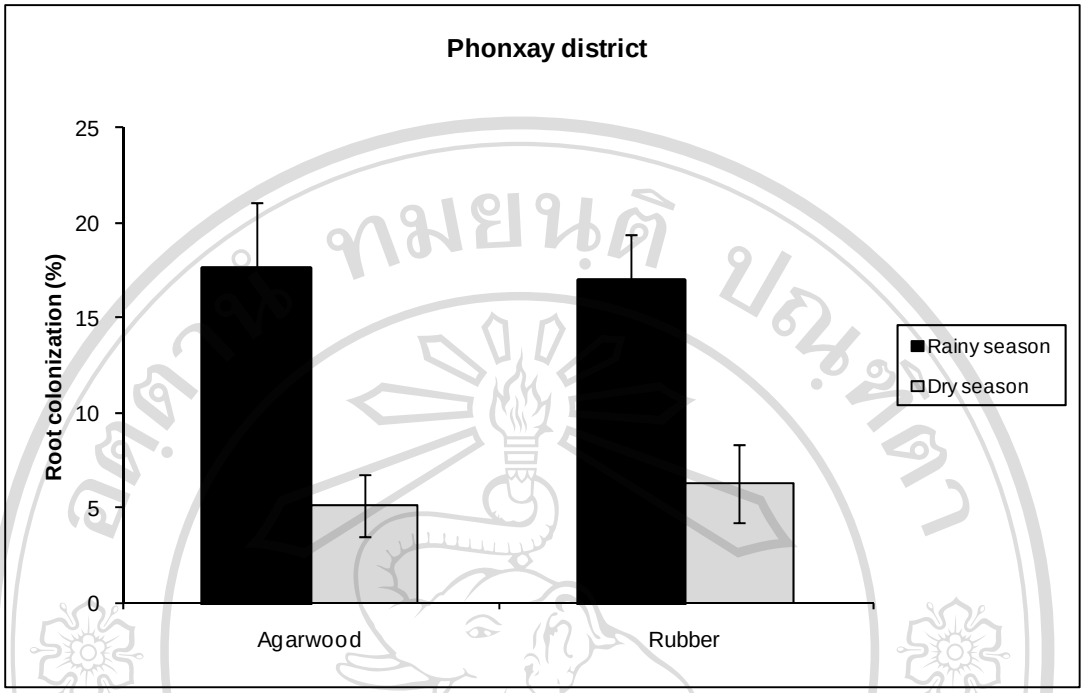

Figure 3.9 Root colonization (% with error bars) of 2 tree crops (agarwood and rubber) in 2 seasons (dry and rainy season) in Phonxay district.

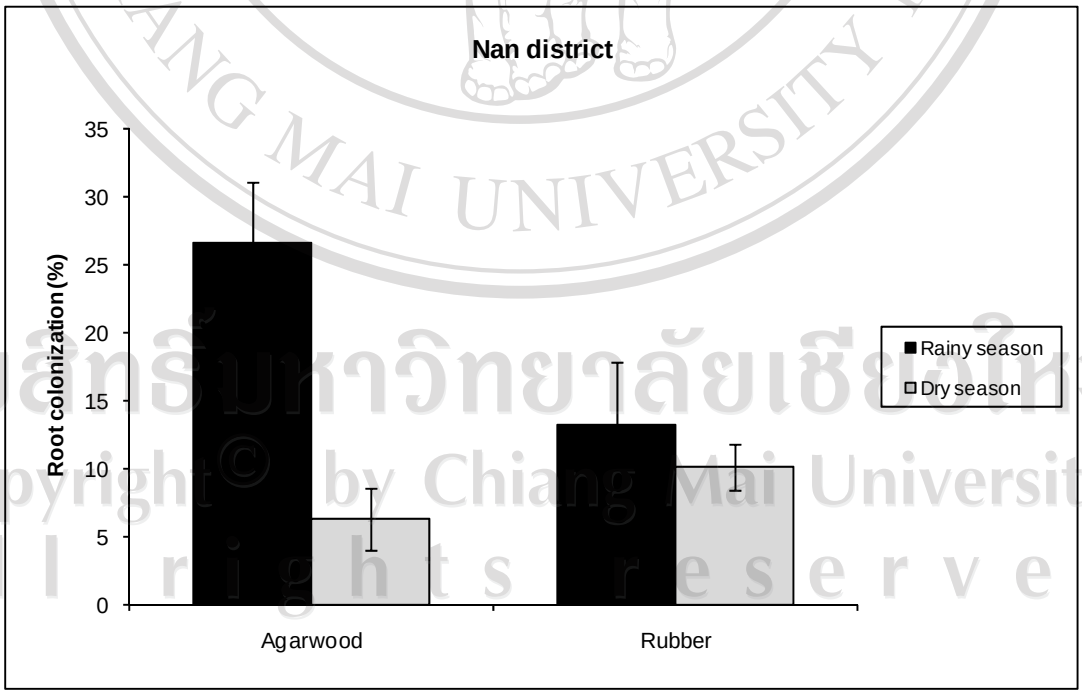

Figure 3.10 Root colonization (%with error bars) of 2 tree crops (agarwood and rubber) in 2 seasons (dry and rainy season) in Nan district.

Leaf phosphorus (P) concentration

Phosphorus (P) concentration in leaf of agarwood and paper mulberry were not significantly different between the two sites, while the P concentration in leaf of the teak and rubber were significantly higher in the Nan site than in the Phonxay district.

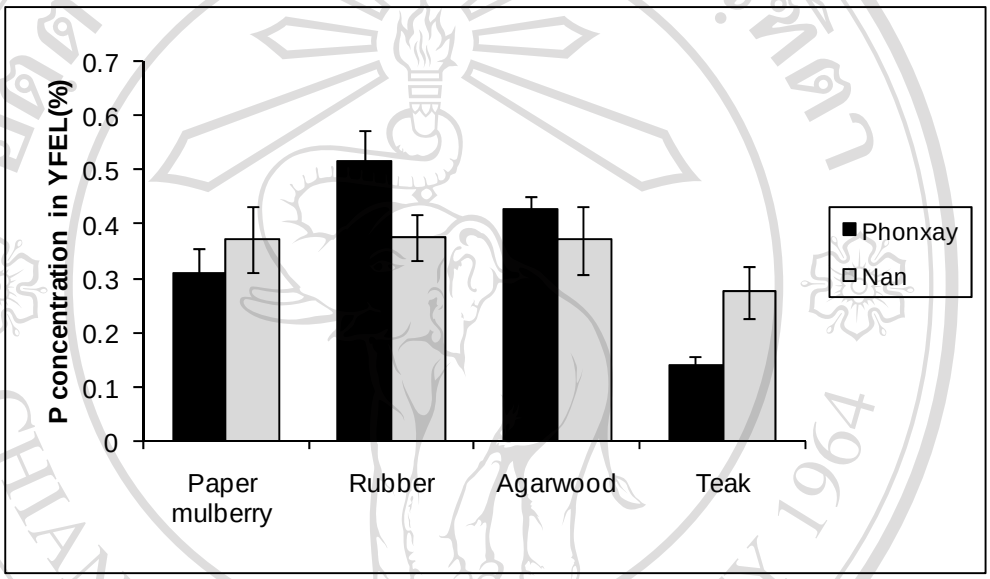

Figure 3.11 P concentration (with error bars) of 4 crops tree (paper mulberry rubber agarwood and teak) at Phonxay and Nan district.

3.4 Discussion

All 4 plants species from the agroforestry systems studied were associated with AM fungi in root colonization and spore population in their rhizosphre, suggesting that they could benefit from the symbiosis. This study confirmed the widespread occurrence of AM fungi in the soil agroforestry (Youpensouk *et al.,* 2004). However, root colonization by AM fungi and spore density varied significantly in deferent tree species. The variation in the percentage of colonization

in the root and AM fungi spore population in agroforestry soil of different tree species recorded in the present study suggested a strong interaction between plant and AM fungi species, which is also dependent on the environment. The higher root colonization by AM fungi and higher spore density in the rainy season than in dry season has been reported from shifting cultivation system in northern Thailand (Youpensouk, 2004), which has similar environment as Luang Prabang in Lao PDR. The root colonization found in the shifting cultivation system in northern Thailand, including fallow enriching tree (*Macaranga denticulata*) and upland rice (Youpensuk *et al*., 2005).

The higher spore density and root colonization in wet season may be the result of the environment in wet season is more suitable for spore germination and infection to root plant. The soil moisture content is a very important factor to determine AM fungi spore germination. Koske (1981) tested effect of soil moisture content on spore germination. This author found that the best soil moisture content for AM fungi spore germination was between field capacity and soil saturation. If soil moisture content was lower than field capacity, the spore germination declined. The dry season, the soil moisture content was low and may be not suitable for spore gemination of AM fungi in the agroforestry system. In contrast, the rainy season, spore germination should be higher than dry season because of higher soil moisture content. The limiting of spore germination because of low soil moisture content in dry season may be the cause of low root colonization in dry season. This similar result was found in upland shifting cultivation in northern Thailand. That root colonization and spore density in root zone of *M. den* were highest at the end of wet season(Youpensuk *et al.,* 2006). *Citrus*

volkameiana that grew in continually moist soil had root colonization at 64 %, but in periodically dry soil the root colonization reduced to 43% (Fidelibus *et al.*, 2000).

There were several studies reported the association between AM fungi and some tree crops. In rubber the root colonization was reported to range between 52 and 81% in Brazil and Bangladesh (Kummar *et al.*, 2000). Mridha (2003) reported 77% root colonization in teak in madhupur forest, Bangladesh. But in the present study, we found only 18% of root colonization of only 18 % in agarwood and 17 % in rubber (Figure 3.9). The low root colonization in this study may be caused of plant phosphorus status (Figure 3.11). From the leaf nutrient analysis in YFEL we found the high phosphorus concentration in rubber, which ranged from 0.4 - 0.55 %. But phosphorus concentration of 0.18 - 0.29% is supposed to be adequate. Yew, F.K and Push parayah, E. (1984). When plants have adequate phosphorus they normally has low root colonization by the AM fungi. Mohammad *et al.*, (2004) found that high P application resulted in a decreased spore number and % root colonization in wheat. Youpensuk (2004) found the same effects in *Macaranga. denticulata* when high P concentrations depressed AM colonization. The adequate P concentration in mature teak tree is 0.14-0.23% (Zech, 1990). The P concentration of 0.1% found in teak in Phonxay is correlated with the higher AM root colonization, and the higher P concentration of 0.3% in Nan was associated with the low root colonization of teak in this location. Higher P concentration could be one of the factors inhibiting root colonization in this study. However, other factors should also be investigated, especially presence of groups of AM fungi in the soil that are best suited to the different tree crops.