CHAPTER 4

BENEFIT OF ARBUSCULA MYCORRHIZAL FUNGI ON GROWTH OF RUBBER SEEDING IN RELATION TO PHOSPHORUS FERTILIZER

4.1 Introduction

Arbuscular mycorrhizal fungi are member of the phylum Glomeromycota (SchuBler *et al.,* 2001) and the number of species of mycorrhizal plants will certainly increase as research progresses. Mycorrhizal colonization increases the uptake of phosphorus (P) and other nutrients from soil by means of the external mycelium. In the tropics, the majority of species from AM fungi due to their role in the phosphorus deficient soils (Smith and Read, 1997). Mycorrhizal plants in low - P can be high dependent on the symbiosis. The low nutrient concentrations of many type of soil provide the appropriate conditions for the development of AM fungi that assist plants in nutrient uptake. Studies examining of the AM fungi status of the nutrient deficiency plants have frequently shown that the plants are colonized by AM fungi (St. john 1980; Bereau *et al.,* 1997; Metccalfe *et al.,* 1998; Onguene and Kuyper 2001).

During phosphorus limitation, plant roots employ several strategies to enhance P uptake, arbuscular mycorrhizal fungi increased P uptake from soil and increased plant growth (Harley and smith, 1983; Mosse, 1973). Little is known on the response of the tree crop rubber to AM fungi in non sterile field soils.

Many studies have demonstrated the importance of mycorrhizal fungi for growth of tree crops under conditions of limiting soil fertility, especially P

(Marschner and Dell, 1994; Clark and Zeto, 2000). Mycorrhizal plants have greater access to soil nutrient reserves because of their enhanced absorption surface and increased array of chemical tools for nutrient acquisition. Beside that, mycorrhizal fungi are known to affect growth of most plant species through various ways. They increase phosphorus uptake, enhance uptake of other plant nutrients by root system and are beneficial in the biological nitrogen fixation of Rhizobium, biological control of root pathogens and drought resistance (Harley and Smith, 1983; Sieverding, 1991; Dela Cruz, 1987; Janos, 1980b).

As result in several other trees crop from agroforestry soil (chapter 3), all species of trees seedlings that formed AM fungi associations formed associations of rubber tree that contained AM fungi have high colonization and spore density much more than other trees crop**.**

The objective of the present study was to evaluate the response to AM fungi in rubber seedlings and to determine a suitable phosphorus level for AM fungi inoculation in seedling nurseries.

4.2 Materials and Methods

A pot experiment was conducted in a greenhouse of the Department of Agronomy, Faculty of Agriculture, ChiangMai University in the winter season (24 November 2007 to 24 March 2008). The experiment was a factorial of 2 factors in RCB design with 4 replications. A pot was an experimental unit. The factors were 2 levels of AM fungi inoculation (uninoculated, AM0, inoculated AM+) and 3 levels of P fertilizer (0, 45.42 or 90.86 mg P/kg; designated as P0, P1 and P2, respectively).

Plant growth medium was prepared from a mixture of sand and soil. Sansai soil (0-30 cm depth) was collected from the MaeHia Experimental Station, Chiang Mai University with the properties in Table 1. The soil was air-dried before mixing with washed river sand in a 3:1 ratio (w/w). The growth medium (7 kg) was put into plastic pots lined with plastic bags. The growth medium was applied with 12.37 mg K/ kg as K_2SO_4 and the required rate of P was applied as KH_2PO_4 (Table 4. 2).

The prepared growth medium was autoclaved at 121°C for an hour. Seed of *Hevea brasiliensis* GT1 (Chinese variety), was supplied by Northern Agricultural and Forestry Center, Luangprabang, Laos P.D.R. (NAFReC). Seeds were surface sterilized with 70% ethanol for 5 minutes and pre-germinated in autoclaved sand for 2 weeks. Seedlings of uniform height (about 12 cm) were transplanted, one seedling per pot, to free-draining plastic pots (26 cm top diameter,17 cm bottom diameter and 24 cm dept) containing 7 kg of the growth medium. Each seedling was inoculated with 100 g of soil inoculum (AM+) or the same weight of autoclaved inoculum (AM0). The soil inoculum was collected from TeeCha village in Mae Hongson Province of Thailand that covered by *Macaranga danticulata*, a fallow enriching tree found associated with a rich diversity of AM fungi (Youpensouk, 2004). The soil innoculum was taken from the field in the wet season of 2007, one hundred grams of inoculum contained 223 AM fungi spores. The spores were mixed species, with *Glomus* and *Acaulospora* as dominant genera. The inoculum was placed at the bottom of each seedling hole in the pot. Each pot was applied with 63 mg N/kg soil monthly as urea (Table 4.2).

Stem diameter, number of branches and plant height were measured monthly since one month after transplanting. Plant height was measured from the ground surface to the top of the canopy. Stem diameter was measured at 1 cm above the ground. At four months after transplanting, all plants were harvested to measure shoot and root dry weight, number of AM fungi spores in the soil and AM fungi root colonization. Shoots were cut at 1 cm above the soil surface. Two hundred grams of soil (0-20 cm depth) was collected from each pot for AM fungi spore counting. Roots were washed free of soil and cut into 1 cm long pieces. Then a root sub-sample (10% of total root fresh weight) was randomly taken from every pot. Root sections of 1 cm were prepared (24 cm per pot) for microscopy and root colonization determined as described in chapter 3. Shoot and root dry weights were determined after drying at 75 ºC for 3 days. The P concentration in shoot was determined by Molybdovanadate Phosphoric Acid method (Murphy and Riley 1962).

Table 4.1 Soil mix properties

Property	Content
OM g/100g	1.98
N g/100g	0.07
P mg/kg	1.9
K mg/kg	40.62
CEC cmol(+)/kg	7.50
Sand %	31
Silt %	54
Clay %	15
Texture	Silt loam

Source: department of soil and conservation, Faculty of Agriculture, Chiang Mai University

Table 4.2 Rate of fertilizer applied

Fertilizer	Mg/kg soil	Kg/ha
KH_2PO_4 (P_0)	0	0
KH_2PO_4 (P_1)	45.42	14.8
KH_2PO_4 (P_2)	90.86	29.6
K_2SO_4 (K)	12.37	18
$CO(NH_2)$ (N)	63	90

Statistical analysis

Analyses of variance were performed on the data and all treatment means tested for significance using appropriate values of least significant differences (LSD).

4.3 Result

Without AM fungi plants dry weight (DW) was increased by applying phosphorus. However, AM fungi inoculation increased plant DW in every phosphorus level. But the effect of AM fungi also depended on P level. The effect of AM fungi was biggest in lowest P level (P0). Increasing P application rate depressed the response to AM fungi of plant dry weight (Figure 4.2 - 4.4).

Without AM fungi number of branches was increased by applying P fertilizer but in AM plant applying P depressed branching. The effect of AM fungi on branching depended on P level. In P0, AM fungi increased branching for 471%. Increasing P level depressed effect of AM fungi. Because in P1, AM fungi increased branching for 167% and in P2 the effect of AM fungi disappeared (Figure 4.5).

Inoculation with AM fungi increased stem diameter. The response to AM fungi depended on P application rate. The response was higher in lower P level. Arbuscular mycorrhiza increased stem diameter for 400 % in P0, 287.5% in P1 and 162.5 % in P2. In AM0 treatment, stem diameter was increased by increasing P level from P0 to P1 but it was stable from P1 to P2. In AM+ stem diameter was depressed by P application (Figure 4.6).

In AM0, applying 14.8 kg P/ha could not increase plant height. To increase plant height, un-inoculated plant needed 29.6 kg/ha of P application. In AM+, P application decreased plants height.

Root colonization and AM fungi spores were not found in AM0 treatment. In AM+, increasing P level decreased root colonization and AM fungi spores. The root colonization was 37.2, 29.5 and 22 % in P0, P1 and P2 respectively

(Figure 4.8). AM spores density were193, 166 and 118 spores per 100 g soil in P0, P1 and P2 respectively (Figure 4.9).

In uninoculated treatments, the P concentration of the plants was lower than in AM inoculated treatments. In AM inoculated treatments, applying P fertilizer significantly increased shoot P concentration in treatment P0 to P2. Arbuscular mycorrhizal fungi inoculation increased P concentration in P0 and P1. Increasing P application rate (P2) with AM fungi depressed the P concentration in plants. Because in P0, AM fungi increased P concentration for 290% and 151% in P1, but no effect of AM fungi to P concentration in P2 (Figure 4.10).

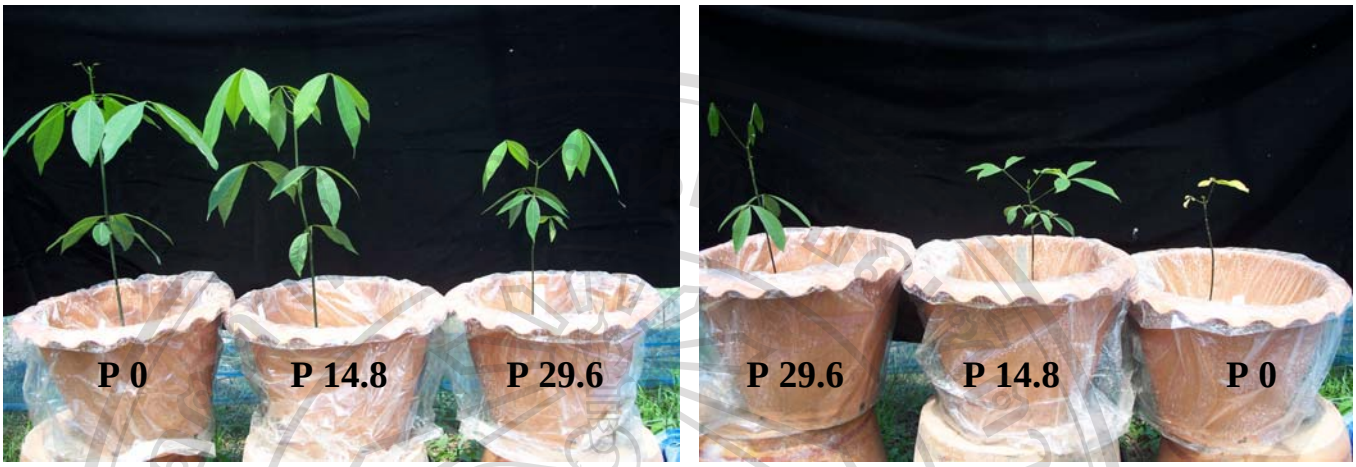

Figure 4.1 Seedlings of four months-old rubber in pot experiment showing the effects of AM fungi inoculation (AM+) and uninoculated (AM0) with different level of phosphorus application rate.

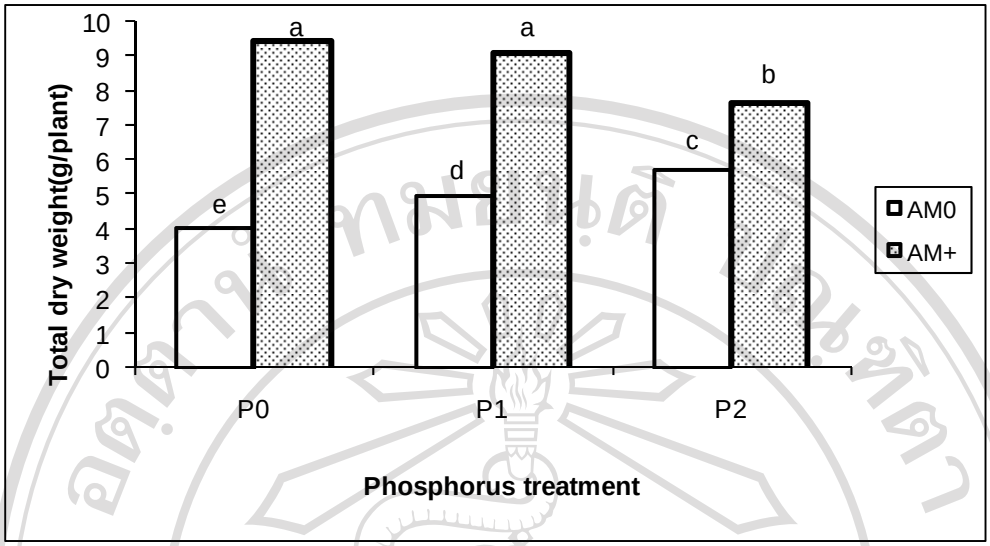

Figure 4.2 Total DW of rubber seedling due to AM fungi inoculation and P application during four months.

Different letters designate significant difference between treatments by tow - way ANOVA.

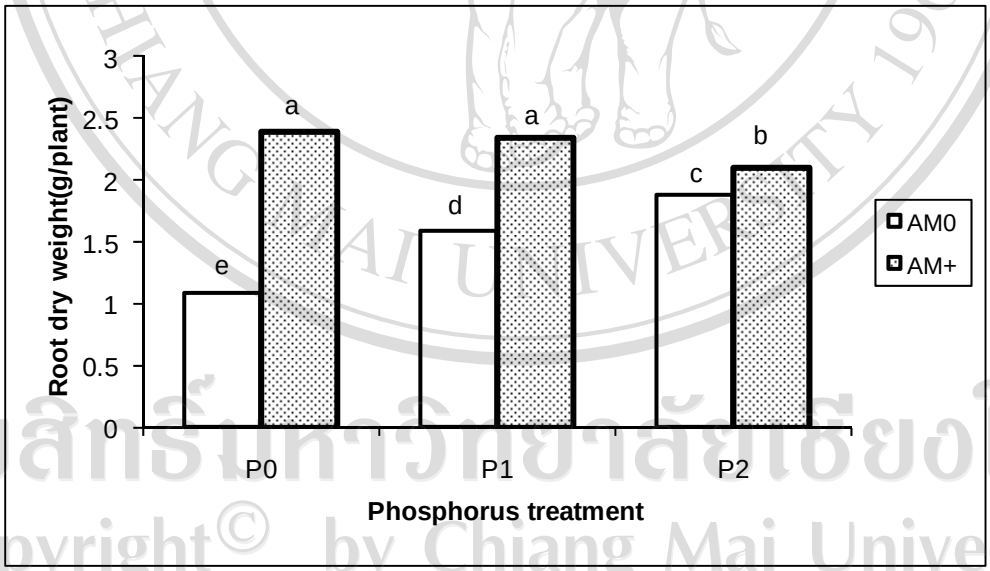

Figure 4.3 Root DW of rubber seedling due to AM fungi inoculation and P application at four months.

Different letters designate significant difference between treatments by tow - way ANOVA

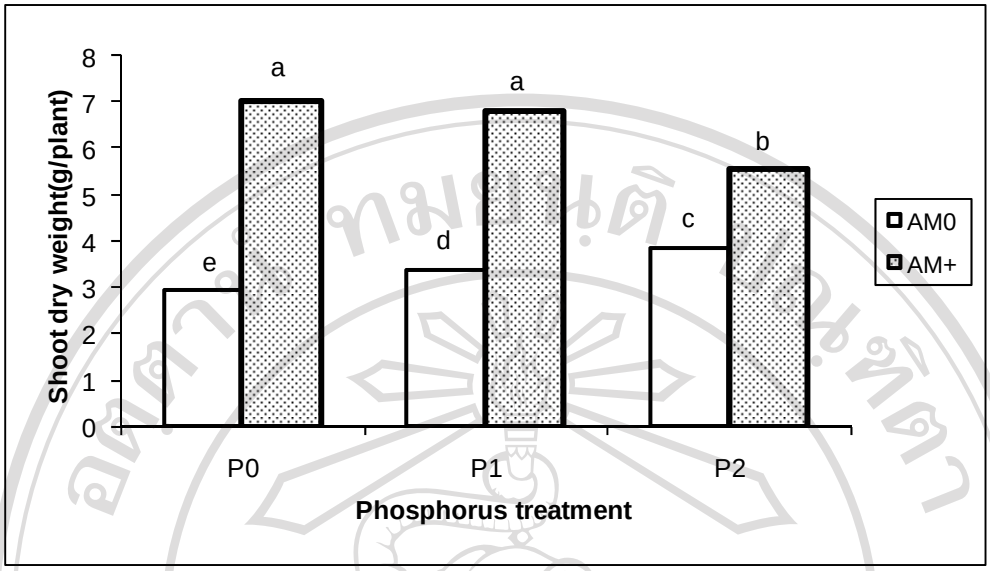

Figure 4.4 Shoot DW of rubber seedling due to AM fungi inoculation and P application At four months.

Different letters designate significant difference between treatments by tow - way ANOVA

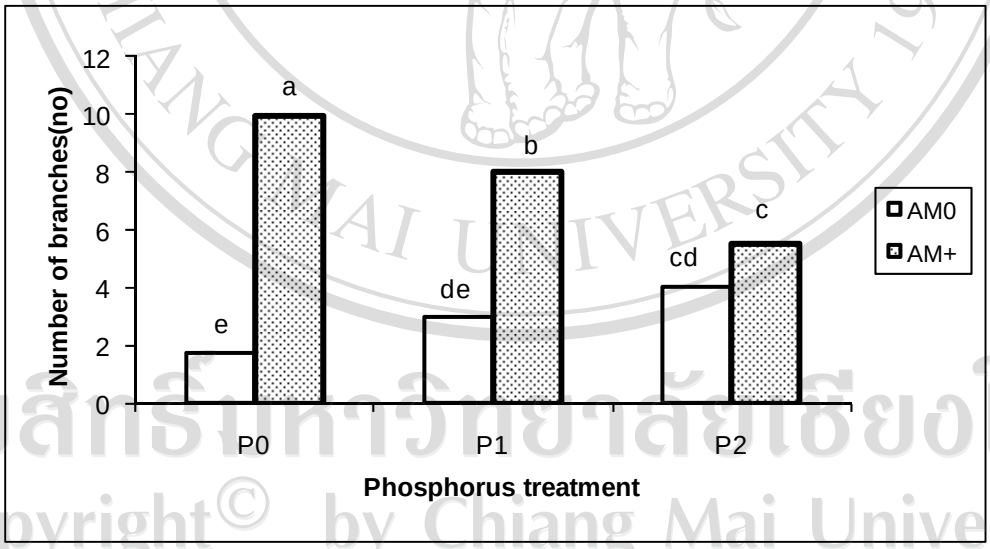

Figure 4.5 Number of branches of rubber seedling due to AM fungi inoculation and P application at four months.

Different letters designate significant difference between treatments by tow - way ANOVA

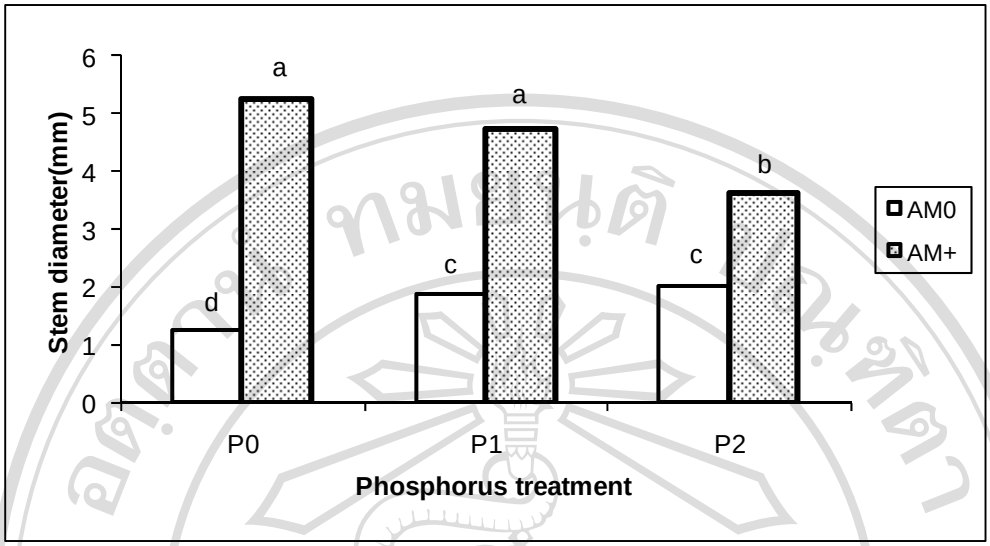

Figure 4.6 Stem diameter of rubber seedling due to AM fungi inoculation and P application during four months.

Different letters designate significant difference between treatments by tow - way ANOVA

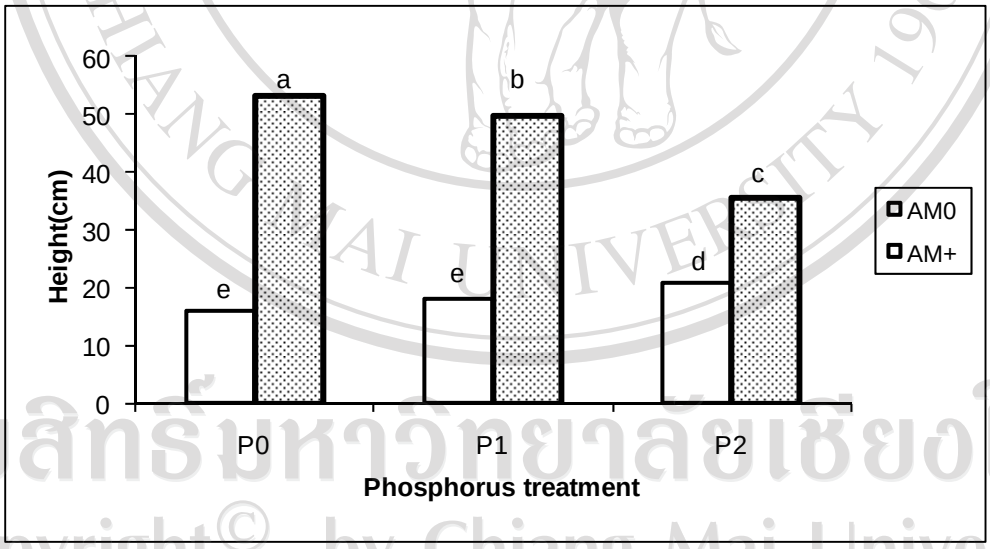

Figure 4.7 Height diameter of rubber seedling due to AM fungi inoculation and P application at four months.

Different letters designate significant difference between treatments by tow - way ANOVA

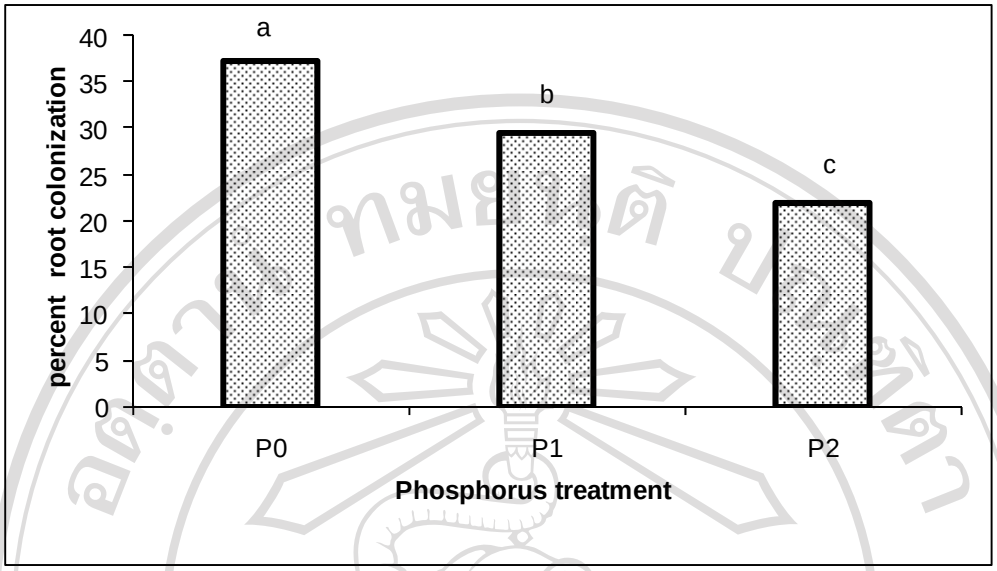

Figure 4.8 Effect of P application on root colonization percentage of rubber seedlings in AM fungi inoculation and P application at four months.

Different letters designate significant difference between treatments by tow - way ANOVA

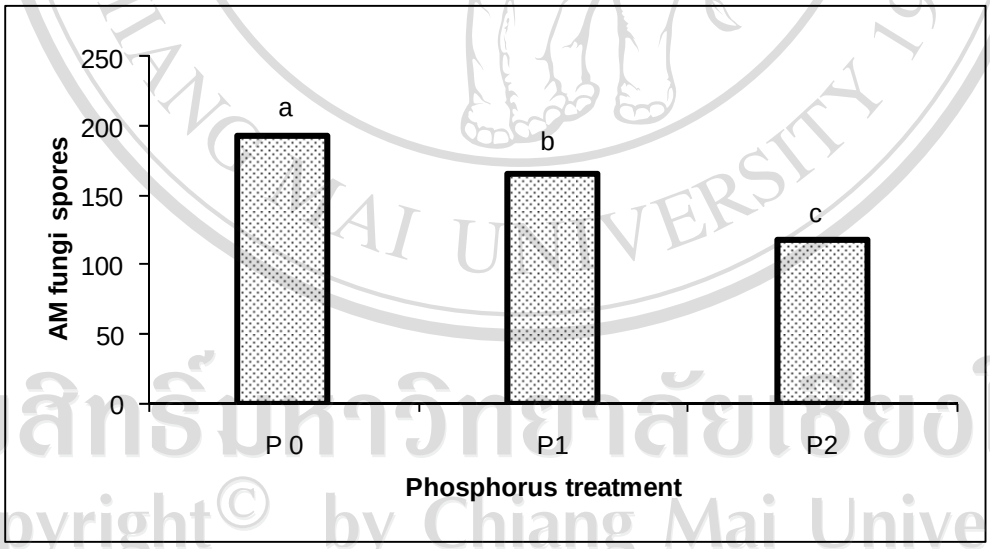

Figure 4.9 Spore number of rubber seedling due to AM fungi inoculation and P application at four months.

Different letters designate significant difference between treatments by tow - way ANOVA

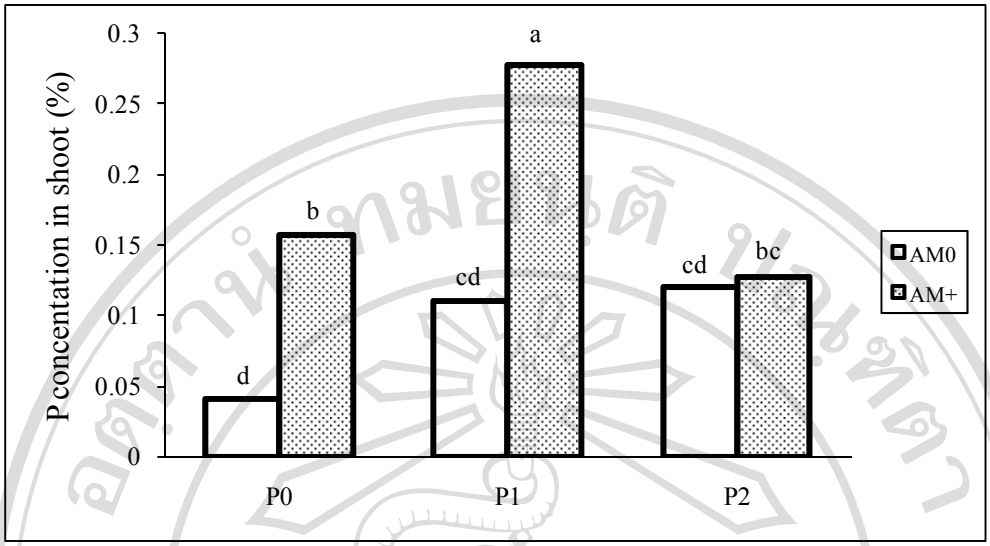

Figure 4.10 Effect of soil inoculation and fertilization on rubber seedling phosphorus concentration.

Different letters designate significant difference between treatments by tow - way ANOVA

Effect of AM fungi on shoot P concentration depended on P application level. Shoot P concentration was increased by AM fungi for 290 and 151 % in P0 and P1 respectively. But there was no effect of AM fungi in P2.

In AM0 treatment, P concentration was continually increased by increasing P level from P0 to P2. But in AM+ P concentration was depressed by increasing P level. The highest P concentration was found at P1 (Figure 4.10).

4.4 Discussion

The current study has shown that phosphorus is the limiting factor and arbuscular mycorrhizal fungi play the importance role in plants growth by enhancing nutrient acquisition.

The current study has shown that rubber seedlings on low P soil can respond to P application. Inoculating rubber seedlings with AM fungi enhanced growth performance and development in a pot experiment with soil low in available P. In this experiment, the AM fungi significantly increased shoot and root dry weight of rubber, number of branches, stem diameter, height, mycorrhizal root colonization and number of spore.

Although AM fungi inoculation was efficient at stimulation growth in this experiment, the magnitude of the responses varied with different levels of available -P. Thus, adding P fertilizer with AM fungi inoculation did not further improve plant growth. The decline in root colonization at high P levels, however, agrees with previous reports (e.g. Bagyaraj and Powell, 1985). Many authors illustrated that application of high P level suppressed root colonization of AM fungi in host plant. The results of this study showed a much smaller effect of P on root colonization by AM fungi.

The percent colonization rates of the rubber roots of 22- 37% were much lower than the other studies. For example, Ikram A, et al., (1991) obtained colonization levels of 42-52 % in inoculated rubber seedlings in nursery study.

In spite of low colonization levels obtained in this study, the effect of AM fungi in stimulating growth of rubber seedlings is clear. Arbuscular mycorrhizal fungi inoculation did not affect high P application rate. It is important to known the response to P application rate and inonculation with AM fungi. This is evident from higher percent root colonization rates of the inoculated vs P application rate at four month. The AM fungi carried in colonized P0 plants higher than P2 plant. The decline

in percent root colonization between P2 and P0 during four months may be due to fact that P level. Root colonization declined in soil with higher available P. With 29.6 kg/ha, root colonization was decreased (Figure 4.8).These results agree with those of some authors on AM fungi inoculation efficiency and P added (Jasper *et al.,* 1989; Stamford *et al.,* 1997). On their work, the substantially increased differences in root colonization of *G. sepium* plant were attributed to AM fungi inoculation whereas, in contrast in *Alnus incana* plants, high P level stimulated root colonization and spore density (Wall *et al.,* 2000). Root colonization was highest at 20 mg P application and reduced with increasing P application and spores density lower when slightly increasing with high P added.

The application of increasing levels of P reduced AM fungi colonization. Similar observation has been reported by Ingleby *et al.,* (2001) on *Calyandra calothyrsus* seedlings. However there authors indicated that high level P fertilizer eliminated growth benefits attributable to AM fungi inoculation In this study even though increased P level with AM fungi inoculation were not enhanced plants growth. The effect of AM fungi inoculation on AM fungi colonization was observed when compared the level P application rate. Plants P concentration had been increased for plant inoculated with AM fungi.

A decline in root colonization may also be a function of the natural biological decline of the AM fungi after four months in a high-P fertile soil. High soil P level reduces AM fungi development (Abbott and Robson 1984; Lui *et al*., 2000).

In general, the higher root colonization by AM fungi and higher P concentration are normally attributed to the tree species and environment factors.

Smith *et al*., (1979) reported that the extent to which typical AM fungi colonize root system varies with species of plant. The extent of AM fungi infection in root zone is also known to be influenced by environmental conditions; the most important being the age of the plants, the level of P in the soil relative to the requirements of the plant and the capacity of the population of AM spores in the soil. The increase of root colonization by AM fungi tended to increase the shoot P concentration. Many researchers have shown benefits of inoculations with AM fungi in perennial crops such as citrus (Youpensouk, 2006), coffee (Tristao *et al*., 2006) and passion fruit (Cavalcante *et al*., 2001).

In conclusion, adding phosphorus fertilizer can secure further improvements in plant yields but inoculating with AM fungi achieved high increases in shoot DW, Root DW stem diameter and number of branches including root colonization.