CHAPTER 5

EVALUATING SOURCES OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULUM TO PROMOTE RUBBER SEEDLING GROWTH

5.1 Introduction

Arbuscular mycorrhizal associations are widespread in the tropics, occurring with a great range of plant species, both annuals and perennials (Douds and Millner, 1999). And many plant species are dependent upon them for growth under normal conditions. Their role in the uptake of nutrients (especially phosphorus) and water, enhancement of N_2 fixation and improvement of soil structure (Douds and Millner, 1999). It is well document that AM fungi can help to improve nutrient and water uptake in poor soils (Marschner and Dell, 1994; Clark and Zeto, 2000). The AM fungi has been shown to improve seedling growth rate in tree in the species (*Bauhinia purpurea, Toona, ciliate, Turpinai pomifera* (Hoge, 2000). The AM fungi help to improve seedling growth survival by enhancing uptake of nutrient and water and increasing root in live span. Some experiments have shown that inoculation in sterile soil can be beneficial to tree growth in the tropics to *M. denticulata* (Youpensouk, 2004), *Acacia abyssinica, A. sieberiana* (Michelson, 1993) and *A. nilotica* (Verma *et al.,* 1994). Both (Ikram *et al.,* 1992); (Michelson, 1993) demonstrated that mixed inocula enhanced plant growth, and (Ahmad and Maziah, 1988) showed that mixed inocula were as good as the best of a range of single-species inocula for *Leucaena leucocephala*, *Albizia falcataria*, *Gmelina arborea* and *Intsia palembanica*. Indeed, mixtures may be particularly beneficial for perennials, given the seasonal range of environmental conditions encountered by them and their changing physiological status with age (Mason and Wilson, 1994).The results of the previous studies bring out clearly that rubber seedlings respond well to AM fungi that inoculation by *M. denticulate* under pot conditions. The pot trail suggests that, inoculated treatment resulted in higher spore density, % root colonization including shoot and root DW when compared with uninoculated treatment. Plant response to AM inoculation was strongest in soil of low P status. In the experiment described here, I assessed the potential of different host plants for a simple method to produce AM fungi inoculum that can be used by farmers in Laos. The plants, including mimosa (*Mimosa invesa*), cowpea (*Vigna unguiculata*), job's tear (*Coix lachrymal-jobi*) and *Macaranga denticulata* were chosen because they are commonly grown in northern Thailand and in similar condition in northern Laos and are known to be associated with AM fungi (Yimyam, 2003; Wongmo, 200x; A. Kongpan, personal communication). The objective of this study was to evaluate four different host plants for the production of AM fungi inoculum and effectiveness of the inoculants to enhance of rubber seedling growth.

5.2 Materials and Methods

The experiment was divided in to two steps

5.2.1 Production of Arbuscular mycorrhizal fungi inoculum

The objective was to produce AM fungi by using four different species are host plants as *Macaranga denticulata*, mimosa (*Mimosa invisa*.), cowpea (*Vigna unguiculata* (L.) Walp.) and Job's tear (*Coix lachrymal-jobi*). The experiment was a

CRD consisting of four treatments, growth medium that containing 4 kg air dry soil in plastic pots (20 cm top diameter, 12 cm bottle diameter and 18 cm depth) that had been autoclaved at 121 ºC for 1 hour. Soil inoculum from root zone of *M. denticulata* collected from beneath mature trees of *M. denticulat* in Tee Cha village Mae Hong Son province, Thailand. Seed of mimosa, cowpea and job's tear and seedlings of *M. denticulata* were planted into pots containing at 5 - 30 plants per pot. One hundred grams of soil inoculum from the root zone of *M. denticulata* that contained are 176 AM fungi spores was inoculated to each pot. Plants were watered once a day with tap water. Pots were placed in green house of the Department of Agronomy, Faculty of Agriculture, Chiang Mai University. Every two weeks soil from the root zone in each pot was examined for AM fungi and spore number assessed. The experiment was harvested two months, the soil from host four plant species were used for inoculation of rubber seedlings.

5.2.2 Main experiment:

A pot experiment was conducted in a greenhouse of the Department of Agronomy, Faculty of Agriculture, ChiangMai University during the rainy season (24 September 2008 to 24 December 2008). The experiment was in CRD with 4 replications. Each replication contained five treatments listed below, with soil from the root zone of four replicated pots mixed together:

1. Inoculated, soil from the root zone of *Macaranga denticulata*

2. Inoculated, soil from the root zone of mimosa

3. Inoculated, soil from the root zone of cowpea

4. Inoculated, soil from the root zone of job's tear

5. Inoculated, soil from the root zone of *Macaranga denticulata* which had been sterized at 121.^{o}C for 1 hour.

Growth medium was prepared from a mixture of sand and soil (3:1). Soil (0-30 cm depth) was collected from the Mae Hia Experimental Station, Chiang Mai University with the properties (Table 5.1)

Table 5. 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

Growth medium were put into plastic autoclavable bags. After that it was autoclaving at 121 ºC for 1 hour. The seed of *Hevea brasiliensis* . GT1 (Chinese variety), from Northern Agricultural and Forestry Center (NAFReC), Luangprabang, Laos P.D.R. was used. The rubber seed was surface sterilized with 70% ethanol for 5 minutes and pre-germinated in autoclaved sand for 2 weeks. The inoculum, soil from the root zone of each of the 4 host plants described above (Table 5.2), plus sterilized soil as inoculated control, was placed in the bottom of the hole in the pot for each seedling.

Seedlings uniform in 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 depth) containing 7 kg of the soil mixture.

Table 5.2 Inoculated with source of AM fungi

Source of AM fungi	Amount of inoculum (g/pot)	Amount of AM spore/100g soil
M. denticulata	110	200
Mimosa	100	237
Cowpea	124	157
Jobs'tear	135	70
Sterized soil (control)	100	-

Plants were watered once every day with tap water. Stem diameter, number of branches and plant height were measured at three months. 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 three months after transplanting, all plants was harvested to measure shoot and root dry weight, number of AM fungi spores in the soil and AM fungi colonization of the roots. Shoots were harvested 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 to free from soil and cut into 1 cm long pieces. Then a root sub-sample was randomly taken from every pot, fixed in 50% ethanol and cleared in 10% KOH before staining with 0.05% trypan blue in lactoglycerol (Brundrett *et al*. 1996). Root colonization percentage was assessed using the intercept method (Brundrett *et al*. 1996). Shoot and root weight 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).

5.3 Results

At the three months, plants from all the inoculation treatments were significantly larger than the uninoculated control (Figure 5.1). The extent of mycorrhizal formation on *M. denticulata* - inoculated plants was similar to the previous experiment, whereas that of the other inoculation treatments showed some differences. This experiment, control plants grown in sterile soil were non-mycorrhizal whereas those grown in sterile soil showed different degrees of root colonization by AM fungi.

Figure 5.1 Three month-old rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated.

In general, rubber plants inoculated with *M. denticulata* and mimosa showed the highest root dry weight (DW) followed closely rubber plants inoculated by inoculum produced in cowpea roots. Lower root DW was found in rubber plants inculated with AM inoculum produced in the roots of job's tear, and lowest in uninoculation control plant (Figure 5.2).

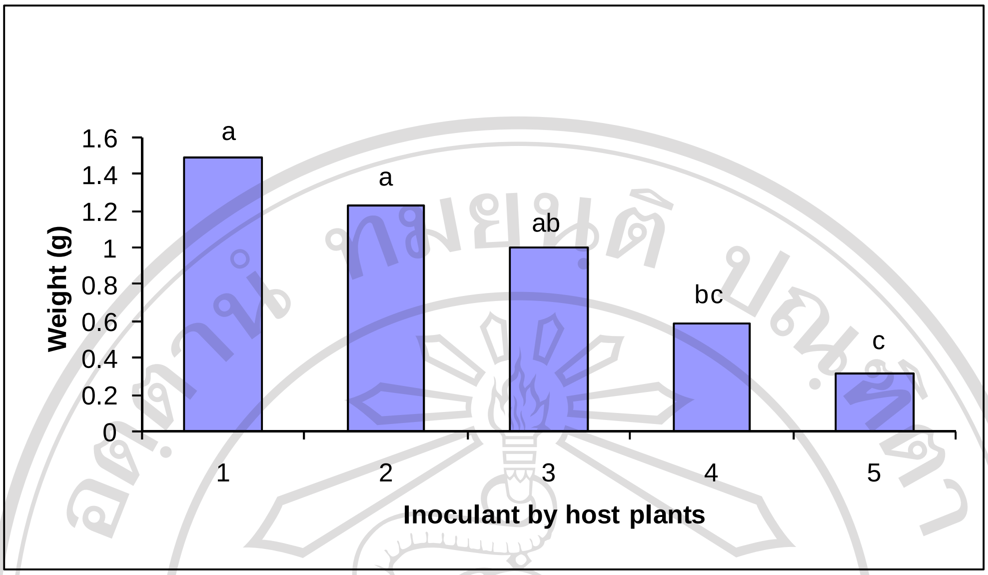

Figure 5.2 Root DW of rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$)

Rubber seedlings inoculated with *M. denticulata* and mimosa showed the highest shoot DW closely followed by those inoculated with inoculum produce in cowpea roots. Lower shoot DW was found in rubber seedlings inoculated with inoculum produced in the roots of job's tear and lowest in uninoculation control plant. (Figure 5.3).

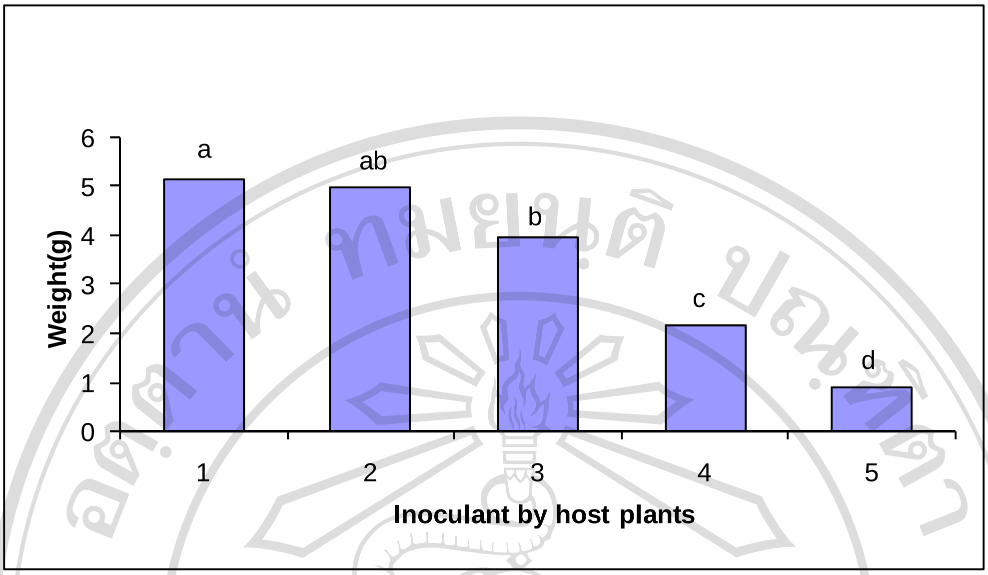

Figure 5.3 Shoot DW of rubber seedlings grown in a sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$)

Plants inoculated with inoculum produced in the roots of *M. denticulata* had the highest number of branches, followed by plants inoculated with inoculum produced in the roots of mimosa, cowpea, job's tears, in that order. The uninoculated rubber seedlings had significantly fewer branches than even those inoculated with inoculum produced in the root zone of job's tear (Figure 5.4).

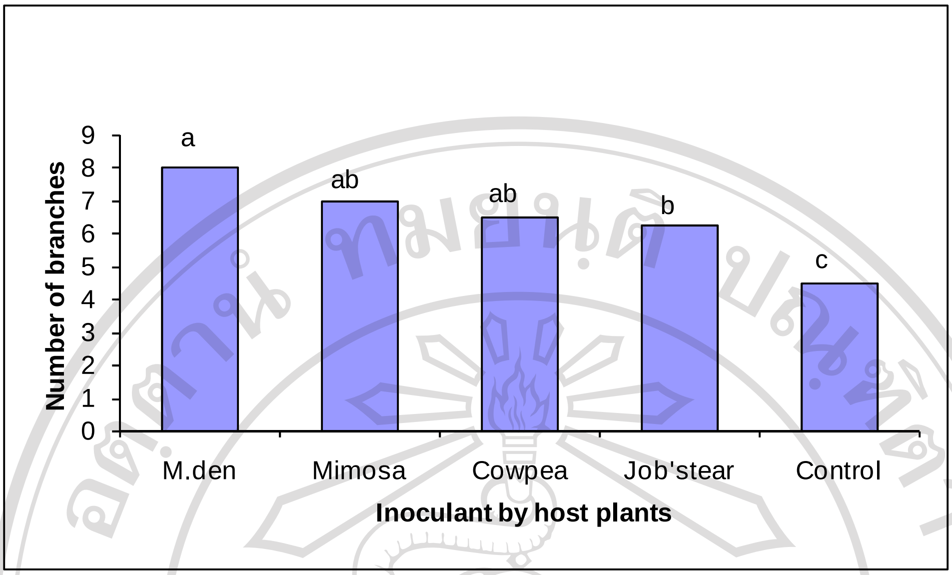

Figure 5.4 Number of branches of rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$)

Rubber seedlings inoculated with inoculum from *M. denticulata*, mimosa and cowpea had the same range of high stem diameter of about 5 mm, compared with significantly ($P < 0.05$) smaller stem diameter of 2-3 mm in those seedlings inoculated with inoculum from job's tear and uninoculated (Figure 5.6).

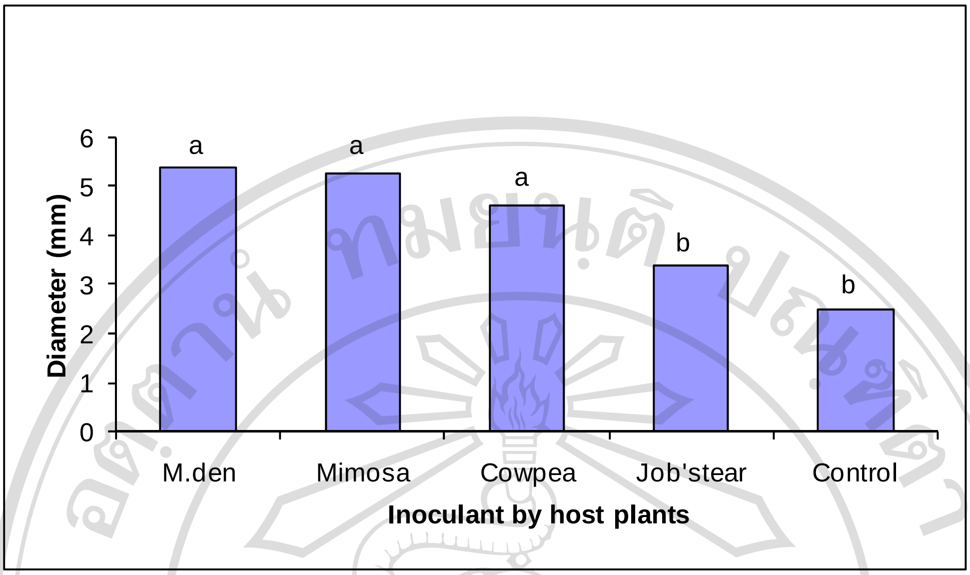

Figure 5.5 Stem diameter of rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$)

Inoculation with AM fungi grew taller than the uninoculated plants, but the effect depended on the host plant on which the inoculum was produced. Rubber seedlings inoculated with inoculum produced on *M. denticulata* (59 cm) and mimosa (55 cm) were tallest. Those inoculated with inoculum produced on cowpea were slightly shorter (46 cm), and shortest were those inoculated with inoculum produced on job's tear (36 cm), which was not significantly different in height from uninoculated plants (27 cm) (Figure 5.6).

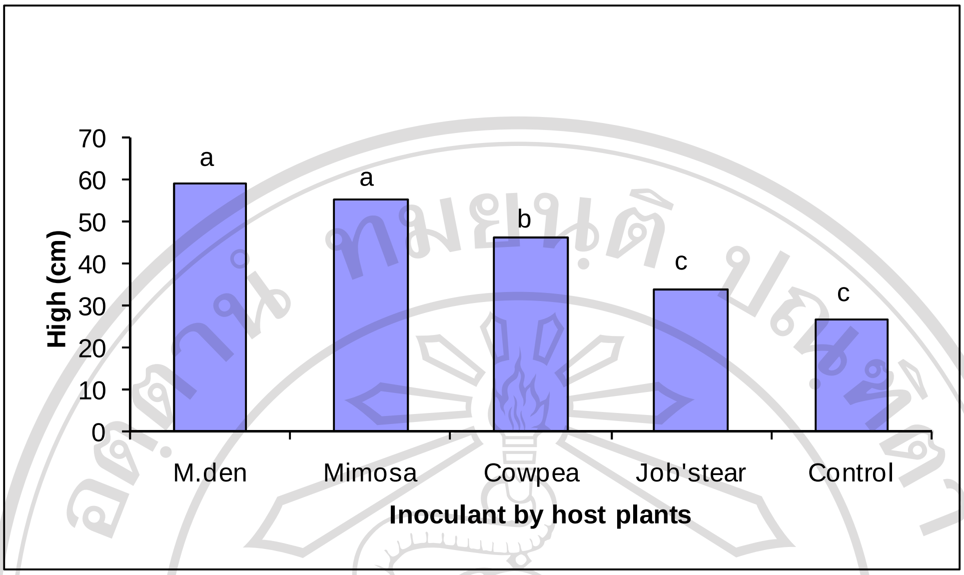

Figure 5.6 Height of seedlings grown in a sterilized soil with AM fungi treatment from four host plant. (1) AM fungi inoculation from *M. denticulata*, (2) AM fungi inoculation from mimosa, (3) AM fungi inoculation from cowpea, (4) AM fungi inoculation from job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$).

The roots of uninoculated rubber seedlings were not colonized by the AM fungi. In general, AM fungi inoculation increased the percent mycorrhizal root colonization and spore numbers in soil. The highest percent root colonization was observed in rubber seedlings inoculated with inoculum produced on *M. denticulata* and mimosa treated plants followed by those treated with cowpea and job's tear (Figure 5.7).

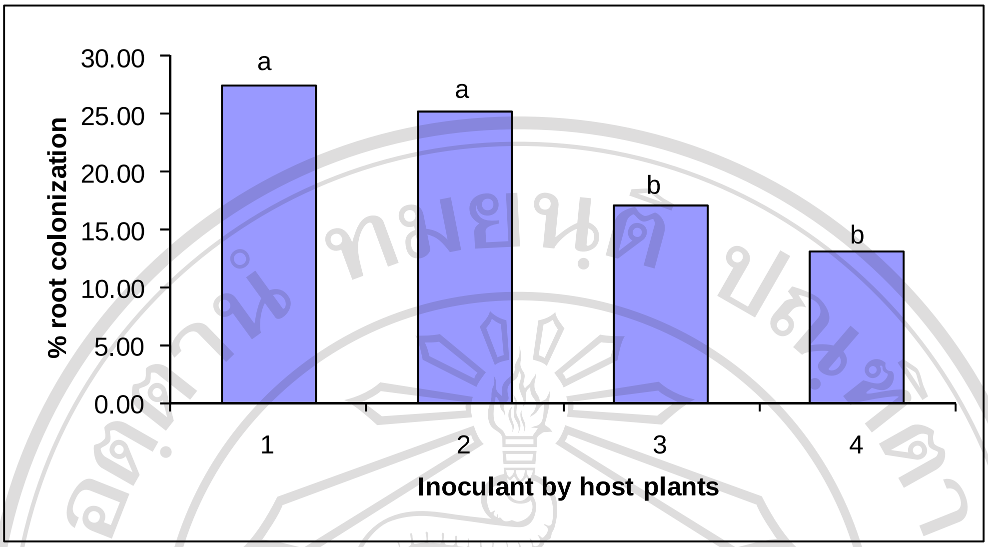

Figure 5.7 Root colonization by AM fungi of rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$).

The number of spores was much lower at only about 120 per 100 g soil with inoculum from the root zone of job's tear while inoculum from *M. denticulata,* mimosa and cowpea produced more than 200 spores per 100 g soil in the root zone of rubber seedling (Figure 5.8). Identified by morphology of their spores I found that *Acaulospora and Glomus* were the dominant genera when AM fungi inoculum were produced on *M. denticulata* and cowpea. In contrast, with inoculum produced on job's tear the proportion of others increased and dominance of *Acaulospora and Glomus* had declined. (Figure 5.8).

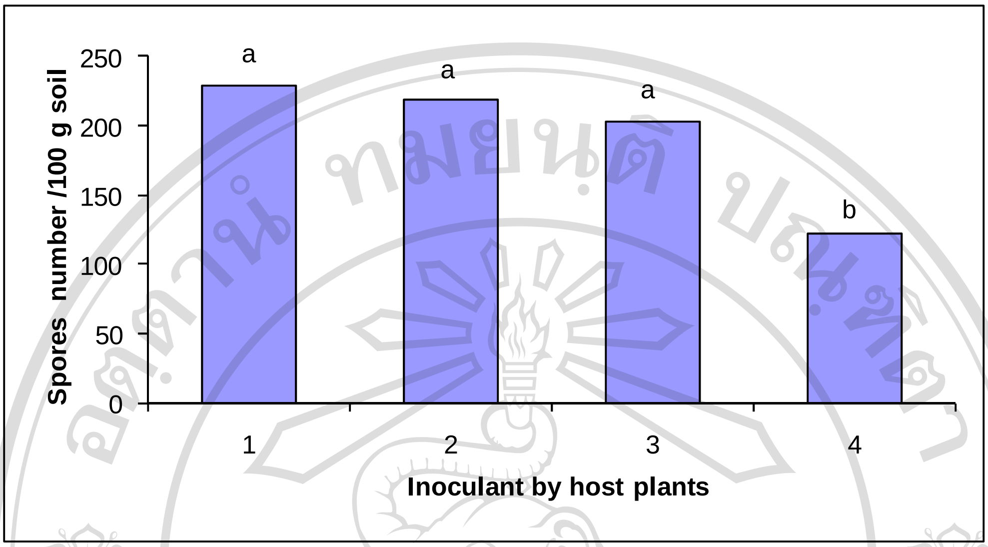

Figure 5.8 Spores density of AM fungi in the root zone of rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$)

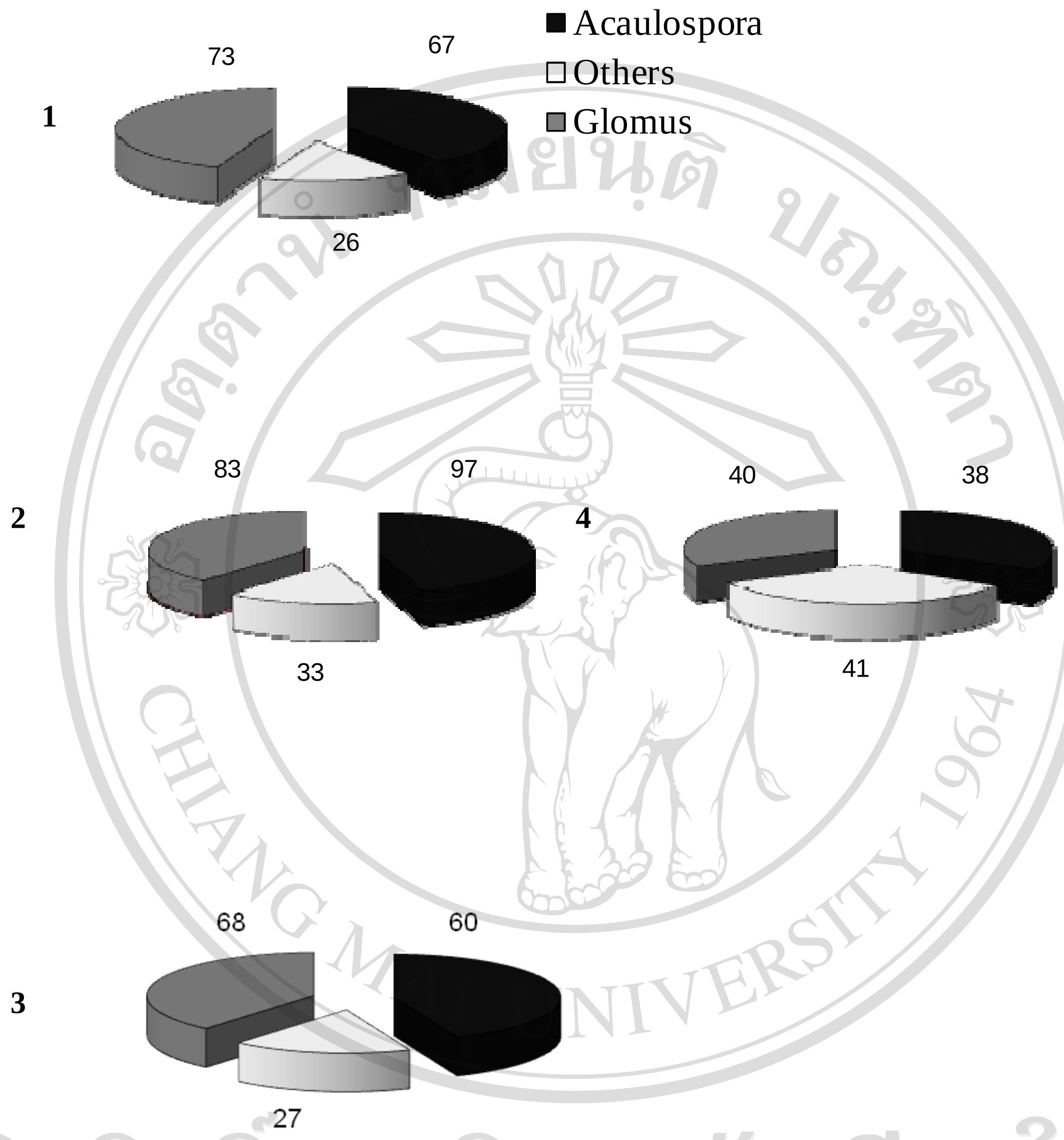

Figure 5.9 The portion of the different genera, with number of spores of each genus per 100g soil. (1) AM fungi inoculation from *M. denticulata*, (2) AM fungi inoculation from mimosa, (3) AM fungi inoculation from cowpea, (4) AM fungi inoculation from job's tear.

Shoot P concentration of the rubber was significantly affected by soil inoculation (Figure 5.10). Soil inoculation increased the nutrient concentration of plants in all treatments except the treatment was inoculated by job's tear. But the effect of AM fungi was biggest in the treatment 1, 2 and 3 that P concentration was increased 489, 572 and 361 % respectively.

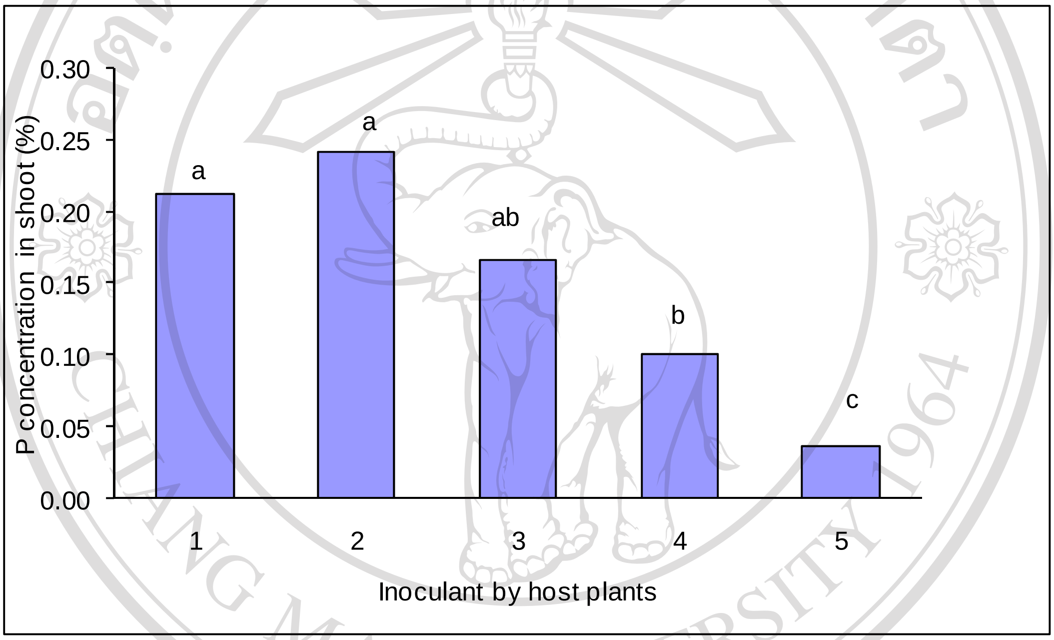

Figure 5.10 Shoot P concentrations of rubber seedlings grown in sterilized soil inoculated with soil from the root zone of four host plants: (1) *M. denticulata*, (2) mimosa, (3) cowpea, (4) job's tear, and (5) uninoculated. (Different letters above bars designate significant difference by LSD, $P < 0.05$)

5. 4 Discussion

For inoculum production, different host plants had different effect on the production of AM spores. Mimosa had the highest performance to multiply AM spore, the second was *M. denticulata* the third was cowpea and job's tear produced the fewest spores. Smits (1994) and Moyersoen (2001) found that AM fungi colonization and spores production depends upon the type of host as well as the duration of infection of these symbiotic organisms. Generally, with increase in the growth period after infection, root colonization of host increase.

The higher mycorrhizal root colonization and plant growth, and confirm the results of Michelson (1993). Inoculation was highly effective in promoting mycorrhizal formation and improvements in growth were obtained with all tree.

The absence of AM fungi colonization in the sterilized control indicates that there was no colonization by contaminants fungi during the experiment, and is also indicative of soil sterilization in effective controlling of AM fungi colonization and growth in the absence of inoculation.

When the effectiveness of inoculums was tested in rubber seedling, all inoculum types could enhance growth of rubber seedlings but the ability to enhance rubber growth was different between the different source of inoculum. Inoculum from *M. denticulata* and mimosa had highest ability to enhance rubber growth. The second was the inoculum from cowpea and inoculums from Job's tear were the least (Figure 5.2-5.4). This was the result of that rubber seedlings inoculated with inoculums from job's tear had lower P status than seedling inoculated with the other inoculums type. It means the different effectiveness between inoculums because of the different in P acquisition. The difference of P acquisition may be the result of different infection ability between inoculums types. The inoculums of *M. dendiculata* and mimosa had higher infection ability than inoculums form cowpea and job's tear (Figure 5.7).

The different in infection ability and effectiveness of inoculums may be caused by the different proportion of AM genus between inoculum sources. The proportion of AM genus was different between inoculum types. We found that in inoculums from *M. denticulata,* mimosa and cowpea that had high effectiveness, had similar proportion of AM Genus. In these 3 inoculum *Acaulospora* and *Glomus* were dominated genus. But in inoculums from job's tear that had less effectiveness than the others, proportion of *Acaulospora* and *Glomus* were less than in the other inoculums. There are some reports that confirm the effect of AM fungi type on effectiveness to enhance plant growth. McGraw and Schenck, (1981), Gracy Sailo and Bagyaraj, (2005) found that species and strains of AM fungi can differ in the extent by which they increased nutrient uptake and plants growth.

Macaranga dennticulata and mimosa are suitable host plant for producing AM inoculums for rubber seedlings because 2 host plants had high ability to multiply AM fungi spores production. And the AM fungi multiplied from these host plant are effective to enhance rubber seedlings growth. The inoculums that had more proportion of *Acaulospora* and *Glomus* had more effective to enhance rubber seedlings growth. Since no information is available on specificity of AM fungi and rubber, it should be further verified if *Acaulospora* and *Glomus* are the preferred genera of AM fungi for rubber.

Hence, the need for selecting efficient AM fungi that can be used for inoculating different mycohhizal plants has been stressed (Abbott and Robson, 1982). This depends on the ability to from extensive and well- distributed hyphae in soil, to from extensive colonization in the root system, and to absorb P from soil. This study has nevertheless demonstrated a very simple method for the production of effective AM fungi inoculum that can be used by small farmers in Lao PDR, Thailand and other developing countries.