# CHAPTER 6

# COMPARING EFFECTIVENESS OF DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ISOLATES TO ALLEVIATE ACID SOIL STRESS IN COWPEA

## 6.1 Introduction

The effectiveness of AMF from Haui Teecha village in alleviating acid soil stress in cowpea was proved in chapter 5. However, the effect was derived from inoculation with soil from the rhizosphere of *Macaranga denticulata*, the fallow enriching tree from farmer's shifting cultivation field, which has been previously shown to be highly diverse. A sampling of the rhizosphere soil in farmers' fields in Haui Teecha found a total of 17 species in 5 genera of AMF associated with upland rice (Youpensuk *et al.*, 2005a) and 29 species in 6 genera were associated with the fallow enriching tree *Macaranga denticulata* (Youpensuk *et al.*, 2004). It had been further discovered that different groups of the AMF had very different effects on growth and nutrient accumulation of the fallow enriching *M. denticulata* (Youpensuk *et al.* 2005b), just as has been previously reported (Green, 1983). Moreover the effectiveness of AMF species may be influenced by soil condition such as soil acidity and available P (Skipper and Smith, 1979). Identification of effective AMF genus and species in acid soil will have practical implications for realizing benefits from AMF in legume production on acidic low P soils. The objective of this chapter is to identify the effective AMF species from Haui Teecha village and to determine the interaction between AMF species, soil pH and soil P on growth of the legume host.

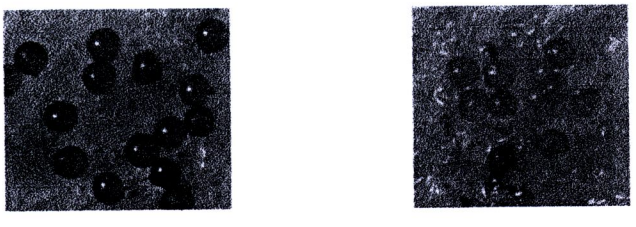
## 6.2 Materials and methods

Three pot experiments with steam sterilized soil were conducted in greenhouses of the Faculty of Agriculture, Chiang Mai University.

*Inoculum production*

Spores of abuscular mycorrhiza fungi (AMF) in soil collected from Haui Teecha village were extracted by wet sieving and sucrose centrifugal method (Brundrett *et al.*, 1996). Single spore of two selected species of *Acaulospora* (AM1, *Acaulospora* sp and AM2, *Acaulospora morrowiae* strain CMU22 – Figure 6.1) were multiplied in pot culture growing spineless mimosa (*Mimosa invisa* Mart) as host, in a growth medium was a mixture of soil and sand in 2:1 ratio. The soil contained 4.2 mg P/kg (Bray II). The growth medium pH was adjusted to be 5 by adding $Al_2(SO_4)_3$. Four months after growing 2 species of AMF were multiplied successfully.

The root zone soil of mimosa was used as AMF soil inoculum in experiment 6.2.1, with 114 spore/ g in AM1 incoculum and 295.5 spore/g in AM2 incoculum. Inoculum in experiment 6.2.2 and 6.2.3 were spores extracted from the mimosa root zone soil with wet sieving and sucrose centrifugal method.



**a** **b**

**Figure 6.1** Spores of AM1 (a) and AM2 (b)

*Growth medium preparation*

Sansai soil was collected (0-30 cm depth) from Mae Hia Agricultural Research Station and Training Center Chiang Mai University. The soil was air dried, ground and then sieved to pass a 5 mm screen. The sieved soil was mixed with washed river sand in a 2:1 ratio (soil/sand, w/w), 3.6 kg the mixture was put in to each of plastic bags. The pH of the mixture was adjusted to be 5 (acid soil) or 6.7 (non acid soil) by adding $Al_2(SO_4)_3$ $18H_2O$ or $CaCO_3$, respectively. The prepared growth medium was sterilized by autoclaving at 121°C for one hour.

*Root colonization measurement*

The root samples were cleared in 10% KOH for 24 hours before staining with 0.05% trypan blue in lactoglycerol for 3 days. Root colonization percentage was assessed using the intercept method (Brundrett *et al.* 1996). Thirty pieces of 1 cm root sections were examined under a compound microscope for each sample. The data in percentage of root colonization was arsine transformed before analysis of variance.

**6.2.1 Comparing the effect of soil inoculants containing two different AMF on cowpea growth in acid soil**

This experiment was arranged in split pot in randomized complete block design with 3 replications. One pot was one experimental unit. Main plots were 3 inoculation treatments, including AM0, AM1 and AM2. Subplot were 2 levels of soil pH including pH 5 (acid soil) and pH 6.7 (non acid soil).The growth medium was the mixture of sand and soil as described above. Five surface sterilized seeds (soaking in 70% ethanol for 5 minute) of cowpea line CP4-2-3-1 were grown in 5 L drainable

plastic pots, each containing 3.6 kg of growth medium with pH 5 or 6.7. Each pot received 33 kg P/ha in form of $KH_2PO_4$. Every seed was inoculated with 1 ml of appropriate rhizobium suspension ($10^9$ cell/ ml). Each pot was inoculated with 20 g soil from the root zone of mimosa containing either AM1 or AM2 or autoclaved soil inoculum (AM0). One week after emergence, seedlings were thinned to 3 plants per pot. At 48 days after sowing (pod setting stage) shoots were cut at above ground level. Roots were washed free of soil particles. Root nodules were collected and counted. Roots were cut into 1 cm segments. Ten percent of root fresh weight of each pot was sampled randomly for measurement of mycorrhizal colonization. Dry weight of shoot, roots and nodules were determined after drying at 75 °C for 3 days.

### 6.2.2 Comparing effects of spore inoculants of two AMF strains on cowpea growth in acid soil with varying available soil P

Spores of AM1 and AM2 multiplied on mimosa as in 6.2.1 were extracted by wet sieving and 50% sucrose centrifugation method (Brundrett *et al.* 1996) before transfer to filter paper (90 cm diameter) 1,000 spore/paper then keep in 4 °C for a week before inoculation. The experiment was arranged in factorial combinations of 3 factors in randomize complete block design (RCB) with 3 replications. The first factor was 3 AMF inoculation treatments including un-inoculated control treatment (AM0), inoculating with spores of AM1 and AM2. The second factor was 2 levels of soil pH including pH 5 (acid soil) and pH6.7 (non acid soil). The third factor was 3 levels of soil P concentration including 7, 11 and 16 mg P/kg (P7, P11 and P16 respectively). the soil for growth medium preparing was collected from Mae Hia agricultural research station and training center Chiang Mai University with the following

properties: 3.8 mg Bray II P/kg and pH (1:1 $H_2O$) 5.9. The growth medium was prepared as described above. Growth medium P concentration was adjusted to be 7, 11 and 16 mg P/kg (P7, P11 and P16) by applying 38, 58, 84 mg $KH_2PO_4$/kg respectively. The plant used was cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Rajathanee), provided by Khon Kaen Field Crop Research Centre. Seeds were surface sterilized with 70% ethanol for 5 minutes then washed three times with sterilized water. Five surface sterilized seeds were sown in free-draining plastic pot contained 3.6 kg of potting medium. The seeds were put on the filter paper containing 1,000 spores of isolate 1 (AM1) or isolate 2 (AM2) or blank filter paper as a control (AM0). Each seed was inoculated with 1 ml of *Rhizobium sp.* suspension ($10^9$ cells /ml) before buried. The suspension provided by Department of Soil Science Faculty of Agriculture Chiang Mai University. Plants were harvested at 8 weeks after sowing (pod filling stage). Shoots were cut at ground level. Roots were washed to free from soil. Root nodules were collected. Roots were cut around 1 cm long. Then a root sub-sample (10% of total root fresh weight) was randomly taken from every pot for mycorrhizal root colonization measurement as described before. Shoot, root and nodule were weighted after drying at 75 °C for 3 days.

### 6.2.3 Effect of spore inoculation with 2 AMF strains on mimosa

Mimosa (*Mimosa invisa*) was used as a test plant for AMF response in a pot experiment at the same time as experiment 6.2.2. The growth medium preparation and AMF and soil pH treatments were same as experiment 6.2.2 but with one level of soil P only (7 mg P/kg). The experiment was arranged in factorial combinations of 2 factors in RCB with 3 replications. The first factor was 3 inoculation treatments

including inoculated with AM1, AM2 and uninoculated control treatment (AM0). The second factor was 2 soil pH levels (pH 5 and 6.7). The growth medium P concentration was adjusted to 7 mg P/kg by applying 38 mg $KH_2PO_4$/kg. Twenty seed of mimosa were sown in free-draining plastic pots each containing 3.6 kg of steam sterilized growth medium. The seeds were put on filter paper containing 1,000 spores of AM1 or AM2. A blank filter paper was used as un-inoculated control (AM0). The seeds were inoculated with 5 ml rhizobium suspension ($10^9$ cell/ml) before being covered with soil . Plants were thinned to 5 plants per pot at 2 weeks after emergence. Plants were harvested at 13 weeks after sowing shoot was cut at above ground level. Ten g. of fresh roots was excavated to measure root colonization by the method described before. Shoot was oven dried for 3 days before weighing. The pot medium in this experiment would be used as soil inoculum in further experiment therefore root biomass was not measured.

## 6.3 Result

### 6.3.1 Comparing the effect of soil inoculants containing two different AMF on cowpea growth in acid soil

No root colonization was found in A0 treatment. Root colonization in inoculated treatments was low (3 to 20 % Table 6.1) and not different between AM1 and AM2. Shoot dry weight and total P content were 15 and 14 % depressed by soil acidity respectively (Table 6.1 and 6.2). Root, total and nodule dry weight, nodule number and shoot P concentration were not affected by soil acidity. There was no effect of AMF on growth and P status of cowpea (Table 6.1 and 6.2).

**Table 6.1** Root colonization shoot root and total dry weight of cowpea inoculated with different AMF in acid and non acid soil

| | Root colonization (%) | Shoot dry weight (g/pot) | Root dry weight (g/pot) | Total dry weight (g/pot) |
|---|---|---|---|---|
| | | **pH 5** | | |
| **AM0** | none | 12.1 | 4.4 | 16.53 |
| **AM1** | 10.3 | 11.3 | 4.1 | 15.27 |
| **AM2** | 20.5 | 11.4 | 5.3 | 16.64 |
| | | **pH 6.7** | | |
| **AM0** | none | 13.4 | 5.2 | 18.62 |
| **AM1** | 3.0 | 15.6 | 5.2 | 20.80 |
| **AM2** | 19.9 | 12.4 | 4.3 | 16.67 |
| | | **F-test** | | |
| **AM** | NS | NS | NS | NS |
| **pH** | NS | * | NS | NS |
| ($LSD_{0.05}$) | | (2.0) | | |
| **AMxpH** | NS | NS | NS | NS |

AM = arbuscular mycorrhizal fungi, pH = soil pH, * = significant at $p<0.05$, NS = non-significant different, the number in parenthesis is least significant difference at $P < 0.05$ ($LSD_{0.05}$)

**Table 6.2** Nodule number nodule dry weight shoot P concentration and total P content of cowpea inoculated with different AMF in acid and non-acid soil

| | Nodule number per pot | Nodule dry weight (mg/pot) | Shoot P concentration (%) | Total P content (mg/pot) |
|---|---|---|---|---|
| | | **pH 5** | | |
| **AM0** | 779 | 486 | 0.071 | 13.9 |
| **AM1** | 643 | 530 | 0.083 | 14.5 |
| **AM2** | 571 | 543 | 0.088 | 16.0 |
| | | **pH 6.7** | | |
| **AM0** | 633 | 537 | 0.082 | 17.1 |
| **AM1** | 714 | 535 | 0.092 | 17.9 |
| **AM2** | 654 | 503 | 0.097 | 17.0 |
| | | **F-test** | | |
| **AM** | NS | NS | NS | NS |
| **pH** | NS | NS | NS | * |
| ($LSD_{0.05}$) | | | | (1.6) |
| **AMxpH** | NS | NS | NS | NS |

AM = arbuscular mycorrhizal fungi, pH= soil pH, * = significant at $p<0.05$, NS = non-significant different, the number in parenthesis is least significant difference at $P < 0.05$ ($LSD_{0.05}$)

### 6.3.2 Comparing effects of spore inoculants of two AMF strains on cowpea growth in acid soil with varying available soil P concentration

Root colonization was not found in AM0 treatment. Root colonization percentage in AM1 and AM2 treatment was not different. Root colonization was not affected by P application but it was enhanced by soil acidity (Table 6.3).

**Table 6.3** Effect of soil acidity on root colonization of cowpea inoculated with AM1 and AM2 in 3 P level.

| | P7 | | P11 | | P16 | | mean |
|---|---|---|---|---|---|---|---|
| | AM1 | AM2 | AM1 | AM2 | AM1 | AM2 | |
| pH 5 | 23.9 | 29.9 | 26.3 | 33.4 | 20.5 | 22.8 | 26.1 A |
| pH 6.7 | 19.4 | 11.8 | 13.7 | 16.1 | 16.8 | 14.7 | 15.4 B |
| F-test | $AM^{NS}$ | pH** | $P^{NS}$ | $AMxpH^{NS}$ | $AMxP^{NS}$ | $pHxP^{NS}$ | $AMxpHxP^{NS}$ |

AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = nonsignificant, ** = significant at $P < 0.01$

Soil acidity depressed shoot, root, total, and nodule dry weight for 41, 28, 37 and 28 % respectively (Table 6.4 to 6.7). Phosphorus application increased shoot, roots, total and nodule dry weight (Table 6.4 to 6.7). Interaction between AMF and soil P was found on shoot and total dry weight Table (6.4 and 6.6). In P7 shoot and total dry weight were not affected by AMF. In P11, AM1 increased shoot and total dry weight for 28 and 23% respectively but no effect of AM2 was found. In P16, shoot and total dry weight were increased for 19 and 14% respectively by AM2 but not affected by AM1 (Table 6.4 and 6.6).

**Table 6.4** Shoot dry weight of cowpea inoculated with different AMF in varied P level of acid and non-acid soil

| | pH 5 | pH 6.7 | Mean |
|---|---|---|---|
| | P7 | | |
| AM0 | 2.69 | 3.28 | 2.98 E |
| AM1 | 2.35 | 4.36 | 3.36 E |
| AM2 | 2.10 | 3.74 | 2.92 E |
| mean | 2.38 b | 3.79 a | |
| | P11 | | |
| AM0 | 3.77 | 5.81 | 4.79 D |
| AM1 | 5.20 | 7.08 | 6.14 C |
| AM2 | 3.27 | 5.93 | 4.60 D |
| mean | 4.08 b | 6.27 a | |
| | P16 | | |
| AM0 | 4.84 | 9.74 | 7.29 B |
| AM1 | 5.77 | 9.94 | 7.86 AB |
| AM2 | 6.10 | 11.27 | 8.69 A |
| mean | 5.57 b | 10.32 a | |

| F-test | AM* | pH** | P** | AMxpH$^{NS}$ | AMxP* | pHxP$^{NS}$ | AMxpHxP$^{NS}$ |
|---|---|---|---|---|---|---|---|
| $LSD_{0.05}$ | 0.55 | 0.45 | 0.55 | - | 0.95 | 0.78 | - |

AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = nonsignificant, * = significant different at $p<0.05$ ** = significant at $P < 0.05$

**Table 6.5** Root dry weight of cowpea inoculated with different AMF in varied P level of acid and non-acid soil

| | pH 5 | pH 6.7 | Mean |
|---|---|---|---|
| | P7 | | |
| AM0 | 1.88 | 2.30 | 2.09 |
| AM1 | 1.69 | 2.31 | 2.00 |
| AM2 | 1.64 | 2.27 | 1.96 |
| mean | 1.74 | 2.29 | |
| | P11 | | |
| AM0 | 2.03 | 2.64 | 2.34 |
| AM1 | 2.82 | 3.32 | 3.07 |
| AM2 | 2.00 | 3.18 | 2.59 |
| mean | 2.28 | 3.05 | |
| | P16 | | |
| AM0 | 2.45 | 3.84 | 3.15 |
| AM1 | 2.76 | 3.78 | 3.27 |
| AM2 | 2.80 | 4.24 | 3.52 |
| mean | 2.67 b | 3.95 a | |

| F-test | $AM^{NS}$ | $pH^{**}$ | P** | $AMxpH^{NS}$ | $AMxP^{NS}$ | $pHxP^{NS}$ | $AMxpHxP^{NS}$ |
|---|---|---|---|---|---|---|---|
| $LSD_{0.05}$ | - | 0.25 | 0.30 | - | - | - | - |

AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = nonsignificant, ** = significant at $P < 0.01$

**Table 6.6** Total dry weight (g/pot) of cowpea inoculated with different AMF in varied P level of acid and non-acid soil

| | pH 5 | pH 6.7 | Mean |
|---|---|---|---|
| | P7 | | |
| AM0 | 4.57 | 5.58 | 5.07 E |
| AM1 | 4.03 | 6.67 | 5.35 E |
| AM2 | 3.74 | 6.01 | 4.88 E |
| mean | 4.11 b | 6.09 a | |
| | P11 | | |
| AM0 | 5.80 | 8.45 | 7.12 D |
| AM1 | 8.02 | 10.40 | 9.21 C |
| AM2 | 5.28 | 9.11 | 7.19 D |
| mean | 6.36 b | 9.32 a | |
| | P16 | | |
| AM0 | 7.29 | 13.58 | 10.44 BC |
| AM1 | 8.54 | 13.72 | 11.13 AB |
| AM2 | 8.91 | 15.51 | 12.21 A |
| mean | 8.24 b | 14.27 a | |

| F-test | AM* | pH** | P** | AMxpH$^{NS}$ | AMxP* | pHxP** | AMxpHxP$^{NS}$ |
|---|---|---|---|---|---|---|---|
| $LSD_{0.05}$ | 0.79 | 0.64 | 0.79 | - | 1.36 | 1.11 | - |

AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = not significant at $P < 0.05$, * = significantly different at $P < 0.05$, ** significantly different at $P < 0.01$

**Table 6.7** Nodule dry weight (mg/pot) of cowpea inoculated with different AMF in varied P level of acid and non-acid soil

| | pH 5 | pH 6.7 | Mean |
|---|---|---|---|
| | P7 | | |
| AM0 | 162 | 217 | 190 |
| AM1 | 166 | 222 | 194 |
| AM2 | 155 | 226 | 191 |
| mean | 161 | 222 | |
| | P11 | | |
| AM0 | 199 | 278 | 239 |
| AM1 | 237 | 317 | 277 |
| AM2 | 203 | 276 | 239 |
| mean | 213 | 290 | |
| | P16 | | |
| AM0 | 242 | 421 | 332 |
| AM1 | 313 | 473 | 393 |
| AM2 | 392 | 426 | 409 |
| mean | 316 | 440 | |

| F-test | $AM^{NS}$ | $pH^{**}$ | P** | $AMxpH^{NS}$ | $AMxP^{NS}$ | $pHxP^{NS}$ | $AMxpHxP^{NS}$ |
|---|---|---|---|---|---|---|---|
| $LSD_{0.05}$ | - | 29.6 | 36.2 | - | - | - | - |

AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = not significant at $P < 0.05$, * = significantly different at $P < 0.05$, ** = significantly different at $P < 0.01$

### 6.3.3 Effect of spore inoculation with 2 AMF strains on mimosa

For the mimosa experiment, effects of soil acidity were seen at 72 days after sowing (Figure 6.2). At 91 days after sowing 12% root colonization was found in AM0 in non-acid soil but in acid soil the contamination was not found. The root colonization with AM1 and AM2 were not different and not affected by soil pH. The root colonization in inoculated treatment ranged between 33 to 61% (Table 6.8). Shoot dry weight was increased equally by AM1 and AM2, by some 380%, with soil acidity having no effect on shoot weight (Table 6.9).

**Table 6.8** Root colonization (%) of mimosa inoculated with different AMF species in acid and non-acid soil at 13 weeks after sowing.

| | pH5 | pH6.7 | mean |
|---|---|---|---|
| AM0 | 0.0 | 12.4 | 6.18 B |
| AM1 | 41.3 | 60.8 | 51.05 A |
| AM2 | 40.8 | 33.4 | 37.08 A |
| F-test | AM** | $pH^{NS}$ | $AMxpH^{NS}$ |

AM = AMF inoculation, pH growth medium pH, ** = significantly different at $p<0.01$, NS = not significant at $P < 0.05$

**Table 6.9** Shoot dry weight (mg/plant) of mimosa inoculated with different AMF species in acid and non-acid soil at 13 weeks after sowing.

| | pH5 | pH6.7 | mean |
|---|---|---|---|
| AM0 | 53.7 | 62.9 | 58.3B |
| AM1 | 246.7 | 333.2 | 290.0A |
| AM2 | 312.3 | 247.4 | 279.9A |
| F-test | AM** | $pH^{NS}$ | $AMxpH^{NS}$ |
| $LSD_{0.05}$ | 94.3 | - | - |

AM = AMF inoculation, pH growth medium pH, ** = significant at $P < 0.01$, NS = not significant at $P < 0.05$

A


B

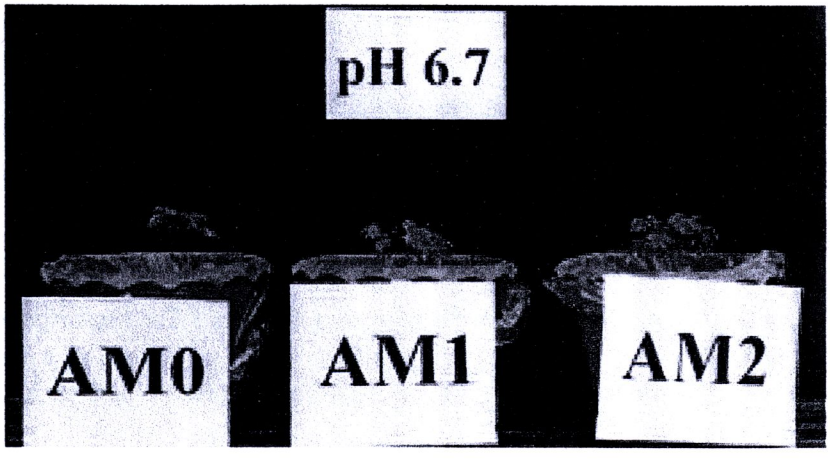



**Figure 6.2** Growth of mimosa inoculated with different AMF in acid (A) and non-acid (B) soil

**6.4 Discussion**

In experiment 6.2.1 there was a slight effect of soil acidity, with 15% shoot weight depression (Table 6.1). Comparing with the result of experiment 4.2.3 at 33 kg P/ha (same P application rate as this experiment) soil acidity depressed shoot weight for 60% and all growth parameters were depressed by soil acidity (Table 4.10). But in experiment 6.2.1 all growth parameters except shoot weight were not affected by soil acidity (Table 6.1). The low root colonization percentage and no effect of AMF was found although the AMF were isolated from the effective inoculum in chapter 5. The hypothesis is "high soil P concentration eliminated effect of soil acidity and AMF on cowpea growth". High rate of P application can alleviate acid soil stress (Haynes and Ludecke, 1981). When P status of host plant was high the benefit of AMF was diminished (Table 5.2 and 5.6). Although the P application rate in experiment 6.2.1 was same as the chapter 5 but it can not guarantee to provide the same soil P concentration. In the experiment 4.2.3, applying 33 kg P/ha provided soil P concentration 10.8 and 11.7 mg P/kg in acid and non acid soil respectively (Table 4.9). But in experiment 6.2.1 applying the same rate of P provided 21.2 and 22.0 mg P/kg in acid and non acid soil respectively. This high soil P made P status in cowpea was in sufficient level. Aziz and Habt (1989) reported that 0.062 % P in shoot is sufficient for cowpea growth at 52 days after sowing. From Table 6.2 shoot P in all treatments was in the sufficient rang. The sufficiency of P in plant was the cause of no response to mycorrhiza (Marschner, 1995). There is a doubt that "why the suitable P rate for testing effect of AMF and acid soil in chapter 5 was too high for experiment 6.2.1". The different soil property between soil lots may be the cause of this problem. Although soils used in all experiments were collected from the same soil series

(Sansai soil) and from the same area and had similar soil P concentration but soil property might be different. Normally soil from the same series has similar property but not exactly same. In chapter 4, 5 and experiment 6.2.1 P level was fixed by P application rate but the application rate was proved it was unsuitable criteria. Soil Phosphorus concentration (Bray II) was used as the criteria in experiment 6.2.2 because it related to available soil P for plant.

The result in experiment 6.2.2 suggested that the P levels are suitable to evaluate effect of soil acidity. The adverse effect of acid soil was recovered. The soil acidity distinctly depressed cowpea growth in all P levels. Using soil P concentration as criteria was better than P application rate. The 2 AMF isolate enhance plant growth in different level of P. The AM1 was effective in low P soil (P11) but AM2 was effective in moderately low P soil (P16). In the extremely low P soil (P7) both of them were un-effective. But the Root colonization and benefit of the fungi in this experiment were less than the experiment in chapter 5. In chapter 5 root colonization ranged from 40 to 57% (Table 5.1) and shoot weight was 73% increased by AMF in acid soil (Table 5.2). But the root colonization in experiment 6.2.2 was between 11% and 30% and shoot weight was just 28% increased by the fungi (Table 6.8 and 6.9). Effect of AMF to make cowpea more tolerance to acid soil disappeared. The soil acidity depress biomass yield in all AM0, AM1 and AM2 treatments (Table 6.6) while in chapter 5 biomass yield of inoculated cowpea was not affected by soil acidity (Table 5.4). The inoculums used in experiment 6.2.2 were single species spores while in chapter 5 was mix species soil inoculum. The diversity of AMF species in the soil inoculum might be the advantage point of the soil inoculum (Bethlenfalvay, 1992). The isolate species used in this experiment may not the most effective species. But

these isolates successfully colonized mimosa root. Forty-one percents root colonization was found in mimosa root at 91 days after sowing and the effect on mimosa growth had been found since 72 days (Table 6.8 and Figure 6.2). This confirming these AMF isolate were highly effective to enhance mimosa growth in acid soil. But the question is why they are less effective in cowpea. The mimosas in experiment 6.2.3 were harvest at 91 days after sowing but cowpeas in 6.2.2 were harvested at 52 days. Mycorrhiza fungi had more time to colonize mimosa root had develop association with mimosa than cowpea. But in chapter 5 cowpeas growth were distinctly increased by AMF at 45 days after sowing. The different result between 2 experiment may caused by the different inoculation method. In chapter 5, 50 g soil inoculum (containing 1,250 AMF spore) was put under 5 cowpea seeds at sowing but in experiment 6.2.2 1,000 AMF spores laying on filter paper was put under 5 cowpea seeds. In experiment 6.2.2 the filter paper asked like a barrier preventing spores to distribute in soil profile. Therefore spore in soil inoculum in chapter 5 had more chance to colonize cowpea root (Figure 6.3). Moreover the soil inoculum has not only spore as an inoculation unit but also AMF hyphe and infected root fragment in soil can ask like infection unit (Brundrett *et al.* 1996). Therefore the effective of different inoculum types was evaluated in Chapter 6.

The effectiveness of 2 AMF isolate depended on P level. In extremely low P soil (P7) both AMF isolates were not effective to improve cowpea growth. In low P (P11) AM1 was effective species but In moderately low P (P16) the effective specie was switched to AM2.

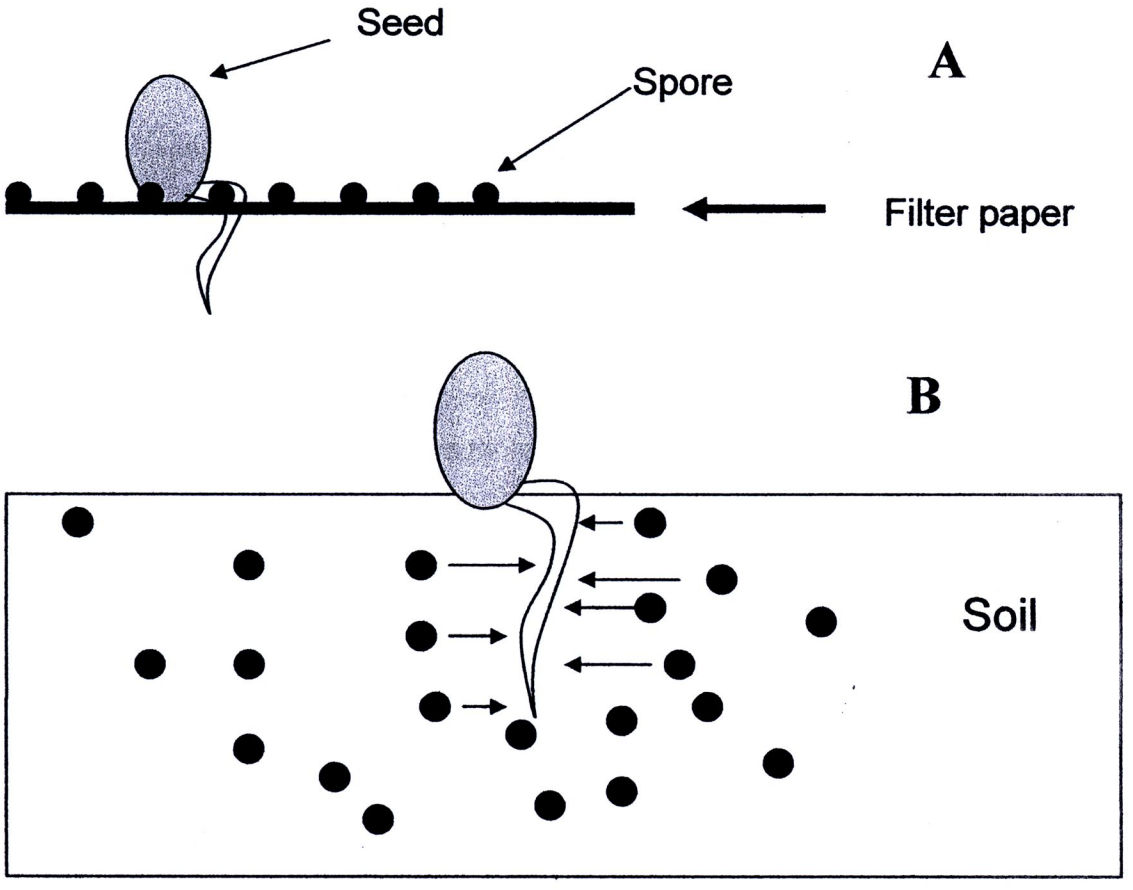



**Figure 6.3** The different of inoculation method in chapter 6 (A) and chapter 5 (B)