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## Effect of some soil chemical properties on the occurrence of arbuscular mycorrhizal fungi in soils low pH growing rice plant in the Mekong Delta, Vietnam

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**Abstract** The results showed that about 7 – 64% of the rice roots were infected by AMF community. The infection structure included 3 main types of hyphae, arbuscules and vesicles. The density of AMF spores presented in the soil samples ranged from 500 to 1635 spores/100g of dry soil. The percentage of the AMF colonization had a positive correlation with soil pH value ( $r=0.72^*$ ), a negative correlation with electric conductivity EC ( $r=-0.87^*$ ), with organic matter content ( $r=-0.77^*$ ), with total phosphorous content ( $r=-0.71^*$ ) and positive correlation with the number of AMF spores ( $r=0.71^*$ ). The research illustrated the symbiosis system between AMF and rice roots and the correlation between the occurrence of AMF and soil pH, EC, organic matter content as well as the total phosphorous content in paddy fields under the soil low pH.

**Keywords:** AMF spores, Arbuscular mycorrhizal fungi, Low-pH paddy soils, Percentage of AMF colonization

### Introduction

In the Mekong delta region of Vietnam, acid sulfate soils occupy approximately 1.6 million hectares mainly concentrated in the areas such as Long Xuyen Quadrangle and the depression of Hau River. Acid sulfate soils with low pH and high toxicity of Al and Fe are the obstacles to rice cultivation. Therefore, several studies have proposed many physical and chemical methods to improve rice cultivation on acid sulfate soils such as irrigation water, liming the soil, and using chemical fertilizers. Along with the recent science and

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technology development, the use of biological products and microbial organic fertilizers in agricultural production is essential. Arbuscular mycorrhizal fungi (AMF) are among the beneficial symbiotic microorganisms that help the growth and development of plants grown under the disadvantageous soil condition.

Arbuscular mycorrhizal fungi form symbiotic relationships with about 280000 of terrestrial plant species (Brundrett, 2009). Many studies showed that AMF can increase the growth of terrestrial plants by increasing uptake of phosphorus and water content (Smith and Read, 2008), reducing the poisonous plants in adverse environmental conditions. However, the soil properties are significantly affected the AMF colonization rates and spore density. High concentrations of nitrogen (N) or phosphorus (P) in the soil can weaken the symbiotic relationship between plants and AMF, significantly reduce the penetration rate of AMF and inhibit spore production. However, moderate P supplementation increased spore production significantly (Lin *et al.*, 2020).

However, the studies on the presence of arbuscular mycorrhizal fungi (AMF) on rice grown in acid sulfate soils are very limited, especially in the acidic soil in the Mekong delta. This study was aimed to investigate some chemical parameters on acid sulfate soils as indicators affecting the occurrence and colonization of AMF in paddy soils and rice roots under the acidic soil condition.

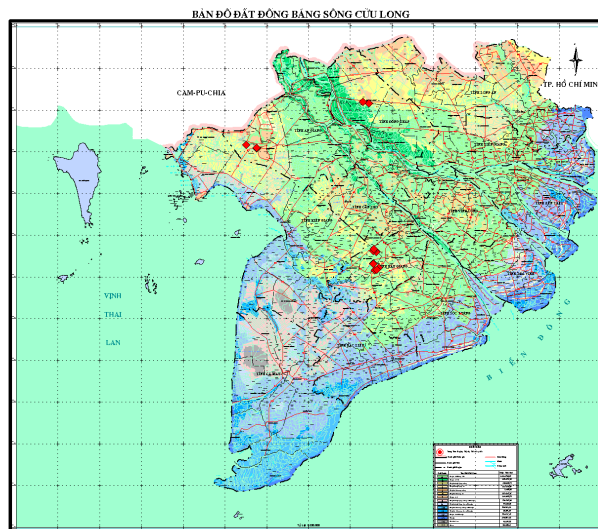
## **Materials and methods**

### ***Materials***

Collection of samples: Twenty nine samples of rice roots and 29 samples of the low pH paddy soil were collected when the rice plants were about 55-65 days old. These samples were taken at An Giang, Dong Thap and Hau Giang provinces during the summer- autumn season. Sample information was showed in Figure 1. The selected fields for sampling were disease-free in the previous crop as well as during the cultivation period, without fertilizer and pesticides application at least 14 days before sampling. At each field, 15 samples of soils (0- 15cm of depth) and 15 samples of rice roots was randomly collected. Soil and root samples were pooled for each type and represented for the field. The samples were kept cool and transported to the laboratory.

### ***Preparation of samples***

Rice root samples were thoroughly washed, removed old roots, roots with abnormal color and roots showing signs of infection. The selected young roots were cut 1 cm length and used for staining. Soil samples were homogenized and removed plant residues and air-dry at room temperature. The soil samples were then ground through a 2 mm sieve used for determined the number of spores and soil pH and EC and 0.5 mm sieve for analyses of total before use.



**Figure 1.** The location of sampling materials at An Giang, Dong Thap, and Hau Giang provinces

***Mycorrhizal colonization in the rice root samples***

Two grams of processed root samples were stained with trypan blue in lactic acid (0.05%) following to the process of INVAM (<http://invam.wvu.edu>) (Xuan *et al.*, 2016) to assess the presence of AM infection in the rice root samples under electron microscope with 40x magnification. The percentage of root colonization was determined according to Lakshman (2014).

***The presence of arbuscular mycorrhizal spore in paddy soils***

One hundred grams of dried soil samples were wet-sieved which used to determine the number of AMF spores following to Gerdemann and Nicolson method (1963). The number of spores was determined by counting directly on the cellulose nirate membrane filters with a diameter of 47mm and a spore size of 45 μm (Whatman, Japan). The morphology of spores was observed under

stereomicroscopy (Carton MS 4573 DSZT-44FT, Japan) and optical microscope (Nikon eclipse E100, USA).

### ***Analysis of soil chemical properties***

Soil samples were measured  $\text{pH}_{\text{H}_2\text{O}}$  and EC (soil: water is 1:2.5), total nitrogen was quantified by using the Kjeldahl method (Kjeldahl, 1883), total phosphorus (P<sub>tot</sub>) was analyzed according to the method of Bray II, ammonium content ( $\text{NH}_4^+$ -N) was extracted with KCl 2M solutions with a soil: solution ratio of 1:10 and determined on a colorimeter at 650 nm, the available phosphorus (P<sub>avail</sub>) content determined following the procedures of Murphy and Riley (1962). The organic carbon content in the soil was determined by the method of Walkley and Black (1934). The C/N ratio was calculated as a fraction of the total organic carbon content and the total nitrogen (N) content.

### ***Data analysis***

Analysis of variance (ANOVA) and comparison of the correlation between the percentage of root colonization and the number of AMF spores with the soil chemical properties by using correlation models and univariate regression of SPSS software (version 22.0).

## **Results**

### ***Presence of arbuscular mycorrhizal fungi in paddy soils***

The AMF colonization: The percentage of AMF colonization in the root samples ranged from 7 - 64%. The density of spores was about 575 to 1635 spores/100g of dry soil. The density of spores tended to increase when the infection rate got greater (Table 1).

### ***Evaluation of the correlation between the occurrence of AMF and the soil chemical properties***

The results of evaluating the correlation between the colonization of AMF and soil chemical properties indicated that the percentage of AMF colonization was correlated with the pH, EC, soil organic matter (%C), total P, and the density of AMF spores in paddy soils (Table 2). There was no

correlation of the AMF colonization with either available phosphorus or ammonium concentration (Table 2).

**Table 1.** The summary of the occurrence of arbuscular mycorrhizal fungi in different chemical properties of paddy soils

| Sample      | Occurrence of AMF in paddy soils |  | Some chemical properties of soil samples |            |      |                  |   |   |
|-------------|----------------------------------|--|--|------------|------|------------------|---|---|
|             | Percent of colonization (%)      | Density of spores (spore/100g of dry soil) | pH <sub>H</sub> <sub>20</sub>            | EC (μS/cm) | %C   | Pavail. (mgP/kg) | NH <sub>4</sub> <sup>+</sup> -N (mg/kg) | Ptot. (%P <sub>2</sub> O <sub>5</sub> ) |
| <b>DT1</b>  | 38                               | 1200                                       | 4.8                                      | 646        | 6.43 | 51.97            | 42.32                                   | 0.12                                    |
| <b>DT2</b>  | 43                               | 1295                                       | 5.2                                      | 246        | 3.81 | 40.17            | 20.61                                   | 0.09                                    |
| <b>DT3</b>  | 53                               | 1045                                       | 4.9                                      | 299        | 3.76 | 22.49            | 23.62                                   | 0.08                                    |
| <b>DT4</b>  | 48                               | 1055                                       | 5.0                                      | 268        | 3.52 | 39.16            | 20.27                                   | 0.08                                    |
| <b>DT5</b>  | 50                               | 1335                                       | 5.0                                      | 348        | 3.52 | 29.08            | 19.23                                   | 0.07                                    |
| <b>DT6</b>  | 64                               | 1465                                       | 4.9                                      | 317        | 3.52 | 28.94            | 27.44                                   | 0.09                                    |
| <b>DT7</b>  | 43                               | 1160                                       | 5.2                                      | 236        | 3.69 | 88.73            | 20.97                                   | 0.08                                    |
| <b>AG1</b>  | 42                               | 1280                                       | 4.4                                      | 710        | 5.28 | 20.86            | 57.92                                   | 0.08                                    |
| <b>AG2</b>  | 46                               | 1465                                       | 4.8                                      | 493        | 5.48 | 23.41            | 15.12                                   | 0.09                                    |
| <b>AG3</b>  | 48                               | 1590                                       | 4.4                                      | 541        | 3.67 | 19.02            | 124.2                                   | 0.05                                    |
| <b>AG4</b>  | 54                               | 1535                                       | 4.9                                      | 380        | 5.53 | 34.72            | 63.43                                   | 0.09                                    |
| <b>AG5</b>  | 33                               | 825  | 3.8                                      | 445        | 7.48 | 2.26             | 21.50                                   | 0.08                                    |
| <b>AG6</b>  | 31                               | 575  | 4.0                                      | 555        | 4.89 | 0.85             | 62.16                                   | 0.12                                    |
| <b>AG7</b>  | 48                               | 820  | 4.4                                      | 387        | 5.55 | 1.93             | 27.09                                   | 0.08                                    |
| <b>AG8</b>  | 37                               | 1030                                       | 4.4                                      | 456        | 8.27 | 10.25            | 9.71                                    | 0.12                                    |
| <b>HG1</b>  | 47                               | 840  | 4.8                                      | 203        | 3.16 | 5.96             | 19.45                                   | 0.09                                    |
| <b>HG2</b>  | 46                               | 825  | 4.8                                      | 180        | 3.23 | 5.80             | 22.01                                   | 0.11                                    |
| <b>HG3</b>  | 47                               | 840  | 4.7                                      | 299        | 3.99 | 9.11             | 31.67                                   | 0.15                                    |
| <b>HG4</b>  | 47                               | 915  | 4.7                                      | 519        | 4.63 | 24.94            | 30.40                                   | 0.10                                    |
| <b>HG5</b>  | 44                               | 1635                                       | 4.5                                      | 944        | 4.14 | 21.92            | 30.53                                   | 0.09                                    |
| <b>HG6</b>  | 34                               | 805  | 4.4                                      | 744        | 5.17 | 18.44            | 35.72                                   | 0.12                                    |
| <b>HG7</b>  | 39                               | 1250                                       | 4.5                                      | 652        | 2.56 | 9.06             | 30.40                                   | 0.11                                    |
| <b>HG8</b>  | 7                                | 600  | 3.9                                      | 2300       | 9.38 | 8.09             | 31.85                                   | 0.16                                    |
| <b>HG9</b>  | 11                               | 610  | 4.2                                      | 1969       | 8.38 | 16.68            | 37.07                                   | 0.22                                    |
| <b>HG10</b> | 9                                | 620  | 4.2                                      | 1543       | 7.48 | 14.66            | 26.50                                   | 0.17                                    |
| <b>HG11</b> | 11                               | 500  | 4.1                                      | 1731       | 7.98 | 10.39            | 35.57                                   | 0.15                                    |
| <b>HG12</b> | 43                               | 910  | 4.3                                      | 1082       | 5.45 | 17.13            | 30.34                                   | 0.11                                    |
| <b>HG14</b> | 25                               | 640  | 4.1                                      | 1452       | 5.86 | 21.87            | 35.52                                   | 0.11                                    |
| <b>HG15</b> | 50                               | 1340                                       | 4.7                                      | 492        | 3.33 | 10.82            | 31.50                                   | 0.16                                    |

**Table 2.** The correlation between the AMF colonization and some soil chemical properties as well as the density of AMF spores

|                  | Correlation coefficients (r) |        |        |         |        |                                 |            |
|------------------|------------------------------|--------|--------|---------|--------|---------------------------------|------------|
|                  | pH                           | EC     | %C     | Pavail. | Ptot.  | NH <sub>4</sub> <sup>+</sup> -N | AMF spores |
| AMF colonization | 0,72*                        | -0,87* | -0,77* | 0,24    | -0,71* | 0,02                            | 0,708*     |

Note: \* significance at the level of 5%

### *Correlation of AMF colonization with some soil chemical properties*

The pH and EC value: The AMF colonization rate was positively correlated with the pH value ( $y = 27.06x - 83.804$ ;  $r = 0.72^*$ ) (Figure 3a). The AMF colonization in rice root samples increased when the soil pH value increased and fluctuated in the range of pH 3.8 - 5.2. The EC value of soil samples was about 154- 2300  $\mu\text{S}/\text{cm}$ . The AMF colonization was negatively correlated with the EC value in the soil samples ( $y = -0.0221x + 54.677$ ;  $r = -0.87^*$ ) (Figure 3b). The AMF colonization decreased when the EC value in the soil increased.

The total phosphorus in paddy soils: The concentration of total phosphorus in soil samples fluctuated from 0.07- 0.22% of  $\text{P}_2\text{O}_5$ . The AMF colonization in the rice root samples exhibited a negative correlation with the total phosphorus contents in the soil ( $y = -285.26x + 70.163$ ;  $r = -0.71^*$ ) (Figure 3c). The AMF colonization increased when the total phosphorus content in the soil was low, so mycorrhizal expansion in the soil could absorb more phosphorus and enhance metabolism inside the roots, thereby helping to provide soluble phosphorus for plant growth. However, there was no correlation between the AMF colonization and the dissolved phosphorus (Figure 3d).

The organic matter in the soil: The results demonstrated that the AMF colonization was negatively correlated with the organic matter content in the soil ( $y = -6.01x + 69.96$ ;  $r = -0.77^*$ ) (Figure 4a). The AMF colonization gradually decreased as the organic matter content in the soil increased.

### *The correlation of AMF colonization and the number of AMF spores*

There was a positive correlation between the AMF colonization and the number of AMF spores ( $y = 0.0299x + 8.3999$ ;  $r = 0.708^*$ ) (Figure 4b). The higher the percentage of the AMF colonization was, the greater the density of spores was formed.

## Discussion

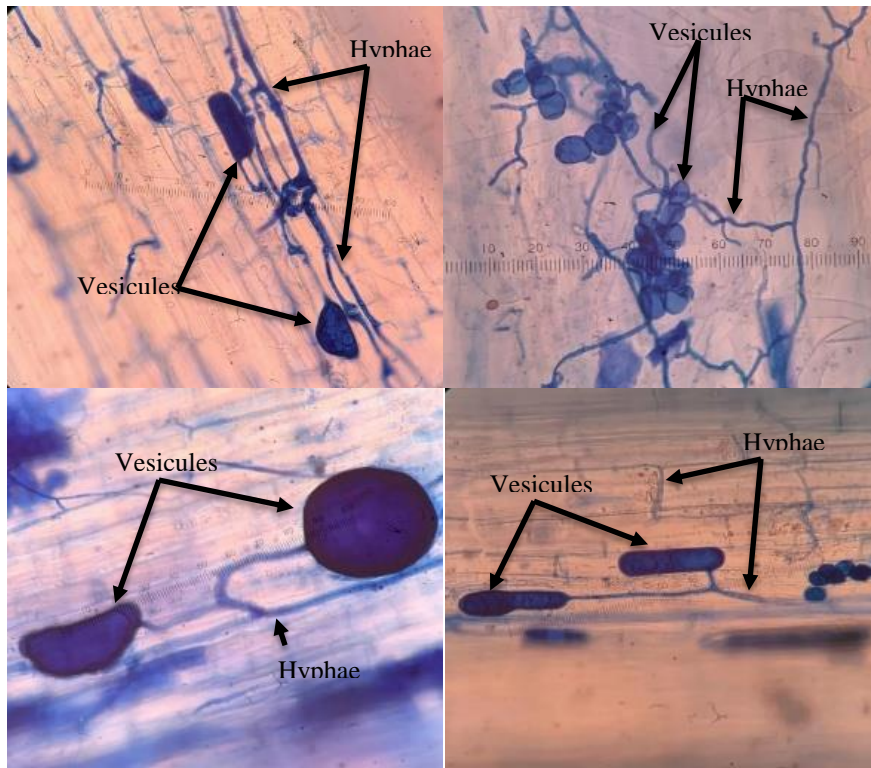
Arbuscular mycorrhizal fungi are considered as functional indicators in agriculture because of their beneficial roles for plant health. Although it has been believed that AMF could not colonize the rice root, the recent studies have positive signals in demonstrating the occurrence of AMF in paddy soils (Sharma *et al.*, 1988; Watanarojanaporn *et al.*, 2013; Wang *et al.*, 2015; Mitra *et al.*, 2021). In this study it was illustrated that the AMF were present in paddy fields with a level of soils low pH. Depending on the practical cultivation, the occurrence of AMF of each the sample was also affected. The gaps in a rate of different position may be explained by the variation of AMF, the soil nutrients, the soil profile as well as application of chemical agriculture. Those factors were proved to have effects on AMF colonization (Gosling *et al.*, 2006; Lumini *et al.*, 2011; Barber *et al.*, 2013).

This result could be prompted to be the pioneer for further study about roles of AMF in paddy soil. Although AMF is obligatory aerobic microorganism in nature, their population can also co-exist with rice root in anaerobic condition based on the ability to observe O<sub>2</sub> through the aerenchyma of rice plant (Watanarojanaporn *et al.*, 2013).

The percentage of AMF colonization in this research had similar results to those of Olubodea *et al.* (2020) who studied about the AMF colonization of rice roots grown in Southwest Nigeria, of which the rate was about 33.6% - 76.2% while the percentage of AMF colonization in the study of Bernaola *et al.* (2018) in the southern of America showed that the AMF colonization was about 1,8% - 61,4%. Under the soil low pH, the colonization of AMF in rice root was also higher than that of winter crop in southern Switzerland with the same culture conditions as well as in the Italian rice field (Mäder *et al.*, 2000; Vallino *et al.*, 2009; Lumini *et al.*, 2011).

During the survey, the AMF colonization in rice roots were formed with three types of colonization of hyphal, vesicular and arbuscular structure. The result was in line with studies of Chen *et al.* (2017) and Bernaola *et al.* (2018). Sarkodee-Addo *et al.* (2020) also detected three different structures of AMF on rice root of which the most common one is hyphae structure following up by arbuscular and vesicular one. The hyphae structure was a common form of AMF that appeared in all samples. On the other hand, the vesicular structure often observed at the high colonization samples. The infection structure helped the roots system expand into bulk soils and enhanced the abilities to uptake water, nitrogen, phosphorous and some trace elements of lead and zinc. Those abilities may help the farmer to save fertilizer. Furthermore, the vesicular structure can also store salt and heavy metals, they may help plants in metal detoxification in adverse conditions (Xuan *et al.*, 2016). The research have

observed the arbuscular structure because of the rare appearance and the degenerated tendency in two weeks after root colonization (Parniske, 2008).



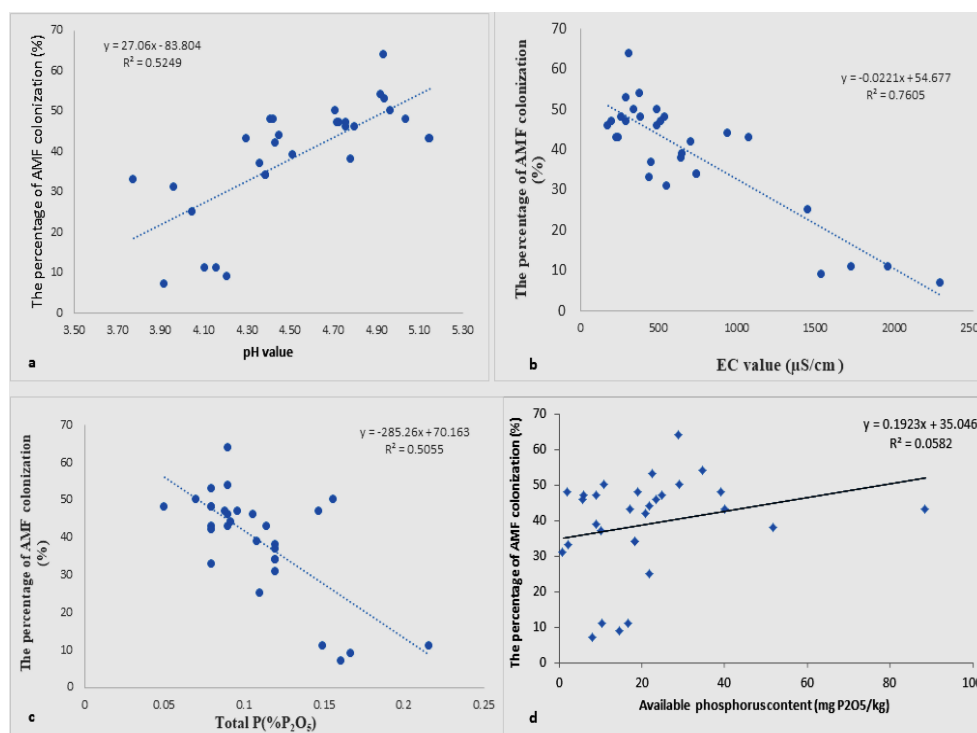
**Figure 2.** Some infection structure of AMF in rice root under 40x magnification

The density of AMF spores in low pH paddy soils tended to have a higher number of spores than that of paddy soils in the northern part of Vietnam (Hang *et al.*, 2012) with a level of 500- 1635 spore/100g of dry soil. Rickerl *et al.* (1994) found twice more spores in wet than in drier soil and they suggested that higher sporulation in wet soil was a stress response of the fungi to the wet condition. This result was also similar to that of studies of Escudero and Mendoza (2005); Mendoza *et al.*, (2005). This is in agreement with Chen *et al.* (2017) who showed that the highest population observed in south-eastern China was  $93 \pm 6$  spores/100 g of dry soil with the pH was 5.3. While the result of Olubodea *et al.* (2020) in south-western Nigeria showed that the spores population in rhizosphere was about 13-174 spores/20g dry soil.

In agricultural practice, soil pH value is one of factors which affect on plant growth, soil nutrient availability and soil microbes. The result in this

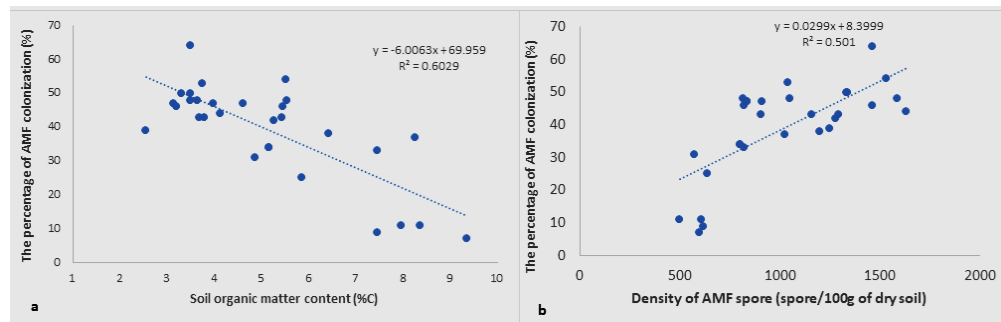


study showed that soil pH affected positively on the percentage of AMF colonization in rice roots. This investigation was consistent with the research experiment of Medeiros *et al.* (1994) concluding that, on sorghum (sorghum), the colonization rate of *Glomus* spp. increased as the pH value gradually increased in the range of 4.0 - 7.0, the colonization rate was low at pH 4.0 and high at pH 5.0; 6.0; 7.0. However, Fusconi and Mucciarelli (2018) found that the positive correlation of AMF colonization with soil pH only occurred on the dicot species when soil pH increased from 7 to 8 under a wetland condition.



**Figure 3.** The correlation between the orurence of AMF and some soil chemical properties

On the contrary of soil pH, the EC value in soil had a negative effect on the AMF colonization in this study. The colonization rate of AMF in rice root was greater when the EC value belowed 1000  $\mu\text{S}/\text{cm}$ . This result was supported by the conclusion of Juniper and Abbott (1993) on the influence of salt concentration in soil on the growth of AMF. The EC value was evaluated by the soluble salt content present in the soil solution, on high EC alkaline soils due to the presence of  $\text{H}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ , and  $\text{SO}_4^{2-}$  ions. With hydrolysis of Al, it releases  $\text{H}^+$  ion, which increases the acidity of the soil, leading to a decrease in soil pH. Therefore, the AMF colonization tends to decrease.



**Figure 4.** The correlation between the AMF colonization and the EC value of the soil

The study found that the soil organic matter (%C) and total phosphorus ( $P_{tot.}$ ) in soils had negative correlation with the percentage of AMF colonization. Møller *et al.* (2013) presented that addition of organic matter to the submerged soil induced sediment anoxia and reduced root growth as a result of reducing the AMF colonization but caused no apparent plant stress. It is believed that the organic matter content in soil low pH tend to be higher than that in alluvial soils. This may cause some disadvantages of the AMF colonization in rice plant.

In the acid sulphate soils extractable aluminum and ferrous ions are main factors affecting rice growth and reducing rice root length consequently reducing rice yield (Huang *et al.* 2016; Onaga *et al.* 2016). In addition, insufficient phosphorus (P) for rice has been observed in the soil low pH level due to the immobilized P that has precipitated with free aluminum and iron ions to form aluminum phosphate ( $AlPO_4$ ) and iron phosphate ( $FePO_4$ ) (Margenot *et al.* 2017). These forms of P are poorly available to plants (Rengel and Marschner 2005). The AMF could help plant uptake P in soil. However, the AMF colonization decreased when the total phosphorus content was high. This means soil phosphorus contents had significantly direct influences on plant and phosphorus contents and AMF infection characteristics. In this study, it was found that the percentage of the AMF colonization was negatively affected by the total phosphorus in paddy soils under the soil low pH level. This result is supported by the study of Abbott *et al.* (1984).

### ***The number of AMF spores in the soil***

The AMF colonization and the number of AMF spores showed positive correlation. This means that the sporulation of AMF depended on the AMF colonization in the paddy soils. In addition, Garc ía and Mendoza (2009); Stevens *et al.* (2010) showed that AMF spores in the submerged soil may

survive for a long period and they may accumulate in paddy soil therefore the density of AMF spores could increase when the percentage of the AMF conlonization increased in the paddy soils.

The result showed that some chemical properties of soil low pH levels have effects on the occurrence and colonization of AMF in paddy soils in the Mekong delta region. The colonization of AMF affected positively on pH value, organic matter content and affected negatively on EC, total phosphorous and density of AMF spores in paddy soils. The results of this investigation will prompt further studies aimed at assessing roles of AMF as growth enhancers especially in soil low pH cultivating rice plants.

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