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Isolation and characterisation of Arbuscular mycorrhizal (AM) fungi spores from selected plant roots and their rhizosphere soil environment

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ABSTRACT

Aims: Arbuscular mycorrhizal (AM) fungi or previously known as the vesicular-arbuscular mycorrhizal (VAM) fungi, is a type of endomycorrhiza that closely associates with most species of plants. Meanwhile, they significantly improve the nutrients uptake in exchange of photosynthates and decrease the stress caused by both biotic and abiotic factors through symbiosis relationship. However, the understanding of indigenous AM fungi species present in its host plants are comparatively inadequate, hence this research study concentrated on indigenous AM fungi population in some selected plants that contribute to agricultural sector in Malaysia and phytochemical properties of soil that affect the colonization rate of AM fungi.
Methodology and results: Bamboo, banana, coconut, sugarcane, papaya, lemongrass, pandan and tapioca plant were selected in this study. The soil and plant roots were sampled and the fungi spores were extracted by applying Wet sieves and decantation techniques then further purified by sucrose density centrifugation. Genera Glomus, Funneliformis, Rhizophagus, Acaulospora and Dentiscutata were isolated and Glomus was determined as the dominant genera followed by Acaulospora in these selected plants. Soil pH were found to be significantly affecting the AM fungi population and the root colonization percentage of AM fungi in the plants analysed.
Conclusion, significance and impact of study: From this study, tapioca recorded the highest percentage of AM fungi root colonization rate with 20.00% in root while banana recorded the lowest rate of 3.33% only. Based on this study, tapioca is recommended for the propagation of AM fungi for biofertilizer usage in agricultural sector in future.

Keywords: Arbuscular mycorrhizal fungi, *Glomus*, *Acaulospora*, root colonization

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are a type of obligate symbiotic fungi belongs to the phylum Glomeromycota and are known as one of the recurring endomycorrhizae around the world (Sharma and Yadav, 2013). The AM fungi are fundamental in soil microbiota and they colonised more than 80% of the terrestrial plants in the natural ecosystem (Hildebrandt *et al.,* 2002; Burni *et al.,* 2011) ranging from sub-polar to tropical latitudes as well as the swampy area in low lands to hill grass of high altitude (Read, 1991). The symbiotic relationship developed between AM fungi and terrestrial plants could be traced back 400 million years ago in *Aglaophyton major* and the spores resembled *Scutellospora* and *Acaulospora* species were well preserved in plant interior (Remy *et al.,* 1994; Dotzler *et al.,* 2009). From this fossil records, it suggested that AM fungi had a vital function in most of the founding of natural terrestrial ecosystem.

Plant growth requires large amount of nutrients and water uptake from the soil throughout the stages. However, they do not interact directly with the soil environment; instead they interact indirectly through the AM fungi (Smith and Read, 2008). The AM fungi are comprised of three major components which involved (1) the roots itself that supply carbon in the pattern of sugars to the fungi; (2) development of arbuscules by repeated dichotomous branching within cortical cells of roots that connect between plant cytoplasm and the fungi and (3) the structure of extraradical hyphae which help in nutrients uptake (Bago *et al.,* 1998; Smith and Read, 2008; Aggangan *et al.,* 2011). These components of AM fungi form close associations with the host plants which significantly improve the mineral nutrients uptake and decrease abiotic and biotic stress. Hence, there is a huge potentials of AM fungi to serve as biofertilizer in the agricultural sector.

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There are dozens of benefits by utilizing AM fungi in agricultural sector. According to research done by Naher *et al.* (2013) on inoculated AM plants, the results showed that they are capable to assist the phosphorus ions uptake in contrast with those uninoculated plants. The extensive hyphae network of AM fungi that extends from the roots allow the host plant to explore a great volume of soil, hence overcome the limitations stipulated by slow dispersion of inorganic phosphate (Pi) in soil (Schachtman *et al.*, 1998). Besides, it also assists in the long-haul translocation of Pi to the host plant in the form of polyphosphate (polyP) (Van der Heijden *et al.*, 2008; Hijikata *et al.*, 2010). Through this hyphae network, the AM fungi that grown under condition of P deficiencies were managed to accumulate a massive number of polyP within hours to raise the phosphorus ions opportunity in plant (Hijikata *et al.*, 2010). This was considered as an adaptive trait that generally emerged in microbes to equip for the P deficiency, known as the 'polyP overcompensation (overplus)' (Harold, 1966) which enable the host plant's phosphate necessity to be filled up to 90% and thus increase the phosphate uptake significantly.

Despite that, AM fungi also assists in the uptake of both inorganic (Govindarajulu *et al.*, 2005) and organic (Hodge *et al.*, 2001) nitrogen ions from soil, increase the plant resistance to the pathogen (Vigo *et al.*, 2000; Akhtar and Siddiqui, 2008; Naher *et al.*, 2013), alleviated plant stress especially on salt tolerance (Evelin *et al.*, 2009; Estrada *et al.*, 2013; Abdel *et al.*, 2014), drought tolerance (Porcel and Luiz-Lozano, 2004; Aroca *et al.*, 2007) and heavy metal's toxicity (Hildebrandt *et al.*, 1999), increase the defence of plants to nematode and root infections in rhizosphere soil (Linderman, 2000), provide resistance to root herbivores (Gange, 2001) and improve the soil texture as well as water relations considerably due to the development of large widespread hyphal network extend from the plant root system (Bethlenfalvay and Schuepp, 1994).

Despite benefits of AM fungal inoculants, the understanding of indigenous AM fungi species that present in their host plants are comparatively inadequate. Hence this study targeted on the indigenous AM fungi population in some selected agriculture plants and to determine the phytochemical properties of soil that significantly affect the root colonization rate and spore numbers of AM fungi.

MATERIALS AND METHODS

Sampling of soils and fine root samples

The rhizosphere soil and fine sample of roots were sampled from lemongrass, pandan, papaya, tapioca, banana, coconut, bamboo and sugarcane around Kota Samarahan, Sarawak (1.4594°N, 110.4989°E). Three samples were collected randomly and respectively for each selected plant at the depth range of 0 to 20 cm. The collected root and soil samples were labelled according to date of collection, location and type of plants respectively.

Isolation of AM fungi spores

Wet sieving and decanting techniques

The samples were air dried for 3 to 4 days before the isolation of AMF spores. The fungi spores were extracted by applying the techniques of decanting and wet sieving as described by Gerdemann and Nicolson (1963) with slight modification. Approximately 10 g of air dried sample was added to 100 mL of tap water and mixed vigorously with glass rod for 30 sec. The suspension was let to settle for 10 to 15 sec to settle down the heavy particles and organic materials to the bottom layer of the flasks. Suspension was decanted gradually through four standard sieves (U.S. Standard Sieve series, DUAL MFG. Co., Chicago). The top sieve had the biggest pores diameter of 300 µm, followed by three sieves with pores diameter of 125 µm, 106 µm and 63 µm respectively. The decantation process was repeated until suspension turned translucent at the upper layer. Each sieving retained on different sieves were decanted into separate petri dishes by using a wash bottle and labelled according to their sizes for further purification process.

Sucrose density gradient centrifugation techniques

The retained that contained high amount of organic materials and AM fungi spores were further purified by sucrose density gradient centrifugation techniques as construed by Daniels and Skipper (1982) with modification. Sieving retained were transferred from petri dish into 50 mL centrifuge tubes respectively with sterile dH$_2$O and centrifuged at 1200 rpm for 3 min. The supernatant and debris that adhered to the wall were removed with caution without disturbing the pallets collected. The pallets were re-suspended in chilled 50% (w/v) sucrose solution and homogenized to create uniform suspension. The suspensions were then centrifuged instantly at 1200 rpm for a minute and supernatant were decanted into the sieves with pore diameter of 63 µm. Spores that trapped by the sieves were carefully rinsed with tap water to remove the excess sucrose, followed by washing using wash bottle into the petri dish labelled according to their sizes. These isolated AM fungi spores were kept for further identification processes.

Enumeration and characterisation of AM fungi spores

The spores isolated were observed and enumerated under compound microscope (LEICA DM 500) in petri dish with 100× magnification power and subsequently inverted research microscope (Olympus IX51) with 400× magnification power. The average number of AM fungi spores was enumerated using formula shown below:

$$\text{Number of spores} = \frac{\text{Number of spores in 10 g of soil}}{10 \text{ g}}$$

The AM fungi spores were identified morphologically based on spore shape, size of spore, subtending hyphae, spore walls, type of hyphae attachment and colour using the identification manual of VA Mycorrhizal Fungi described by Schenk and Perez (1990) together with the identification keys from International Culture Collection of Vascular-Arbuscular Mycorrhizal Fungi website.

Staining of AM fungi roots

The structure of AM fungi in roots such as arbuscules, hyphae and mycelium were hard to be observed without proper staining methods as construed by Phillips and Hayman (1970). Roots collected were washed gently to get rid of the soil particles without disturbing mycelium and spores attached to the roots. These root samples were excised into ten fragments with 1 cm height each and transferred into the beaker that contained 10% (w/v) KOH solution which were sufficient to mask the root fragments. The beaker was then incubated in 90 °C water bath for approximately 1 to 2 h and allowed to chill to ambient temperature after the incubation period. Approximately 30% (v/v) hydrogen peroxide were added to the beaker and the root fragments were further incubated for an hour at ambient. The cleared root fragments were cleaned thoroughly with distilled water for few times to eliminate the excess hydrogen peroxide on the root's surface. These cleared root fragments were then acidified and stained by boiling in Pelican ink-vinegar solution which contained 5% (v/v) acetic acid for a few hours (Vierheilig *et al.,* 1998). After that, the root fragments were rinsed thoroughly with acidified tap water (few drops of vinegar) to remove the excess stain. The fragments were now ready for observation under microscope.

Evaluation of AM fungi root colonization

The stained root fragments were observed under inverted research microscope (Olympus IX51) with 200× magnification power to score for any structures associated to AM fungi. The root colonization percentages were calculated by an average of three replicates using the modified frequency distribution method as proposed by Biermann and Lindermann (1983) shown below:

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized root segments}}{\text{Overal number of root segments evaluated}} \times 100$$

Physical and chemical analysis of soil samples
Soil texture analysis

Soil texture is an important element to be studied as it affects physical, chemical and biological processes in soil environment (Bhat *et al.,* 2011). It was studied by applying the 'Feel Method' as detailed by Thien (1979) shown in Figure 1.

Soil pH analysis

The pH of soil samples was analysed as it can provide vital information on the nutrients abundancy in soil, the activities of microorganism and the physical state of soil (Motsara and Roy, 2008). The soil pH was identified by practicing a modified method described by Jackson (1967). Soil samples were grinded by using pestle and mortar respectively into fine powder and distilled water was added at a ratio of 1:2 to form soil suspension. The suspension was disturbed continuously for 30 min using a glass rod and leave aside for 1 h. Electrode end of pH meter was inserted into the soil suspension and the pH was registered. The electrode was washed with sterile dH_2O before pH analysis of each sample.

Statistical analysis

The correlation relationship among the soil pH, root colonization rate of AM fungi and number of spores was studied to analyse the effect of soil pH. The data collected were subjected to Pearson's correlation analysis by using statistical software IBM SPSS vr21.0 and extend of correlation were based on the standard whereby: (a) $r < 0.20$: insignificant correlation; (b) $0.20 < r < 0.40$: poor correlation; (c) $0.40 < r < 0.70$: intermediate correlation; (d) $0.70 < r < 0.90$: strong, active correlation; (e) $r > 0.90$: absolute strong, active correlation.

RESULTS AND DISCUSSION

Few genera of AM fungi spores were isolated and characterized from the crop plants studied as shown in Table 1 and Figure 2. The identification of the spores was primarily referred to the colour and shape of the spores, the number of wall layers and any other type of structures that associated with the AM fungi.

From Table 1, AM fungi isolated from the crop plants were *Glomus* spp., *Acaulospora* spp., *Funneliformis* spp., *Rhizophagus* spp. and *Dentiscutata* spp. Among various AM fungi species isolated, genera *Glomus* spp. was found to be the predominant species followed by *Acaulospora* spp. in this research study. In tropical country, the humid environment of tropical ecosystem was sustained by a fast material recycling process (Read, 1994). According to Bever *et al.,* (1996), *Acaulospora* and *Glomus* could produce higher number of spores than other AM fungi species in the similar environs. In addition, the size of spores produced are smaller, hence, lead to a shorter time in sporulation process (Hepper, 1984). Thus, *Glomus* and *Acaulospora* with fast sporogenous characteristics could adapt to tropical ecosystem in a better way than the other AM fungi (Hepper, 1984).

On top of that, Zhao *et al.,* (2001) and Ananthakrishnan *et al.,* (2004) also proclaimed that genera *Glomus* and *Acaulospora* are capable to dominate the tropical soils than the other mycorrhiza genus due to the wide host range of choices as well as better capability to survive in vast environment. The symbiotic relationships of AM fungi although are not host

specialized but are kinds of host-preferred (Smith and Read, 2008). The host preferential of AM fungi have important weight on the ecology of plant (Bever, 2002). The preferential plant reacted to promote the growth of selected AM fungi by allocate the photosynthate preferentially (Bever *et al.,* 2009). These responses of plants will alter the composition and diversity of AM fungal communities in soil environs and further exert enormous outcome on the diversification along with the composition of plants in consequences and in vice versa (Van der Heijden *et al.,* 1998; Bever, 2002). In this research study, it suggested that *Glomus* and *Acaulospora* spp. were the most preferential AM fungi by the selected host plants and thus can be easily found within the rhizosphere soil environment of studied host plants.
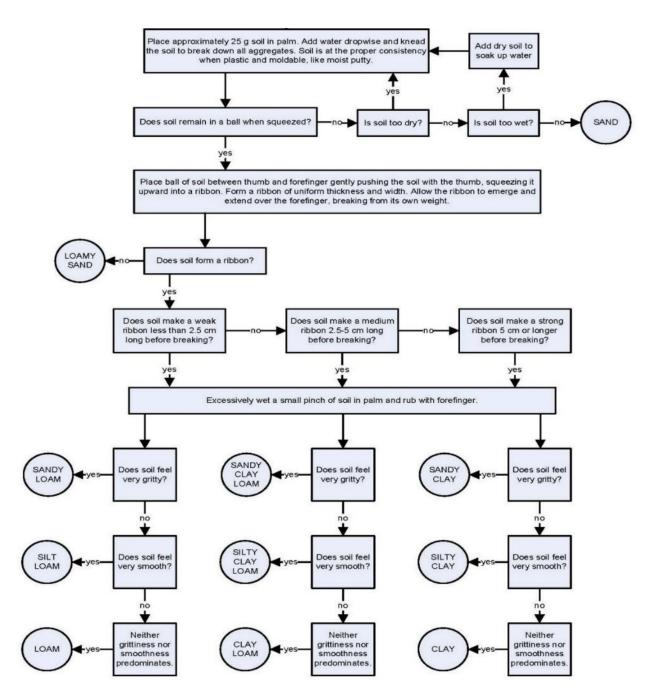

Figure 1: Soil texture by feel method flow chart by Thien (1979).

Table 1: The genus of AM fungi and spore numbers isolated from the selected type of plant's rhizosphere soils and the root colonization of the AMF.

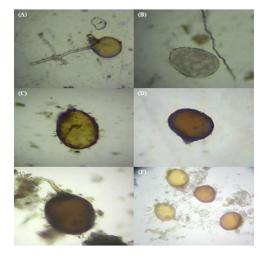| Type of Plant	Genus of AMF spores isolated	Number of spores/g in soil samples	Root Colonization (%)
Lemongrass	*Glomus* spp.	2.8	10.00
Papaya	*Glomus* spp., *Acaulospora* spp.	5.7	16.67
Pandan	*Glomus* spp., *Funneliformis* spp.	4.3	13.33
Tapioca	*Glomus* spp., *Acaulospora* spp., *Rhizophagus* spp.	6.2	20.00
Bamboo	*Glomus* spp., *Acaulospora* spp.	3.2	6.67
Banana	*Glomus* spp., *Acaulospora* spp., *Funneliformis* spp., *Dentiscutata* spp.	4.8	3.33
Coconut	*Glomus* spp., *Acaulospora* spp., *Rhizophagus* spp.	2.7	13.33
Sugarcane	*Glomus* spp., *Acaulospora* spp., *Rhizophagus* spp.	2.3	6.67

Figure 2: The AM fungi that isolated from the studied plant shown in 400× magnification power. *A. Glomus* spp. (globulose shape, light yellow in colour and hyphae attach to the spore); B, *Rhizophagus* spp. (white colour subglobulose spore with many spores seen in it); C, *Acaulospora* spp. (cicatrix in middle of spore); D, *Dentiscutata* sp. (globose shape and dark-brown colour); E, *Funneliformis* spp. (funnel-like spore and dark brown in colour) and F, Different colour and shape of *Glomus* spp.

The population dynamic of AM fungi was determined by collection and enumeration of the spores present in soil samples. However, there are variation exists in term of spore numbers present in soil samples irrespective of the locality of soil samples (Hindumathi and Reddy, 2011). From Table 1, it showed that rhizosphere soil of tapioca plant contained the highest number of spore count which is about 6.2 spores per gram of soil sample followed by papaya (5.7 spores), banana (4.8 spores), pandan (4.3 spores), bamboo (3.2 spores), lemongrass (2.8 spores) and coconut (2.7 spores). In contrast, sugarcane recorded the lowest number of spore count which is approximately 2.3 spores per gram of soil sample. These findings suggested that the population dynamic of AM fungi are varies in every plant species although the soils were collected from the same region. According to Khakpour and Khara (2012), AM fungi's spore numbers that exists in soil environs were greatly influenced by numerous abiotic and edaphic factors. Comparable phenomenon was discovered by Nasrullah *et al.* (2010) whereby the number of AM fungi spores varied significantly across different locations affect by the physiochemical status of the soil.

Apart from spore numbers, root colonization rate of AM fungi in studied plants was calculated and tabulated in Table 1. In this study, tapioca plant achieved the highest root colonization rate which is 20% compare to the other studied plants. This was followed by papaya (16.67%), pandan (13.33%), coconut (13.33%), lemongrass (10%), bamboo (6.67%) and sugarcane (6.67%). Although banana plant had higher spore numbers in rhizosphere soil, but the rate of AM fungi colonization in banana plants had recorded the lowest (3.33%) among the studied plant. From this study, it can be deduced that the root colonization rate is low among all studied plant samples. The low AM fungi root colonization rate could be the consequences of field usage that greatly changed the soil ecosystem and nature of the soil. From the prior study by Stabler *et al.* (2001), it reported that the urban expansion had lowered the AM fungi root colonization rate compare to the undisturbed environment. The association between sporulation and colonization of AM fungi was different since the association depends on soil nutrient contents, host plants and mycorrhizal species (Stutz and Morton, 1996) while the colonization of AM fungi associated with several factors such as reliance of host plants on mycorrhiza, the host plant phenology, alterations in soil microenvironment along with the unknown characteristics of the AM fungi host plants (Eom *et al.,* 2000). Identical results were addressed by Baraka *et al.,* (2012) that concluded the reaction of plants towards mycorrhization depend on the nutrients in soil environments and type of AM fungi exist in plant. The other factors that may cause variations were the plant root exudates (sesquiterpene) (Akiyama *et al.,* 2005) as well as soil moisture (Simpson and Daft, 1990).

In this study, the rhizosphere soil was analysed for the physiochemical properties as it will exert significant impacts on dispersion and abundance of AM fungi (Reddy *et al.,* 2007). The physiochemical properties analysed in this study is the texture and pH of the rhizosphere soil and the result was tabulated in Table 2.

Table 2: Physiochemical and pH of rhizosphere soil collected among the studied plant species.

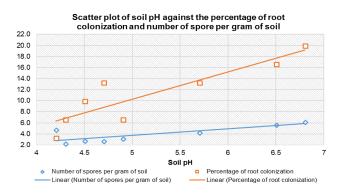| Type of Plant	Forming of a ball	Forming of Ribbon	Soil Feel	Type of Soil	Soil pH
Lemongrass	Yes	Yes	No noticeable feel	Clay Loam	4.5
Papaya	Yes	Yes	Gritty	Sandy Loam	6.5
Pandan	Yes	Yes	Gritty	Sandy Clay Loam	5.7
Tapioca	Yes	Yes	Gritty	Sandy Loam	6.8
Bamboo	Yes	Yes	Smooth	Silt Loam	4.9
Banana	Yes	Yes	Gritty	Sandy Loam	4.2
Coconut	Yes	Yes	Smooth	Silt Loam	4.7
Sugarcane	Yes	Yes	Smooth	Silt Loam	4.3

Figure 3: The scatter plot of soil pH against the percentage of root colonization and number of spores in soil.

The texture of rhizosphere soil significantly affects the holding capacity of water in soil, aeration, movement of roots and availability of nutrients, thus soil aggregates which are better in quality and stable are considered as better option for AM fungi to colonize (Motsara and Roy, 2008). In this study, the texture of the rhizosphere soils was estimated by the 'Feel Method'. The 'Feel Method' described by Thien (1979) was employed due to its rapid results although the accuracy were largely depended on the experience as well as subjective. From the results shown in Table 2, there are four different soil textures obtained from the 'Feel method'. The tapioca plants that had the topmost spore numbers in rhizosphere soil and rate of root colonization among the studied plants. Its rhizosphere soil is sandy loam in texture. According to Motsara and Roy (2008), sandy soil was typically more porous and dry, warmer and less fertile to the other soil texture which can help to stimulate the association and colonization of mycorrhiza in the soil environment. This result was in consensus to the study by Carrenho *et al.* (2007) that recorded AM fungi in sandy soil had the topmost colonization percentage compare to the others.

The pH of rhizosphere soil of studied plant samples was analysed as the soil pH has substantial impact on the nutrient availability in plants which is able to change the AM fungi's distribution in soil environment (Sundar and Sabari, 2011). Results of the pH readings of rhizosphere soil were tabulated in Table 2. It showed that rhizosphere soil of the tapioca plants recorded the highest reading of pH which is 6.8 (slight acidic) while the rhizosphere soil of the banana was observed with the lowest pH reading among the studied plants which is 4.2 (acidic). All rhizosphere soil samples showed acidic pH due to the nature of tropical soil and it was counterfeited by the management history of the soil. The rhizosphere soil of tapioca plants showed the highest pH recorded a higher spore number in soil and root colonization of AM fungi among the studied plants as the soil pH was ideal for the sporulation and colonization of AM fungi. In contrast, the banana plants with its rhizosphere soil as the sandy loam yet recorded the lowest root colonization rate, suggested that pH of the rhizosphere soil is the most significant effects that greatly influence the rate of colonization of AM fungi. Acidic soil was not suitable for AM fungi as they typically have poor structure of soil, lower water capacity and root penetration which affect the spore numbers and colonization in the studied rhizosphere soil (Motsara and Roy, 2008).

The correlation between type of rhizosphere soil, pH of rhizosphere soil, spore numbers in rhizosphere soil and colonization rate was identified and tabulated as shown in Table 3. From the result in Table 3, the r value +0.807 indicated a positive and strong correlation between soil pH and the spore numbers in rhizosphere soil. Meanwhile, the r value of soil pH against the percentage of root colonization is +0.890, also indicated a strong, active correlation. The scatter plots of correlation were plotted as shown in Figure 3. From this Pearson correlation, it suggested that the pH of the rhizosphere soil had significant effects on both spore numbers in soil and rate of colonization in roots whereby the higher rhizosphere soil pH will show higher number of spore in rhizosphere soil and higher rate of root colonization. Similar observation and findings was observed by Trindade *et al.* (2006) and Bhat *et al.* (2011) that soil pH had positive and steady correlation with the colonization rate of AM fungi in soil.

Table 3: Pearson correlation among type and pH of soil, spore numbers and root colonization percentage of AM fungi in rhizosphere soil.

		Number of spores per gram of soil	Type of soil	Percentage of root colonization	Soil pH
Number of spores per gram of soil	Pearson Correlation	1	-.510	.578	.807[*]
	Sig. (2-tailed)		.196	.133	.016
	N	8	8	8	8
Type of soil	Pearson Correlation	-.510	1	-.236	-.267
	Sig. (2-tailed)	.196		.573	.522
	N	8	8	8	8
Percentage of root colonization	Pearson Correlation	.578	-.236	1	.890[**]
	Sig. (2-tailed)	.133	.573		.003
	N	8	8	8	8
Soil pH	Pearson Correlation	.807[*]	-.267	.890[**]	1
	Sig. (2-tailed)	.016	.522	.003	
	N	8	8	8	8

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

CONCLUSION

Various indigenous AM fungi species such as *Glomus*, *Acaulospora*, *Funneliformis*, *Rhizophagus* and *Dentiscutata* were isolated from the crop plants and *Glomus* genera was identified as the predominant genera followed by *Acaulospora* in these selected plants. In this study, tapioca plant achieved the highest root colonization rate and spore number present in soil whereas banana recorded the lowest root colonization rate despite the greater spore number that present in soil. This findings suggested that tapioca plant appeared to be the most suitable host plant to propagate the AM fungi. Apart from that, soil pH induced the most significant influence (positive correlation) on AM fungi by affecting the root colonization rate in crop plants and spore number in rhizosphere soil environs. Hence, the pH of rhizosphere soil should be monitored at slight acidic level for a good dispersion of AM fungi.

AM fungi are widely documented for its contributions towards the natural ecosystem and host plants. Therefore, it is necessary to further study and analysed the AM fungi in detailed to enable full utilization of fungi. The potentials of these AM fungi in agricultural as field is that they can be used biofertilizer, biological protectors and biological control agents could trigger a revolution in traditional agriculture practices towards sustainable agricultural system in future.

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REFERENCES

Abdel Latef, A. A. H. and Miransari, M. (2014). Chapter 2: The role of arbuscular mycorrhizal fungi in alleviation of salt stress. *In:* Use of Microbes for the Alleviation of Salt Stresses. Miransari, M. (Ed.). Springer, New York, US. ISBN: 978-1-4939-0720-5.

Aggangan, N., Kim, Y., Moon, H. and Han, H. (2011). Selection of arbuscular mycorrhizal inoculants for improved growth of somatic embryo-derived *Liriodendron tulipifera* (Magnoliaceae) plantlets. *Philippine Journal of Science* **140(1), 59-67.**

Akhtar, M. S. and Siddiqui, Z. A. (2008). Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. *In:* Mycorrhizae: Sustainable Agriculture and Forestry. Siddiqui, Z. A., Akhtar, M. S., and Futai, K. (eds.). Springer Science+Business Media BV, Dordrecht, Netherlands. ISBN: 978-1-4020-8769-1.

Akiyama, K., Matsuzaki, K-i. and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435, 824-827.**

Ananthakrishnan, G., Ravikumar, R., Girija, S. and Ganapathi, A. (2004). Selection of efficient arbuscular mycorrhizal fungi in the rhizosphere of cashew and their application in cashew nursery. *Scientia Horticulturae* **100(1-4), 369-375.**

Aroca, R., Porcel, R. and Ruiz-Lozano, J. M. (2007). How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* **173(4), 808-816.**

Bago, B., Azcon-Aguilar C, Goulet, A. and Piche, Y. (1998). Branched absorbing structures (BAS): A feature of the extraradical mycelium of symbiotic

arbuscular mycorrhizal fungi. *New Phytologist* **139(2), 375-388**.

Baraka, M. A., Abd-Elmoneim, T. S., Ramadan, El-S. M. and Mohamed, A. F. (2012). Biodiversity and classification of arbuscular mycorrhizal fungi (Glomales) in Ismailia Governorate. *Agricultural Research Journal Suez Canal University* **12(1), 83-90**.

Bethlenfalvay, G. J. and Schüepp, H. (1994). Arbuscular mycorrhizas and agrosystem stability. *In:* Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Gianiazzi, S. and Schüepp, H. (eds.). Birkhäuser Basel, Basel, Switzerland. ISBN 978-3-0348-8504-1. **pp. 117-131.**

Bever, J. D., Morton, J. B., Antonovics, J. and Schultz, P. A. (1996). Host-dependent sporulation and species diversity of Arbuscular Mycoorhizal fungi in a mown grassland. *Journal of Ecology* **84(1),71-82.**

Bever, J. D. (2002). Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil* **244(1-2), 281-290.**

Bever, J. D., Richardson, S., Lawrence, B. M., Holmes, J. and Watson, M. (2009). Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* **12(1), 13-21.**

Bhat, S. H., Darzi, A. B., Dar, M. S., Ganaie, M. M. and Bakhshi, S. H. (2011). Correlation of soil physic-chemical factors with VAM fungi distribution under different agroecological conditions. *International Journal of Pharma and Bio Sciences* **2(2), 98-106.**

Biermann, B. and Linderman, R. G. (1983). Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. *New Phytologist* **95(1), 97-105.**

Burni, T., Hussain, F. and Sharief, M. (2011). Arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of *Mentha Arvensis* L., and *M. Longifolia* huds. *Pakistan Journal of Botany* **43(6), 3013-3019**.

Carrenho, R., Trufem, S. F. B., Bononi, V. L. R. and Silva, E. S. (2007). The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta Botanica Brasilica* **21(3), 723-730.**

Daniels, B. A. and Skipper, H. D. (1982). Methods for the recovery and quantitative estimation of propagules from soil. *In:* Method and Principles of Mycorrhizal Research. Schenck, N. C. (Ed.). American Phytopathological Spciety, St. Pau, Minnesota. **pp. 29-35.**

Dotzler, N., Walker, C., Krings, M., Hass, H., Kerp, H., Taylor, T. N. and Agerer, R. (2009). Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycological Progress* **8(1), 9-18.**

Eom, A.-H., Hartnett, D. C. and Wilson, G. W. T. (2000). Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* **122(3), 435-444**.

Estrada, B., Aroca, R., Maathuis, F. J., Barea, J. M. and Ruiz-Lozano, J. M. (2013). Arbuscular mycorrhizal fungi native from a Mediterranean saline area enhance maize tolerance to salinity through improved ion homeostasis. *Plant, Cell and Environment* **36(10), 1771-1782.**

Evelin, H., Kapoor, R. and Giri, B. (2009). Arbuscular mycorrhizal fungi in alleviation of salt stress: A review. *Annals of Botany* **104(7), 1263-1280.**

Gange, A. C. (2001). Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist* **150(3), 611-618.**

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46(2), 235-244.**

Govindarajulu, M., Pfeffer, P. E., Jin, H., Abubaker, J., Douds, D. D., Allen, J. W., Bücking, H., Lammers, P. J. and Shachar-Hill, Y. (2005). Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435(7043), 819-823.**

Harold, F. M. (1966). Inorganic polyphosphates in biology: Structure, metabolism, and function. *Bacteriological Reviews* **30(4), 772-794**.

Hepper, C. M. (1984). Isolation and culture of VA mycorrhizal (VAM) fungi. *In:* VA Mycorrhiza. Powell, C. L. and Bagyaraj, D. J. (eds.). CRC Press, Boca Raton, USA. **pp. 95-112**.

Hijikata, N., Murase, M., Tani, C., Ohtomo, R., Osaki, M. and Ezawa, T. (2010). Polyphosphate has a central role in the rapid and massive accumulation of phosphorus in extraradical mycelium of an arbuscular mycorrhizal fungus. *New Phytologist* **186(2), 285-289.**

Hildebrandt, U., Kaldorf, M. and Bothe, H. (1999). The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology* **154(5-6), 709-717.**

Hildebrandt, U., Janetta, K. and Bothe, H. (2002). Towards growth of arbuscular mycorrhizal fungi independent of a plant host. *Applied and Environmental Microbiology* **68(4), 1919-1924.**

Hindumathi, A. and Reddy, B. (2011). Occurrence and distribution of arbuscular mycorrhizal fungi and microbial flora in the rhizosphere soils of mungbean [vigna radiata (L.) wilczek] and soybean [glycine max (L.) Merr.] from Adilabad, Nizamabad and Karimnagar districts of Andhra Pradesh state, India. *Advances in Bioscience and Biotechnology* **2(4), 275-286.**

Hodge, A., Campbell, C. D. and Fitter, A. H. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413(6853), 297-299.**

Jackson, M. L. (1967). Soil chemical analysis. Prentice Hall Inc, New Delhi, India.

Khakpour, O. and Khara, J. (2012). Spore density and root colonization by arbuscular mycorrhizal fungi in some species in the northwest of Iran. *International Research Journal of Applied and Basic Sciences* **3(5), 977-982.**

Linderman, R. G. (2000). Effects of mycorrhizas on plant tolerance to diseases. *In:* Arbuscular mycorrhizas:

Physiology and function. Koltai, H. and Kapulnik, Y. (eds.). Springer, Dordrecht, South Holland. ISBN 978-90-481-5515-6. **pp. 345-365.**

Motsara, M. and Roy, R. N. (2008). Guide to laboratory establishment for plant nutrient analysis (Vol 19). Food and Agriculture Organization of the United Nations, Rome. ISBN 978-92-5-105981-4.

Naher, U. A., Othman, R. and Panhwar, Q. A. (2013). Beneficial effects of mycorrhizal association for crop production in the tropics: A review. *International Journal of Agriculture and Biology* **15(5), 1021-1028.**

Nasrullah, Sharif, M., Rubina, K. and Burni, T. (2010). Occurrence and distribution of arbuscular mycorrhizal fungi in wheat and maize crops of Malakand division of north west frontier Province. *Pakistan Journal of Botany* **42(2), 1301-1312.**

Phillips, J. H. and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55(1), 158-161.**

Porcel, R. and Ruiz-Lozano, J. M. (2004). Arbuscular mycorrhizal influence on leaf water potential, solute, accumulation and oxidative stress in soybean plants subjected to drought stress. *Journal of Experimental Botany* **55(403), 1743-1750.**

Read, D. J. (1991). Mycorrhizas in ecosystem. *Experientia* **47(4), 376-391.**

Read, D. (1994). Plant-microbe mutualisms and community structure. *In:* Biodiversity and Ecosystem Function. Schulze, E.-D. and Mooney, H. A. (eds.). Springer Press, New York, USA. **pp. 181-209.**

Reddy, B. N., Hindumathi, A. and Raghavender, C. R. (2007). Occurrence and systematics of arbuscular mycorrhizal fungi associated with sorghum. *Journal of Phytological Research* **20(1), 11-22.**

Remy, W., Taylor, T. N., Hass, H. and Kerp, H. (1994). Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of America* **91(25), 11841-11843.**

Schachtman, D. P., Reid, R. J. and Ayling, S. M. (1998). Phosphorus uptake by plants: From soil to cell. *Plant Physiology* **116(2), 447-453.**

Schenk, N.C. and Perez, Y. (1990). Manual for identification of VA mycorrhizal fungi. (3rd Ed.). US: Synergistic Publications. ISBN13: 9780962598036.

Sharma, A. and Yadav, M. (2013). Isolation and characterization of vesicular arbuscular mycorrhiza from barley fields of Jaipur district. *International Journal of Agricultural Science and Research* **3(1), 151-156.**

Simpson, D. and Daft, M. (1990). Interactions between water-stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum. *Plant and Soil* **121(2), 179-186.**

Smith, S. E. and Read, D. (2008). Mycorrhizal symbiosis. Academic Press, London, UK.

Stabler, L. B., Martin, C. A. and Stutz, J. C. (2001). Effect of urban expansion on arbuscular mycorrhizal fungal mediation of landscape tree growth. *Journal of Arboriculture* **27(4), 193-202.**

Stutz, J. C. and Morton, J. B. (1996). Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. *Canadian Journal of Botany* **74(12), 1883-1889.**

Sundar, S. K. and Sabari, V. M. (2011). Microbial diversity and phytochemical profile of Solanum nigrum L from two different sites of Kannyakumari district, Tamil Nadu, India. *International Journal of Research in Biotechnology and Biochemistry* **1(1), 8-10.**

Thien, S. J. (1979). A flow diagram for teaching texture by feel analysis. *Journal of Agronomic Education* **8, 54-55.**

Trindade, A. V., Siqueira, J. O. and Stürmer, S. L. (2006). Arbuscular mycorrhizal fungi in papaya plantations of Espírito Santo and Bahia, Brazil. *Brazilian Journal of Microbiology* **37(3), 283-289.**

Van der Heijden, M. G. A., Bargett, R. D. and Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11(3), 296-310.**

Van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. and Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396, 69-72.**

Vierheilig, H., Coughlan, A. P., Wyss, V. and Piché. Y. (1998). Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* **64(12), 5004-5007.**

Vigo, C., Norman, J. R. and Hooker, J. E. (2000). Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathology* **49(4), 509-514.**

Zhao, Z. W., Xia, Y. M., Qin, X. Z., Li, X. W., Cheng, L. Z., Sha, T. and Wang, G. H. (2001). Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza* **11(3), 159-162.**