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RESEARCH PAPER

Effect of arbuscular mycorrhiza fungi on the growth, nutrient uptake, root infectivity and soil colonisation of rough lemon (Citrus jambhiri) seedlings

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Abstract

The effect of Arbuscular mycorrhiza (AM) fungi on growth, nutrient uptake and root infectivity was determined in Rough Lemon (*Citrus jambhiri*) seedlings raised under four phosphorus regimes in sand culture and also in sand/nitrosol sterile and unsterile conditions. Inoculation with AM fungi increased the plant height, leaf number and stem girth in relation to un-inoculated seedlings grown under equivalent P concentrations. An increase in plant height, leaf number and stem girth also occurred in both inoculated sterile and un-sterile sand/nitrosol media in relation to un-inoculated sterile and unsterile media. Arbuscular mycorrhiza also increased the leaf area and the root, leaf and stem fresh and dry weights and also caused an increase in the uptake of phosphorus and potassium in the leaf tissues. It also favoured mycorrhizal infectivity of roots and increased the root absorptive surface area. This study indicates that AM fungi improves the capacity of tropical fruit to absorb and utilize plant nutrients possibly by increasing the effective root surface area from which available form of nutrients are absorbed and also by increasing access of roots by bridging the depletion zones. Inoculating seedlings with arbuscular mycorrhizal fungi helps to alleviate the adverse effects of global warming and climate change. As a low cost technology, arbuscular mycorrhizal inoculation is recommended as part of the regular practise for incorporating into nursery media used for tropical fruit seedling propagation.

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Introduction

Most tropical soils are nutrient deficient. Phosphorus is the most critical nutrient limiting plant growth in the tropics, mainly because most tropical soils fix phosphorus (Sieverding, 1991). This problem is further compounded by widespread degradation of the fertile topsoil and arid or semi-arid nature of tropical lands. Low input subsistence farming is also widely practiced in most tropical regions.

Among the technologies holding much promise to managing tropical soil fertility and improving plant nutrition is the arbuscular mycorrhiza (AM) fungal inoculation. The benefits of AM inoculation on growth and productivity of fruit seedlings are widely documented. Arbuscular mycorrhizal inoculation has been reported to enhance uptake of plant nutrients, especially phosphorus and zinc (Wamocho, 1998; Fidelibus *et al.*, 2001; Rutto *et al*., 2002a; Muok and Ishii 2006, Chebet *et al*., 2020) and also nitrogen, potassium, calcium and magnesium to a lesser extend (Rutto *et al*., 2002b; Muok and Ishii 2006). Arbuscular mycorrhiza fungi also improves tolerance to drought stress (Rutto *et al*., 2002b), causes a faster recovery after moisture stress (Fidelibus *et al*., 2001), increases the transpiration and photosynthetic rates (Yano-Melo *et al*., 1999) and confers tolerance to flooding (Rutto *et al*., 2002b; Muok and Ishii 2006) and high soil salinity (Muok and Ishii 2006, Chebet, 2020). Other reports indicate that arbuscular mycorrhizal inoculation antagonize against parasitic soil borne pathogens and pests (Elsen *et al*., 2003), improves the ability of plants to withstand transplanting shock and improves general plant performance after transplanting (Wamocho, 1998).

Under tropical conditions, AM fungi could be highly beneficial to perennial crops, which require nursery production before transplanting to the field (Wamocho, 1998). However, although arbuscular mycorrhiza associations, and their fungal propagules (spores, mycelium and infected roots) are widespread in the tropics (Sieverding, 1991), propagules can be lost or their species changed through site disturbance, inhibiting the renewal of vegetation cover, or changing its composition (Mason and Wilson, 1994).

In fruit orchards in Kenya, the AM fungal spores and the mycorrhizal infection of fruit tree roots are low (Wamocho, 1998). Likewise, naturally occurring mycorrhiza formation in fruit/tree nurseries are sparse, even in unsterilized soils, leading to poorly mycorrhizal or potentially-poorly performing seedlings being transplanted (Michelson, 1992). Limited research have been undertaken on the role of AM fungi on the growth, nutrient uptake and root infectivity of tropical fruit species, unlike in temperate fruit species. As a result, experiment was undertaken to determine the role of AM fungi in in passion fruit (*Passiflora edulis* var *edulis*), rough lemon (*Citrus limon*) and papaya (*Carica papaya* var *Solo*).

Materials and methods

Treatments and experimental design

Papaya, passion fruit and lemon seeds were germinated in sterile sand and uniform seedlings selected and transplanted to polythene pots (20cm in diameter and 25cm depth) in a polyethylene-covered greenhouse. The experiment was laid out in sterile sand as a randomized 2 x 3 factorial design consisting of 2 kinds of AM inoculation (AM inoculated and un inoculated) and 4 phosphorus concentrations (0, 0.44, 0.88 and 1.68mg/ml) with 6 replicates per treatment. The plants were watered one a week with 300mls of half strength Hoagland's nutrient solution (Millner and Kitt, 1992) modified to the respective P concentrations.

An experiment was also laid out in low nutrient nitrosol and sand media (1:1 vol/vol) as a 2 x 2 factorial design consisting of 2 kinds of AM inoculation (AM inoculated and un-inoculated) and 2 media conditions (sterile and non-sterile) with 6 replicates per treatment. The AM inoculum contained approximately 200 spores of a mixture of *Glomus caledonium, G. etunicatum, Gigaspora magarita* and *Scutellospora sp* (Plantworks Inc., UK). To ensure uniformity, similar quantities of autoclaved inoculum were added to the non-mycorrhizal pots.

Plant growth measurements

Weekly measurements were taken on plant height, leaf number and stem girth, starting two weeks after inoculation.

Biomass and nutrient analysis

At seedling harvest, measurements were taken on leaf area and leaf, stem and root fresh and dry weights. Oven-dried shoots were then ground and 1 gram from each seedling weighed and dry-ashed by heating for 5 hours at 550°C in a muffle furnace. The ash was taken up in 20% HCl and the solution made up to 20mls with distilled water. Two hundred microliter aliquots from these solutions were further distilled to 10mls before analyzing for Ca,mg and K by atomic absorption spectrophotometry. Phosphorus, as molybdate-reactive P was measured by blue colorimetry at 730nm using a spectrophotometer.

Evaluation of root infection levels

At seedling harvest, root tips (1 ± 0.2cm) were excised and cleared by autoclaving in 10% KOH followed by staining in 0.05% tryphan blue, glycerol and lactic acid (1:1:1) solution. The frequency of mycorrhizal infection was noted per field (10 grids) for 10 fields, using the grid intersect method (Giovannetti and Mosse, 1980). To convert the data into percent infection, the frequency of infection as a fraction of the total number of grids observed was multiplied by 100 (Wamocho, 1998).

Statistical analysis

The data obtained was subjected to ANOVA, using Genstat software. All treatment means were tested for LSD and the means separated by Duncan's multiple range test (Little and Hills, 1978).

Results

Plant Height

There was no significant difference in plant height in seedlings subjected to varied phosphorus concentrations in the first 12 weeks from transplanting. On 16th, 20th and 24th week after transplanting, mycorrhizal lemon seedlings subjected to 0.44, 0.88ppm and 1.68 ppm P had the highest plant height but plant height increase waned in mycorrhizal, 1.68 ppm P such that from 28th to 32nd week, mycorrhizal seedlings subjected to 0.44 and 0.88 ppm P had the highest plant height. There was no significant difference in plant height between mycorrhizal plants that were not supplied with P (0ppm P) and non mycorrhizal plants subjected to 0.44, 0.88 and 1.68 ppm P (Fig. 1).

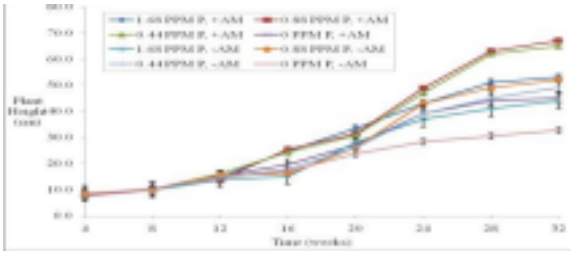

Fig. 1. Effect of arbuscular mycorrhiza fungi and P on the plant height (cm) of rough lemon (*Citrus jambhiri*) seedlings

In low nutrient sand: soil media, arbuscular mycorrhizal seedlings had higher plant height than to non-mycorrhizal seedlings in both sterilized and unsterilized media (Fig. 2.0). There was no significant difference in plant height between the mycorrhizal treatments, whether in sterilized or un-sterilized media. Non-mycorrhizal seedlings raised in sterilized media had significantly higher plant height than non mycorrhizal seedlings raised in unsterilized media in lemon seedlings (Fig. 2).

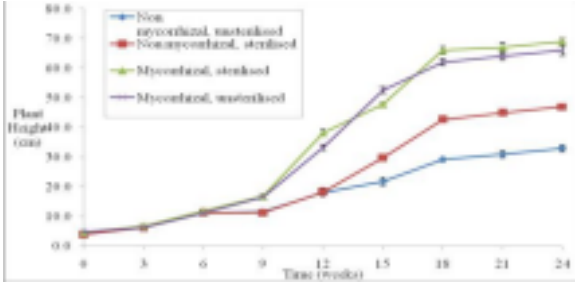

Fig. 2. Effect of arbuscular mycorrhiza fungi and media condition on plant height (cm) of rough lemon (*Citrus jambhiri*) seedlings

Plant biomass

Mycorrhizal rough lemon seedlings raised in both sterilized and unsterilized media had significantly higher leaf number, leaf and root fresh and dry weights and leaf area than non-mycorrhizal plants under both sterilized and unsterilized media. There was no significant difference between all lemon treatments in stem girth and stem fresh weights. There was no significant difference in all parameters between mycorrhizal plants raised in either sterilized or unsterilized media. Non mycorrhizal plants raised in sterilized media had significantly higher leaf and root fresh weight compared to non-mycorrhizal plants raised in unsterilized media (Table 1).

Table 1. Effect of arbuscular mycorrhiza fungi on the leaf number, stem girth, biomass and leaf area of rough lemon (*Citrus jambhiri*) seedlings

Treatment	Leaf No.	Fresh Weight (g)		Dry Weight (g)		Leaf Area (cm^2)	
		Leaf	Stem	Root	Leaf	Stem	Root
-AM, -ST	26.5b	4.5c	6.7a	11.8c	1.1b	1.3b	1.7b	217.4b
-AM, +ST	29.6b	4.8b	7.1a	12.4b	1.3b	1.4ab	2.3b	260.3b
+AM, -ST	35.3a	5.1a	7.2a	15.2a	1.8a	1.5a	3.1a	326.0a
+AM, +ST	39.0a	5.2a	7.2a	15.5a	1.8a	1.4ab	3.4a	344.4a
LSD($p \leq 0.05$)	5.3	0.2	0.6	0.4	0.3	0.1	0.5	44.8
CV (%)	10	14.4	9.7	11.5	10.8	7.4	7.8	9.1

Mycorrhizal root colonisation

Mycorrhizal seedlings had significantly higher root colonisation than non-mycorrhizal seedlings (Table 2.0). There was no significant difference in% root colonisation between mycorrhizal seedlings held in both sterilized and non-sterilized media (Table 2). Non-mycorrhizal plants held in unsterilized media had low mycorrhizal colonisation% while that held in sterilized media did not have any root colonisation (Table 2.0).

Table 2. Effect of media sterilization on mycorrhiza spore number at the beginning and at the end of the experiment period.

	Spores per 25 gram soil sample	
	Beginning	End
	Papaya	Lemons
+AM, +ST	0	676 ± 29	898 ± 48
+AM, -ST	68 ± 8[z]	777 ± 36	856 ± 39
-AM, +ST	0	0	0
-AM, -ST	57 ± 17	158 ± 16	183 ± 31

[z]Means ±SE (N=6)

Leaf Nutrient content

Mycorrhizal seedlings had significantly higher N, P and K% compared to non mycorrhizal seedlings. There was no significant difference in Ca and mg content between all treatments. Mycorrhizal seedlings had significantly higher P and K% compared to non mycorrhizal seedlings. There was no significant difference in N, Ca andmg% between all treatments (Table 3.0).

Table 3. Effect of arbuscular mycorrhiza fungi and planting media on the% leaf nutrient content of rough lemon (*Citrus jambhiri*) seedlings

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
-AM-ST	2.0 ± 0.1[z]	0.2 ± 0.05	2.1 ± 0.2	2.8 ± 0.1	1.6 ± 0.1
-AM+ST	2.0 ± 0.1	0.3 ± 0.07	1.9 ± 0.2	3.1 ± 0.2	1.7 ± 0.2
+AM-ST	2.3 ± 0.1	0.4 ± 0.05	2.6 ± 0.1	3.0 ± 0.1	1.6 ± 0.1
+AM+ST	2.3 ± 0.2	0.4 ± 0.04	2.6 ± 0.1	3.1 ± 0.1	1.6 ± 0.2

[z]Means ±SE (N=6)

Discussion

Results from this study indicate that AM fungal inoculation improves growth of lemons, passion fruits and papaya. The improvement occurred through increase in plant height, leaf number and leaf area, increased biomass accumulation (fresh and dry weights) and improved nutrient uptake. Many researchers have also reported the benefits of arbuscular mycorrhiza on growth and biomass accumulation in plants. Mycorrhiza inoculation was found to increase the plant height, stem diameter and leaf number of sweet corn in USA (Tas, 2014). Similar observations were made by Al-Karaki (2013) in sour oranges and Suri and Choudhary (2013) in soybeans.

The improved performance of mycorrhizal seedlings can be attributed to improved efficiency of phosphorus uptake as evidenced by increased phosphorus accumulation in the leaves. In papaya study in India, leaf petiole of mycorrhizal plants recorded higher total phosphorus (0.42 – 0.63%) as compared to control (0.35%) plants (Kadhe and

Rodrigues, 2009). A significant increase in shoot P concentration was also observed when *L. usitatissimum* was inoculated with *Glommus mosseae* or *G. intraradices* and their combination (Rydlová *et al*., 2011). The experiments were set up in either sand or a mixture composed of sand and nitrosol (1:1 vol/vol), both of which had low nutrient content. Research shows that under such conditions, AM fungi provides a very effective pathway by which P can be scavenged from large volumes of soil and rapidly being delivered to cortical cells within the root (Smith and Smith, 2011). This was attributed to individual fungal hyphae having much smaller diameters than roots, therefore allowing access to narrower soil pores and increasing the soil volume explored (Schnepf *et al*., 2011).

In this study, mycorrhizal seedlings had greater root mass compared to un-inoculated seedlings, as indicated by greater root fresh weight. Likewise, the extent of mycorrhizal root infection was significantly greater in inoculated seedlings than in un-inoculated seedlings. It is expected that this greater mass of mycorrhizal roots corresponded to greater absorptive surface area for nutrients and water.

In experiments undertaken in sand culture under various P levels, mycorrhizal inoculation combined with moderate amount of P provided the highest growth response. Mycorrhizal plants subjected to high P content (1.68 ppm) initially had the highest increase in plant height. However, there was a reduction in plant height in the high P experiment at the end of the experiment period. At the end, there was no significant difference between the myorrhizal plants that received high P and the non-mycorrhizal plants that received similar high P or slightly lower P amount (0.44 and 0.88 ppm P). This indicates that the high phosphorus content in the presence of arbuscular mycorrhiza became deleterious to plant growth. A study in sunflower also found that treatment combination of mycorrhiza and 200kg P/ha and nonmycorrhizal 200kg P/ha combination did not show significant difference in terms of seed yield of sunflower (Vaseghmanesh *et al*., 2014).

In this study mycorrhizal inoculation increased the leaf nitrogen content in rough lemon seedlings. Nitrogen uptake was also significantly increased in mycorrhizal chickpea plants in Pakistan (Yaseen *et al*., 2012). Like in the case of phosphorus, the major benefit of mycorrhiza in increasing uptake of N to plants was by availing greater soil exploration and supply to host roots (Sundar *et al*., 2010).

In this study potassium uptake was increased in lemon, papaya and avocado seedlings. This is consistent with pawpaw study in India which showed that total potassium content of leaf petiole was higher in mycorrhizal plants and ranged from 2.68 - 4.39% as compared to non-mycorrhizal plants (2.26%) (Kadhe and Rodrigues 2009). Uptake of K was also increased by AMF inoculation in cowpea and sorghum (Bagayoko *et al*., 2000). This can be attributed to greater soil exploration and increasing supply to host roots. Further increased K levels in mycorrhizal plants may be attributed to the fact that AM fungi binding soil particles to each other and to the roots, which is beneficial for the nutrient uptake (Estrada-Luna *et al*., 2000).

In the study in sand: nitrosol media, mycorrhizal plants did not differ significantly, in all measured parameters, whether in sterilized or unsterilized media. This indicates that mycorrhizal inoculation played a greater role in the observed plant performance than media sterilization. Un-inoculated seedlings in this study performed poorly in both sterilized and un-sterilized media. However, un inoculated seedlings held in sterilized media performed better that those held in unsterilized media. This could be attributed to elimination of all organisms in the media by sterilization. This can be an advantage through elimination of harmful micro organisms in the media and could have contributed to the improved performance of un-inoculated seedlings in sterilized media.

On the other hand, lack of media sterilization can be an advantage because beneficial micro-organisms are not eliminated. In the un-sterilized seedlings, a small percentage of mycorrhizal root infection was observed. This was expected to have proved beneficial by antagonizing against harmful microbes in the media as reported by Elsen *et al.* (2003).

The presence of mycorrhizal infection in the roots of un-inoculated seedlings raised in un-sterilized media suggests the availability of AM fungi in native soils in the tropics. In this study, unsterilized media had a small quantity of mycorrhizal spores at the beginning of the experiment. This is an indication of the low level of mycorrhization of native soils in Kenya and explains why non mycorrhizal seedlings performed poorly This confirms the report by Wamocho (1998) that in fruit orchards in Kenya, AM fungal spores and the mycorrhizal infection of fruit tree roots are low. Likewise, evidence from a survey of 41 tree species in five nurseries in Ethiopia and Somalia suggest that naturally mycorrhizal formation, even in unsterilized soils can be sparse (Michelson, 1992).

References

Al-Karaki GN. 2013. The effect of arbuscular mycorrhizal fungi on the establishment of sour orange (*Citrus aurantium*) under different levels of phosphorus. VII. International symposium on mineral nutrition of fruit crops book series. Acta Horticulturae **984,** 103-108.

Chebet D, Kariuki W, Wamocho L, Rimberia F. 2020. Effect of Arbuscular mycorrhizal inoculation on growth, biochemical characteristics and nutrient uptake of passion fruit seedlings under flooding stress. International Journal of Agronomy and Agricultural Research (IJAAR) **16(4),** 24-31.

Chebet DK. 2020. Effect of Arbuscular Mycorrhizal inoculation on the growth and performance of tropical fruit seedlings under saline, flooding and nutrient stress. PhD Thesis, Jomo Kenyatta University of Agriculture and Technology, Kenya pages 55-119.

Cruz AF, Ishii T, Kadoya K. 2000. Effect of arbuscular mycorrhizal fungi on tree growth, leaf water potential and levels of 1-aminocyclopropane-1-carboxylic acid and ethylene in the roots of papaya under water stress conditions. Mycorrhiza **10,** 121-123.

Elsen A, Baimey H, Swennen R, De Waele D. 2003. Relative mycorrhizal dependency and mycorrhiza nematode interaction in banana cultivars differing in nematode susceptibility. Plant and Soil **256,** 303-313.

Estrada BE, Aroca R, Barea JM, Ruiz-Lozano JM. 2013. Native arbuscular mycorrhizal fungi isolated from a saline habitat improved maize antioxidant systems and plant tolerance to salinity. Plant Science **201,** 43-51.

Fidelibus MW, Martin CA, Stutz JC. 2001. Geographic isolates of *Glomus* increase root growth and whole-plant transpiration of *Citrus* seedlings grown with high phosphorus. Mycorrhiza **10,** 231-236.

Khade SW, Rodrigues BF. 2009. Studies on Effects of Arbuscular Mycorrhizal (Am.) Fungi on Mineral Nutrition of *Carica papaya* L. Notingham Botanicaland Horticulture Agrobot. Cluj **37(1),** 183-186.

Mason PA, Wilson J. 1994. Harnessing symbiotic associations: vesicular-arbuscular mycorrhizas, p 165-175 In: Leakey, R.R.B., Newton, A.C. (Eds.), Tropical trees: potential for domestication and the rebuilding of forest resources. HMSO, London.

Michelson A. 1992. Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia. For. Ecol. Manage **48,** 335-344.

Millner PD, Kitt DG. 1992. The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. Mycorrhiza **2,** 9-15.

Muok OB, Ishii T. 2006. Effect of arbuscular mycorrhizal fungi on tree growth and nutrient uptake of *Sclerocarya birrea* under water stress, salt stress and flooding J. Jap. Soc. Hortic. Sci **75,** 26-31.

Rutto LK, Mizutani F, Asano Y, Kadoya K. 2002a. Effect of inoculation with arbuscular mycorrhizal (AM) fungus on phosphorus nutrition in loquat seedlings. Bull. Exp. Farm Fac. Agr., Ehime Univ **24,** 1-7.

Rutto LK, Mizutani F, Asano Y, Kadoya K. 2002b. Effect of root-zone flooding on mycorrhizal and non-mycorrhizal peach seedlings. Scientia horticulturae **94,** 285-295

Rydlová J, Püschel D, Sudová R, Gryndler M, Mikanová O. Vosátka M. 2011. Interaction of arbuscular mycorrhizal fungi and rhizobia: Effects on flax yield in spoil-bank clay. Journal of Plant Nutrition and Soil Science **174,** 128-134.

Schnepf A, Leitner D, Klepsch S, Pellerin S, Mollier A. 2011. Modelling phosphorus dynamics in the soil-plant system. In Bünemann EK, Obserson A, Frossard E, eds, Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling pp 113-133 Heidelberg: Springer.

Sieverding E. 1991. Vesicular-arbuscular mycorrhiza management in tropical ecosystems. GTZ. Eschborn, p 371.

Smith SE, Smith FA. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystems scales. Annual Review of Plant Biology **63,** 227-250.

Sundar SK, Palavesam A, Parthipan B. 2010. Effect of native dominant AM fungus and PGPRs on growth and biochemical characteristics of medicinally important Indigofera aspalathoides Vahl. ex. DC. International Journal Biology and Biotechnology **7,** 59-67.

Suri VK, Choudhary AK. 2013. Effects of vesicular arbuscular mycorrhizae and applied phosphorus through targeted yield precision model on root morphology, productivity, and nutrient dynamics in soybean in an acid alfisol. Comm Soil Science Plant Analysis **17,** 2587-2604.

Tas B. 2014. Effect of the Mycorrhiza Application on the Agronomical Properties of Sweet Corn Varieties. Journal of Agriculture and Allied Sciences **3(2),**

Vaseghmanesh T, Kordlaghari KP, Neia M, Kelidari A. 2014. The response of yield components of sunflower to mycorrhiza inoculation and phosphorus fertilizer Annals of Biological Research **4(3),** 101-104.

Wamocho LS. 1998. Studies on the use of vesicular arbuscular mycorrhizal fungi for fruit production in Kenya. PhD Thesis, Jomo Kenyatta University of Agriculture and Technology.

Yano-Melo AM, Saggin Jr OJ, Maia LC, Melo NL. 1999. Effects of arbuscular mycorrhiza fungi on the acclimatization of micropropagated banana plantlets. Mycorrhiza **9,** 119-123.

Yaseen T, Burni T, Hussain F. 2012. Effect of Arbuscular Mycorrhizal inoculation on nutrient uptake, growth and Productivity of chickpea (*Cicer arietinum)* varieties. International Journal of Agronomy and Plant Production ***3*(9),** 334.345.