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Arbuscular Mycorrhizal Fungi (AMF) and NPK Fertilisation Rate on the Growth of Soursop (*Annona muricata* L.) Seedlings

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ABSTRACT

Soursop (*Annona muricata* L.) has been increasingly cultivated in Malaysia. In view of the importance of the crop, there is a need to understand the effects of agronomic management such as NPK fertiliser application and the inoculation of arbuscular mycorrhizal fungi (AMF) on soursop growth and nutrient uptake. Therefore, this study aimed to determine the effects of AMF and fertiliser on the growth and nutrient uptake of soursop seedlings. The experiment was conducted under glasshouse condition in UPM, Serdang, Selangor, Malaysia using completely randomised design (CRD) with five treatments which comprised AMF inoculations with full and half dose of NPK 15:15:15 fertilisation. The treatments were: T1- Control (without AMF and NPK fertiliser); T2- AMF only; T3- AMF with 50% NPK fertiliser; T4- AMF with full amount (100%) NPK fertiliser; and T5- full amount (100%) NPK fertiliser only (without AMF). Plant growth, soil microbial population AMF development, 'nutrient' status of the plants and soils were determined after the 8th week of planting. Soursop seedlings grown in soils treated with 100% NPK 15:15:15 fertiliser (T5) had the highest chlorophyll content, root volume, N uptake and soil N and K. Surprisingly, inoculation of AMF (T2) had similar effects to that of NPK 15:15:15 fertiliser (T5) on plant P uptake. Mycorrhizal spore production even at low numbers (66 spores/10 g soil) indicated probable symbiotic interaction with soursop seedling roots at the nursery stage.

Key words: Arbuscular mycorrhizal fungi, fertiliser rate, soursop, seedlings, symbiosis.

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INTRODUCTION

Malaysia's tropical climate allows various fruits to be grown throughout the year. Soursop (*Annona muricata* L.), a native fruit tree to South America, has been increasingly planted in Malaysia owing to its nutritional value and great economic potential and high demand, either for medicinal or food products. However, soursop cultivation in Malaysia is still new and information on soursop cultivation on Malaysian soils is limited.

Soursop can grow in soils ranging from sandy to clay loam, with well-drained deep soil favouring their growth as good aeration enables development of roots (Hashim and Khalid 1993; Pinto and Silva 1994; Pinto *et al*. 2005). Pinto and Silva (1994) and Pinto *et al.* 2005 suggest that suitable soil pH for soursop is between 6.0 and 6.5, meanwhile Mohd Khalid *et al*. (1993) suggest that soil pH of between 5.0 to 6.5 is favourable for soursop growth. Soursop seedlings require 1-2 g of mixed fertiliser (15N:15P:15K) once a month (Hashim and Khalid 1993) and they are normally left to grow in polybags containing potting mix (growth media) until the age of 6 months before being transferred to the field.

Inoculation of beneficial microorganisms such as mycorrhiza can be explored for soursop cultivation at the nursery stage in Malaysia. Arbuscular mycorrhizal fungi (AMF) has been generally known for its symbiotic relationship with over 90% of plant species (Bonfante and Genre 2010). Through this symbiosis, AMF improves phosphorus uptake, increases drought tolerance and disease resistance of the host plants. The AMF can increase the solubilisation of phosphorus (P), which is normally fixed in most tropical soils, and can increase the uptake of nutrients and water by plants thus improving plant vigour, yield and bioactive compound contents (Beltrano and Ronco 2008; Solaiman *et al*. 2014; Caser *et al*. 2019).

Previous studies on soursop gave positive results when AMF was applied on soil. Application of AMF to soursop seedlings has been reported to increase growth (Silva *et al*. 2008; Chu *et al*. 2001), and increase plant tolerance to lesions by nematode, *Pratylenchus coffeae* (Angélica 2003). Silva *et al*. (2008) found that soursop seedling growth was improved with 10% organic manure with AMF (*Acaulospora longula* Spain & Schenck and *Gigaspora albida* Schenck & Smith). However, *G. albida* did not experience symbiosis with soursop roots when the soil was fertilised. The potential for mycorrhiza to be used as a management tool for soursop cultivation with NPK fertiliser is not known.

Since the agronomic aspects of soursop cultivation in Malaysia using soil beneficial microorganisms are not well understood, the current study was undertaken with the objective of determining the effects of AMF combined with NPK 15:15:15 fertiliser application on soursop growth, soil nutrient availability and plant uptake as well as AMF development.

MATERIALS AND METHODS

Experiment Setup

The experiment was carried out under glasshouse conditions located at the Complex 11A, Faculty of Agriculture, UPM Serdang, Malaysia using 4-month old soursop seedlings. The plants were grown in a growth media containing two amounts of fertiliser (50% and 100%) and inoculated with and without AMF (*Glomus mossea*). The experimental design was a completely randomised design (CRD), replicated four times. The treatments were T1- control; T2- AMF only; T3- AMF +50% NPK (15:15:15); T4- AMF +100% NPK (15:15:15); T5- 100% NPK (15:15:15) only. Topsoil, sand and organic matter used for planting media were air dried and sieved to 2-mm size and autoclaved to remove all spores and inoculants before planting. Topsoil, sand and organic matter were mixed and 1.6 kg were placed in each of the 20 polybags (polibag size of 8 in x 8 in) with the ratio of 2:1:1. The topsoil, sand and organic matter were analysed for soil pH (H_2O) and C, N, P and K were determined before planting. The soil mixtures were sterilised at a temperature of 121˚C in an autoclavefor 1 h.

The roots of the 4-month-old soursop seedlings (purchased from nursery) were washed and cleaned to remove the soil from previous planting and transferred into the polybag according to the treatment with one plant per polybag. For the control, no AMF and fertiliser were applied. Approximately 20g of AMF (60 spores/10g) were added into the AMF treatments while 20g of autoclaved soil were added into the treatments without AMF in order to provide the same soil conditions. The treated seedlings were evaluated after 60 days of transplanting.

Plant Growth and Nutrient Analysis

Soursop plant height was measured by using a measuring tape. Plant chlorophyll was measured using a Minolta SPAD 502 Chlorophyll Meter by selecting fully opened leaves from the top of th shoot. Fresh leaves, stem and root were weighed to obtain their fresh weight. The fresh roots were rinsed with tap water to clean off the soil particles and analysed using WinRHIZO Root Scanner Analyser for root analysis. Leaves, stem and root were dried in the oven using a separate brown paper at a temperature of 50˚C for about 3-4 days. Dry weight of leaves, stem and root were determined and recorded. The nutrient content in plant tissue was analysed by using Kjeldal procedure (Horneck and Miller 1998) for nitrogen concentration while dry ashing method (Jones and Warner, 1969) was used to extract phosphorus and potassium in plant tissue.

Determination of Soil Nutient

Total N was determined by using the Kjeldahl method (Bremner 1960) and the soil filtrates were determined using Auto-Analyzer. Soil available P was determined by using Bray 2 method (Bray and Kurtz 1945) while exchangeable K for every sample was determined by using the modified shaking method (Schollenbereger and Simon 1945).

Determination of AMF Spore and Soil Microbial Population

The number of AMF spores in soil was determined by using the wet sieving and decanting technique (Schenck 1982). About 10 g of fresh soil sample were suspended in 100 mL of water. The suspension was decanted through a 250 µm at the top, 106 µm at the middle and 45 µm at the bottom. Microbial population such as bacteria, fungi and actinomycetes were determined by using total plate count technique (Parkinson *et al.* 1971). After incubation at 28˚C in the incubator for 2-4 days, colonies formed were counted and population was calculated as colony forming units (cfu) per dry soil. Only petri dishes containing 30-300 colonies were counted. The microbial population per 10 g of soil was determined using the following formula:

$$\text{Number of microorganisms g}^{-1}\text{ dried soil} = \frac{\left(\frac{(\text{Colony number per petri dish} \times \text{dilution factor})}{\text{Total weight of fresh soil}}\right)}{\left(1 - \frac{\text{moisture content \%}}{100\%}\right)}$$

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (Ver 9.4; SAS Institute Inc., Cary, NC, USA, 2013) and mean separations using Tukey's honestly significant difference (HSD) test at α=0.05.

RESULTS AND DISCUSSION

Properties of Growth Medium

The mixtures of soil, sand, topsoil and organic soil were analysed separately for pH and nitrogen (N), phosphorus (P) and potassium (K) contents prior to planting. The results are shown in Table 1.

TABLE 1
Properties of growth medium (N, P, K concentration and pH)

Treatment	Nutrient concentration			pH
	N (%)	P (mg/kg)	K (mg/kg)	
Topsoil	0.058	24.3	0.06	6.98
Sand	0.016	141.3	NA	6.71
Organic matter (OM)	0.496	1044	1.67	7.29
Growth media mixture(2:1:1)	0.192	499.5	0.45	7.46

NA=Not available

Data presented in Table 2 show that AMF treatments with and without fertiliser application had significant effects (P≤0.05) on plant height, chlorophyll content and root volume. Inoculation of AMF with 50% NPK 15:15:15 fertiliser (T3) showed the highest plant height compared to 100% NPK fertiliser (T4) and control (T1). This implies that AMF is able to function symbiotically in soil and stimulate plant growth when lesser amounts of fertiliser (half dosage) were applied. Plant chlorophyll content was highest in treatment with 100% fertiliser (40.69 SPAD unit), followed by plants in soil inoculated with AMF combined with 50% fertiliser (39.37 SPAD unit). However, no significant effects (P ≥ 0.05) were noted for shoot dry weight and root dry weight.

Treatment with 100% fertiliser produced a higher root volume (4.51 cm3) compared to control. Similar results were obtained by Hodge *et al.* (2000) who also showed that the application of AMF enhanced root development and that AMF was capable of promoting root growth. In general, nutrient uptake by plant roots play the most important role in nutrient efficiency which also depends on root size and morphology (Gutshick 1993).

TABLE 2
Plant and root growth measurements in response to AMF and fertilisation

Treatment	Plant height (cm)	Plant biomass (g)		Chlorophyll content (SPAD units)	Root volume (cm^3)
		Shoot	Root		
T1 (Control)	25.13 bc	1.16a	0.35 a	36.80 bc	2.61 b
T2 (AMF)	27.80 ab	1.34a	0.46 a	35.18 c	3.53 ab
T3 (AMF + 50% fertiliser)	**28.83 a**	1.15a	0.31 a	39.38 ab	2.18 ab
T4 (AMF + 100% fertiliser)	24.13 c	1.20a	0.30 a	35.43 c	2.21 b
T5 (100% fertiliser)	28.13ab	1.43a	0.45 a	**40.69 a**	**4.51 a**
Trt (Pr > F)	0.0394*	0.4086^{ns}	0.0723^{ns}	0.5289^{ns}	0.0052**

Means with the same letter in a column are not significantly different at P>0.05* by Tukey's HSD test.
*Significant (P≤0.05); **Very Significant (P≤0.01); ns – not significant (P>0.05)

The AMF and fertilisation treatments had a significant effect (P≤0.05) on N concentration in plant as shown in Table 3. Treatments T3 and T5 showed higher N concentrations in plant at 3.11% and 2.78%, respectively, compared to

control (1.67%). This could be related to the high chlorophyll content of both treatments. The high uptake of N from the soil led to accumulation of N in plant tissue is a result of higher plant photosynthetic activities as reflected by the higher chlorophyll content. However, no significant effects (P>0.05) were noted for AMF or fertiliser on P concentration and K concentration in soursop plants.

TABLE 3
Plant nutrient concentration and uptake in response to AMF and fertilisation

Treatment	Nutrient concentration (%)			Nutrient uptake (g/plant)		
	N	P	K	N	P	K
T1 (Control)	1.67 c	0.24 a	3.50 a	3.03 b	0.31 c	4.82 a
T2 (AMF)	1.91 b	0.25 a	3.13 a	3.18 b	**0.44 a**	5.61 a
T3 (AMF + 50% fertiliser)	**2.78 a**	0.27 a	3.27 a	3.71b	0.39 ab	5.23 a
T4 (AMF + 100% fertiliser)	2.20 b	0.22 a	3.29 a	3.32 b	0.36 ab	5.00 a
T5 (100% fertiliser)	**3.11 a**	0.23 a	3.45 a	**5.85 a**	**0.44 a**	6.49 a
Trt (Pr > F)	<0.0001****	0.2951^{ns}	0.3353^{ns}	0.0098*	0.0481*	0.3511^{ns}

Means with the same letter in a column are not significantly different at P>0.05* by Tukey's HSD test.
*Significant (P≤0.05); **Very Significant (P≤0.01); ns – not significant (P>0.05)

TABLE 4
Soil properties in response to application of AMF and fertiliser

Treatment	Soil N (%)	Soil P (mg/kg)	Soil K (mg/kg)	pH
T1 (Control)	0.07b	343.00 b	0.44 bc	7.42 a
T2 (AMF)	0.08 b	358.88 b	0.45 abc	7.56 a
T3 (AMF + 50% fertiliser)	0.07 b	371.70 ab	0.41 c	7.58 a
T4 (AMF + 100% fertiliser)	0.08 b	**400.17 a**	0.47 ab	7.59 a
T5 (100% fertiliser)	**0.15 a**	336.93 b	**0.48 a**	7.41 a
Trt (Pr > F)	0.0144*	0.0191*	0.6872^{ns}	0.0783^{ns}

Means with the same letter in a column are not significantly different at P>0.05* by Tukey's HSD test.
*Significant (P≤0.05); **Very Significant (P≤0.01); ns – not significant (P>0.05)

The uptake of N by soursop plant in the treatment with 100% fertiliser (T5) was significant ($P \leq 0.05$) with highest uptake (5.85g/plant) compared to other treatments. In comparison to N uptake, 100% fertiliser (T5) and AMF (T2) or combination treatments (T4, T5) gave significantly ($P \leq 0.05$) higher P uptake compared to control. This indicates that nutrient uptake by soursop plant is more affected by NPK fertiliser than AMF symbiosis. In contrast, K uptake of plant, however, was to be insignificantly affected ($P \geq 0.05$) by the treatments. Indirectly, AMF could have improved the plant growth processes via root area exploration for greater water and nutrient uptake (Azizah 1999).

Significant effects ($P \leq 0.05$) of 100% NPK fertilisation treatments were exhibited in soil N, P and K (Table 4). Soil supplied with 100% fertiliser gave higher N concentration in soil (0.15%) compared to AMF treatments and control. Treatment with 100% fertiliser gave the highest exchangeable K in soil (0.48mg/kg) compared to control (0.44mg/kg) and the lowest in treatment with AMF and 50% fertiliser (0.41mg/kg). In contrast, P in soil was found to be the highest in treatment inoculated with AMF and combined with 100% fertiliser (400.17mg/kg) compared to others. This indicates that AMF is able to increase P availability in soil which is in line with previous findings (Sadhana 2014). However, there were no significant effects on soil pH between the treatments.

The soil inoculated with AMF showed the highest AMF spores (66 spores/10g soil) followed by the treatment with 50% fertiliser (51 spores/10g soil) and AMF with 100% fertiliser (27 spores/10g soil). The AMF sporulation was better in soil without fertiliser application. This observation was similar to the finding by Ortas (2003). Although the percentage of root infection could not be observed, the high number of spores in soil indicates positive AMF develoment resulting in a better symbiotic association with soursop plants which subsequently benefited the host plant. The AMF alone or in combination with 50% fertiliser flourished the fungal population compared to AMF with 100% fertiliser treatment and fertiliser alone.

Soil treated with 100% fertiliser had the least total bacterial population compared to control or other AMF inoculated treatments. This finding is similar to the study of Medina *et al.* (2003) where the application of mycorrhiza increased the population density of bacteria in the rhizosphere. Mycorrhizosphere effects that could occur include the stimulation of growth and activities of AMF related microorganisms like mycorrhizal helper bacteria (MHB) and phosphate solubilising bacteria (PSB). Mycorrhizal treatment had significant effects on the fungal and bacterial population but not on the actinomycetes population. The data are presented in Table 5.

TABLE 5
Microbial population including mycorhizal development in response to application of AMF and fertiliser

Treatment	Number of AMF spore counts / 10 g of soil	Fungal population	Bacterial population (Log $_{10}$ cfu/g)	Actinomycetes population
T1 (Control)	5 d	2.73 a	5.73 a	4.02 a
T2 (AMF)	**66 a**	2.65 a	5.67 a	3.97 a
T3 (AMF + 50% fertiliser)	51 b	2.50 a	5.62 a	3.73 a
T4 (AMF + 100% fertiliser)	27 c	2.10 b	5.74 a	3.66 a
T5 (100% fertiliser)	0 d	1.80 b	4.62 b	3.78 a
Trt (Pr > F)	<0.0001****	0.0005***	0.0002***	0.1035^{ns}

Means with the same letter in a column are not significantly different at P>0.05* by Tukey's HSD test.
*Significant (P≤0.05); **Very Significant (P≤0.01); ns – not significant (P>0.05)

DISCUSSION

Application of 100% NPK 15:15:15 fertiliser (T5) had significant effects on soursop growth in terms of plant height, chlorophyll content and root volume. Plant chlorophyll content, significantly improved in 100% NPK 15:15:15 fertiliser treatment. Mycorrhizal alone (T2) had similarly lower root volume and was similar in effect to that in soils treated with AMF + 50% fertiliser (T3). This result shows that AMF is able to function symbiotically in soil and stimulate plant photosynthesis when lesser amounts of fertiliser (50% fertiliser) are applied.

The application of AMF alone has been previously shown to enhance root development (Hodge *et al.* 2000). In general, the uptake of nutrients by the roots played the most important role in nutrient efficiency (Gutshick 1993) which also depended on root size and morphology. The application of AMF alone and full rate fertiliser only gave a significantly higher root volume compared to other treatments. The higher root volume indicates a wider area of soils for the roots to explore that help with the uptake of nutrients into the roots (Grant et al. 2005). The underlying mechanisms and factors resposible for AMF inability to function well in this study could be due to the amount of available nutrients in

the applied fertilisers. This might also be due to late inoculation of AMF as the soursop seedlings were only inoculated at 16 weeks, thereby reducing optimal AMF colonisation of plant roots.

Nutrients such as N, P and K composition in soil were greatly influenced by fertilisation as found in the highest concentrations when using 100% fertiliser treatment. N and K concentrations were highest in soils treated with full rate fertiliser. Meanwhile, the highest P concentration in soil was found in AMF with full rate fertiliser treatment compared to treatments with AMF alone and AMF with half rate fertiliser. This result indicates that AMF is able to increase P availability in soil which is in agreement with previous findings by Sadhana (2014). In addition, the increased amounts of soil nutrients might be due to the increase in microbial population (Rachel and Randy 2011).

Application of AMF species *Glomus mosseae* is envisaged to improve the uptake of poorly mobile nutrients such as N and immobile nutrients like P (Barea et al. 2005). However, in this study, the highest significant N and P nutrient uptake by soursop plants was observed in the treatment with full rate (100%) fertiliser. This result indicates that nutrient uptake by soursop plants responded more to NPK fertiliser than to AMF symbiosis. In terms of nutrient concentration in plant tissues, the application of AMF did not affect P and K but it did for N uptake. Indirectly, AMF could have improved the plant growth processes via root area exploration and root volume for more water and nutrients uptake (Azizah 1999). The concentration of N in plant tissue was highest when half rate fertiliser combined with AMF was applied to soils. This could be related to the high chlorophyll content of both treatments and the uptake of N by plants in full rate fertiliser treatment. The high uptake of N from the soil accumulated N in plant tissue, a result of higher plant photosynthetic activities due to high chlorophyll content in plant leaves.

Inoculation of AMF significantly affected the soil microbial population and the number of AMF spores in soil. Normally, AMF hyphal glomalin and plant root exudates are able to stimulate the microbial population in soil (Johannson *et al.* 2004). These AMF sporulated better in soil without fertiliser application. This is in line with Ortas (2003). Although the percentage of root infection could not be observed, the high spore numbers in soil as shown in Table 5 indicate positive AMF development that resulted in a better symbiotic association with soursop plants which benefited the host plants. Inoculation of AMF was able to stimulate the population of bacteria in soil compared to application of 100% fertiliser which is in line with the study of Medina *et al.* (2003) which found that the application of mycorrhiza increased the population density of bacteria in the rhizophere. The densities of AMF spores and hyphae have been observed to decrease under different soil and climatic conditions by the addition of mineral P fertiliser (Douds and Millner 1999; Kahiluoto *et al.* 2001). The presence of mycorrhizophere effects could be due the stimulation of growth and activities of AMF-related microorganisms such as mycorrhizal helper bacteria (MHB) and phosphate solubilising bacteria (PSB). These bacteria could have modified

the AMF mycelium environment and benefited AMF in terms of enhancing its development and formation especially mycelium thus promoting root growth and soil conditions such as pH and P availability (Barea *et al*. 2005).

Fertilisers applied to soil at the highest amount (100%) either alone or with AMF did not stimulate bacterial and AMF spores. This could be due to less favourable soil conditions for AMF mycelium development that created non conducive mycorrhizospheric effects on other soil microbes. The inconsistency in shaping AMF communities could be affected by AMF species identity (Wang *et al.* 1993), plant community composition (Smith and Read 2008) and soil pH (Rousk *et al*. 2010). However, treatments with AMF with full rate fertiliser did not stimulate the fungal population. The only mycorrhizal treatments that stimulated fungi population were treatment with AMF with 50% fertiliser and AMF. This may have been a result of the high rate of fertiliser application which may have led to changes in soil microbial population over the short period which in turn disrupted the relationship between AMF and plants (Bradley *et al*. 2006).

Most of the previous studies on soursop and its symbiosis with AMF involved *Acaulospora* sp. and *Gigaspora* sp. To date, there has been no *Glomus* sp. study reported on soursop in the tropics (Silva *et al.* 2008). Therefore, the results found in this study especially on non-availability of the effects of AMF root infection might be due to the plant not establishing a successful symbiotic association with AMF species tested; this warrants further research. Further, the application of only 20 g of AMF inoculation (60 spores/10g) of the *Glomus mosseae* species to soursop seedlings might not have been sufficient,and that too it was applied at the late stage of 4 months. Therefore, the effect of the infection might have been delayed and the extent of AMF colonisation may have been insufficient to prove the effectiveness of AMF application on soursop plant growth.

CONCLUSIONS

Soursop seedlings grown in soils treated with 100% NPK 15:15:15 fertiliser (T5) had the highest chlorophyll content, root volume, N uptake and soil N and K. Mycorrhizal alone (T2) had similar root volume compared to that in AMF + 50% fertiliser (T3). Mycorrhizal spores and soil microbial population were not stimulated in soils with 100% fertiliser. Interestingly, inoculation of AMF (T2) had similar effects to that of NPK 15:15:15 fertiliser (T5) on plant P uptake. Mycorrhizal development even at low spore production (66 spores/10 g soil), indicated probable symbiotic interaction with soursop seedlings roots at the nursery stage. It is recommended that more trials on inoculation of AMF species on soursop be carried out and that they should be inoculated in the earlier stage of seed germination rather than older plant seedlings to ensure succsesful colonisation and symbiosis.

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