Phosphorus Requirement for Colonization by Arbuscular Mycorrhizal Fungi (AMF) and Effect of AMF Inoculants on Growth of Perennial Crops and Agroforestry Trees

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Abstract: In most tropical soils, phosphorus is deficient and high costs of phosphorus fertilizer made it difficult for smallholder farmers to use it when needed. Arbuscular mycorrhizal fungi is known to improve particularly P in P deficient soils. However, response of plant species to mycorrhizal fungi inoculation and application of different rates of P varies. Therefore, this study was conducted to investigate the effect of phosphorus (P) concentrations on arbuscular mycorrhizal fungi (AMF) colonization and growth of two perennial crops (*Catha edulis* and *Ensete ventricosum*) and four multipurpose agroforestry trees (*Cordia africana, Croton macrostachyus, Erythrina brucei* and *Millettia ferruginea*). The experiment was conducted in a glasshouse. The treatment consisted of 0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg P/g substrate and three species of AMF. The experiment was laid out in CRD design in a factorial arrangement. The results showed that plant growth parameters (shoot length and dry weight) and P uptake increased significantly after inoculations with AMF, namely *Rhizophagus clarus,* and *Rhizophagus intraradices*, and the mixed AMF species. Results on effect of P application on total mycorrhizal dependency (MD) of the studied crops and agroforestry tree species showed that maximum (41.71%) MD value was recorded for *Rhizophagus clarus* in khat (*Catha edulis Forsk.)*, followed by 34.85 and 34.45% MD values for the same *Rhizophagus clarus* in Birbira (*Millettia ferruginea)* and Bisana (*Croton macrostachyus)*, respectively. The next MD values, ranging from 2.57% for *Catha edulis* to 30.67% in *Ensete ventricosum*, were recorded for inoculation with the mixed AMF species. The least MD values of 3.51, 16.46, 10.51, 7.71, 4.34, and 14.32 were recorded for treatments with *Rhizophagus intraradices* for all plant species (*Catha edulis, Cordia africana, Croton macrostachyus, Ensete ventricosum, Erythrina brucei and Millettia ferruginea)* under the study respectively. Optimum P concentrations for maximum benefits from the AMF symbiosis in the aforementioned six plant species varied from 0.005 to 0.02 mg P g^{-1} substrate and the corresponding peaks of arbuscules, vesicles, percent colonization, and spore count per 50 cm^3 sand were noticed at similar P concentrations. Thus, the current research results revealed that the recorded plant growth peaks were attributed to AMF colonization of the perennial crops and agroforestry trees. Therefore, inoculating plant species with a suitable AMF inoculant could result in a benefit comparable to high P fertilizer input and lead to a significant cost saving from expenditure on inorganic P fertilizer. The information obtained on minimum P requirement for perennial crops and shade trees in Sidama agroforestry can form the basis for further pot/field experiments involving integration of chemical fertilizers with AMF

Keywords: Agroforestry; Crops; Inoculation; Phosphorus; Root colonization; Spore density; Trees.

1. Introduction

Agroforestry, a land use system/technology in which trees are deliberately planted on the same unit of land with agricultural crops, has been recognized as one of the most promising strategy for rehabilitating degraded areas and broadly practiced in Sidama Zone of Southern Nations, Nationalities and Peoples' Region (SNNPR), Southern Ethiopia. Some multipurpose shade trees play a vital role in the rural economy of the region. To meet the future demand for these trees and the perennial crops growing under these shade trees, their growth and productivity has to be hastened from the nursery stage onwards and their requirements for major fertilizers, like phosphorus, should be known. According to Tilman *et al.* (2002) and Foley *et al.* (2005), inappropriate and untimely application of fertilizers in agricultural fields generates several environmental pollution and soil problems.

Wrage *et al.* (2010) and de Carvalho *et al.* (2010) reported that side effects due to the practices of agro-ecosystem simplification, where the ecosystem services provided by the soil are increasingly bypassed. The perceived need for seeking alternatives to the current

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agricultural practices has resulted in an enhanced interest in agroforestry systems (Ingleby *et al*., 2007), which can conserve natural resources, improve environmental quality, rehabilitate degraded and deforested lands, and provide multiple outputs to meet the daily demands of the rural population (Pande and Tarafdar, 2004; Muleta *et al*., 2008). Under agroforestry, the needs for ecological sustainability can be reconciled with the needs for sustainable food production (Young, 1997).

Arbuscular mycorrhizal fungi (AMF) can rehabilitate degraded lands subjected to agroforestry systems (Mutuo *et al*., 2005; Cardoso and Kuyper, 2006). The common mycorrhizal network may further enhance the benefits of agroforestry through vertical niche expansion of AMF (Simard and Durall, 2004; Cavagnaro *et al*., 2005; Theuerl and Buscot, 2010). The low biomass production of agroforestry tree species in degraded areas can, therefore, be circumvented by the use of AMF (Shukla *et al*., 2009).

The key function of AM fungi is the exploration of the soil beyond the range of roots for better plant growth and nutrition (Oehl *et al*., 2002; van der Heijden *et al*., 2006). According to Jakobsen *et al.* (2005) and Ma and Rengel (2008), AMF have the potential to make crop cultivation successful in soils with low P level through effective exploitation of the P sources. The P level has been shown to significantly influence AMF colonization of crops and agroforestry trees (Koide, 1991; Covacevich *et al*., 2007).

To manage plant growth and productivity of agro-ecosystems, particularly agroforestry systems, knowledge of P requirement levels of the trees and crops practiced in a given area is mandatory. Therefore, the present study was conducted with the specific objective to investigate the effect of phosphorus (P) concentrations on arbuscular mycorrhizal fungi (AMF) colonization and growth of two perennial crops and four multipurpose agroforestry trees that grow in Sidama agroforestry practices.

2. Materials and Methods

2.1. Description of the Study Area

The study was carried out in the greenhouses of Hawassa University and Hawassa College of teacher education in the capital city of SNNPR located at about 275km form Addis Ababa.

2.2. Experimental Materials

In this study, seeds of selected plant species in Sidama agroforestry were used. Three native species of AM fungi isolated and purified were used as AMF inoculants. Dominant AMF were isolated from the rhizosphere soil of field grown trees, perennial and annual crops by wet sieving and decanting techniques of Gredman and Nicolson (1963).

2.3. Experimental Procedures

Trap culture was set using *Sorghum bicolor* as a host plant. After 5 months of growth, trap cultures were examined for efficiency of the isolates. Then the most vigorous species were selected for further culturing. To get the pure culture three successive inoculations on the same trap plant has been carried out.

Morphological taxonomic identification of spores (color, size, shape, cell wall layers, hyphal attachment, germination shield, etc) was checked to be matched with the description provided by the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM, 2006). Materials and inocula used in this study consisted of soil along with chopped root bits of *Sorghum bicolor*, spores, and extrametrical mycelia from trap culture pots.

2.3.1. Effect of P Concentrations on Plant Growth and P uptake due to AMF Inoculants

Experimental procedures 1: To study the effect of P concentrations on plant growth and P uptake after inoculation with AMF, separate experiments were carried out with two perennial crops and four most common multipurpose shade trees in the agroforestry. The trials consisted of six P concentrations (0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg P g^{-1} substrate) and three mycorrhizal treatments and un-inoculated plants (control). Thus, a total of 24 treatment combinations were involved per plant species, and each experiment was replicated three times. Seeds of *Catha edulis, Cordia africana, Croton macrostachyus, Ensete ventricosum, Erythrina brucei and Millettia ferruginea* were surface-sterilized with 2% sodium hypochlorite (NaOCl), washed five to six times with sterile distilled water and germinated at 30 ^{0}C in 20 cm top diameter plastic pots filled with 2 kg sterilized river sand.

At the time of sowing, 50 g of mycorrhizal inocula was applied to the hole in the pots where pre-germinated seedlings were individually transplanted. Phosphorus was applied to the pots at 0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg g^{-1} rates as KH_2PO_4. The potted plants were grown in greenhouse and were watered daily. One seedling was maintained per pot and half-strength Hoagland's solution in deionized water was applied at weekly interval. The composition of the Hoagland's solution was (0.51 g/L KNO_3, 0.246 g/L $Ca(NO_3)_2$, 0.245 g/L $MgSO_4.7H_2O$, 1.43 g/L H_3BO_3, 0.91 g/L $MnCl_2.7H_2O$, 0.11 g/L $ZnSO_4.5H_2O$, 0.04 g/L $CuSO_4.5H_2O$, and 0.04 g/L $H_2MoO_4.H_2O$). Pots were arranged in completely randomized design (CRD) and to reduce the risks of cross contamination, kept on separate benches, with a space of 40 cm between each treatment.

Data collection: Seedlings were harvested three months after transplanting and were analyzed for shoot length and dry weight by standard methods (Tanwar *et al*., 2013). Phosphorus uptake was recorded using molybdenum blue method according to Jackson (1973).

Mycorrhizal dependency (MD) was calculated according to Plenchette *et al.* (1983) as follows:

MD (%) = [(M-NM)/M] X 100

Where: M is the total dry biomass of mycorrhizal plant; NM is the total dry biomass of non-mycorrhizal plant.

2.3.2. Effect of P Application on AMF Colonization on Perennial Crops and Trees

Experimental procedure 2: To study the effect of P application on AMF colonization of the two perennial crops and the four component plants of the agro-forestry, 24 treatment combinations for each plant (1 plant X 6 p rates X 3 AMF species plus control) were replicated four times and six plants were maintained per replicate/pot (one *Ensete ventricosum* was grown per pot because of its broad canopy and large pseudostem).

Data collection: Two plants per pot (2 plants from 2 pots in the case of *Ensete ventricosum*) were harvested 1, 2, and 3 months after sowing to monitor formation of arbuscules and vesicles, and the colonization index was calculated and the spore concentration per 50 cm^3 sand was counted. Fine roots were cleared with 10% KOH and stained with acid fuchsin (0.01% in lactoglycerol) as reported by Phillips and Hayman (1970), and then colonization rates of arbuscules and vesicles were recorded. Colonization percentage was determined by gridline intersection method of McGonigle *et al.* (1990). Sporocarp and spores were isolated according to Gerdemann and Nicolson (1963), and were counted (mean of 40 counts for each subsample under field vision of the stereomicroscope was taken as number of spores/100g dry soil).

2.2. Data Analysis

All the data on plant growth were subjected to a one-way analysis of variance (ANOVA) for testing the effects of AMF inoculation and P application, and their interactions. The means were compared and ranked using Duncan's Multiple Range Test (DMRT) at 5% probability level. The means of the experiments were analyzed statistically using a general linear model for analysis of variance of completely randomized designs (CRD). Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 20.0).

3. Results

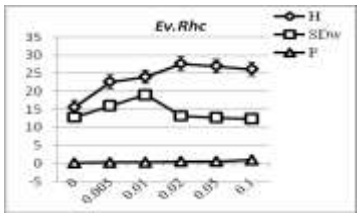
3.1. Plant Growth, Shoot Dry Biomass Yield, and P Uptake

The results on effect of AMF inoculation (*Rhizophagus clarus, Rhizophagus intraradices* and the mixed species) and P (0, 0.005, 0.01, 0.02, 0.05, and 0.1 mg P g^{-1} substrate) application on growth and P uptake by *Catha edulis, Cordia africana, Croton macrostachyus, Ensete ventricosum, Erythrina brucei, and Millettia ferruginea* are presented (Figure 1). Most of the peaks of shoot lengths, and dry weights of these plant species occurred within the P concentrations ranges of 0.005 to 0.02 mg g^{-1}. For the un-inoculated plant species, such peaks increased with increase in P concentrations. For the two AM fungi separate and mixed inoculations studied, these peaks indicated that the optimum P concentrations for maximum benefits from the AMF symbiosis in plant species lied mostly within the ranges from 0.005 to 0.02 mg g^{-1} P concentrations (Figure 1).

For shoot length, the optimum P concentration for most effective AMF inoculants, *Rhizophagus clarus*, *Rhizophagus intraradices* and the two-mixed species in *Ensete ventricosum, Cordia africana, Erythrina brucei and Croton macrostachyus* was 0.02 mg P g^{-1} substrate. For *Catha edulis* inoculated with *Rhizophagus Clarus*, both plant height and shoot dry weight increased with increase in P concentration and in *Millettia ferruginea* there was a slight height increase in treatments inoculated with *Rhizophagus intraradices*; however, increase in shoot dry weight at 0.01 mg g^{-1} P concentration was consistent (Figure 1) with the other four plant species mentioned above.

Thus, except *Catha edulis,* which was inoculated with *Rhizophagus clarus* and that positively responded to increasing P concentrations, inoculating abovementioned perennial crops and agroforestry trees, with a suitable AMF inoculant (at lower P concentration) could be as effective as high inputs of recommended P fertilizer application. A similar benefit is expected in case of other tree seedlings, as the optimum P concentration for all selected agroforestry shade trees studied with different AM fungi for maximum benefit from the symbiosis was low (0.005–0.02 mg P g^{-1} substrate). Since different AM fungi can transport and transfer different amounts of P to plants, their effects on plant growth can also be different. Despite this fact, however, in the current study the two species from Glomeromycota and the mixture of the two-species produced similar results in the greenhouse as compared to the un-inoculated, which was given similar P concentrations with other treatments.

In all perennial crops and agroforestry trees studied, the AMF inoculants used, namely *Rhizophagus clarus* and *Rhizophagus intraradices* and the mixed species resulted in significantly ($p \leq 0.05$) increased shoot length, dry weight, and P uptake. Results obtained with *Rhizophagus clarus* were almost at par with the mixed species. Compared to *Rhizophagus clarus*, lower responses were recorded with *Rhizophagus intraradices* (Figure 1).



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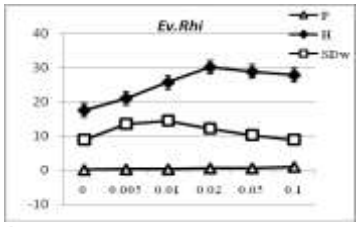

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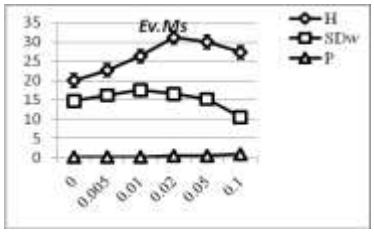

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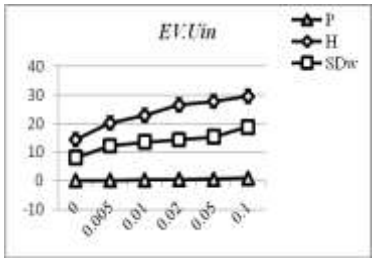

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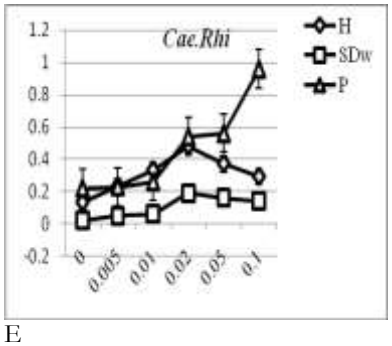

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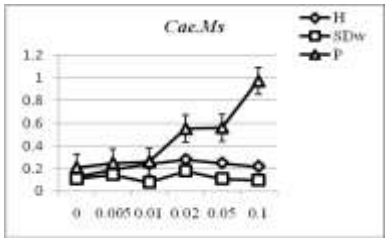

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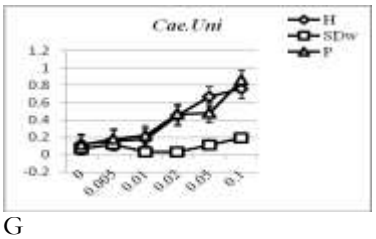

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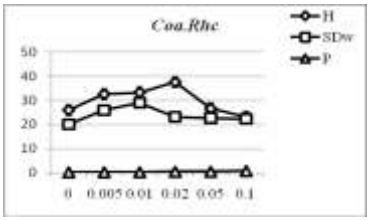

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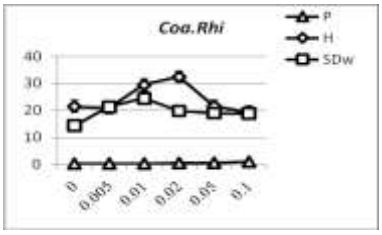

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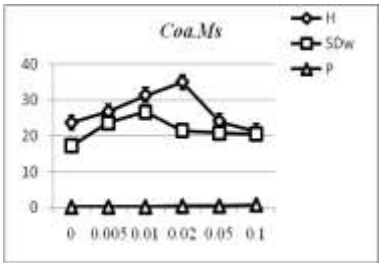

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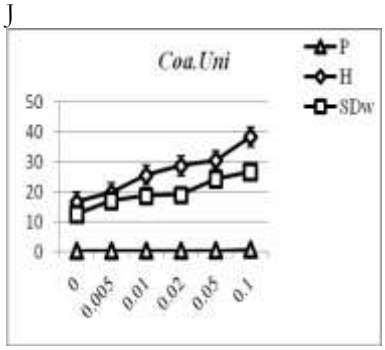

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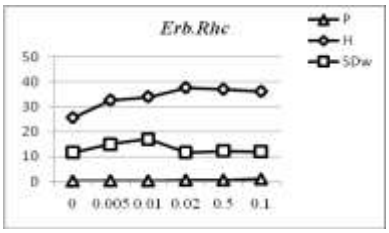

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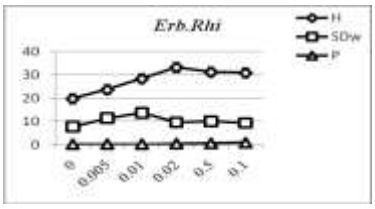

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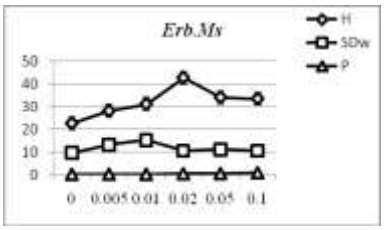

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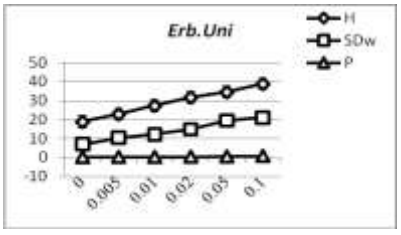

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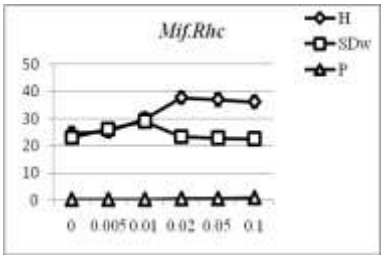

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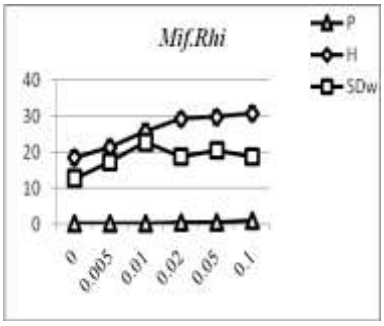

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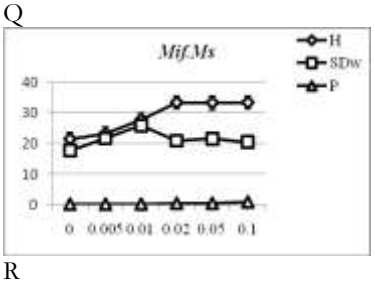

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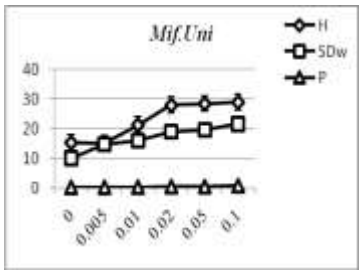

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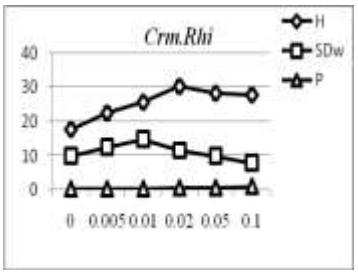

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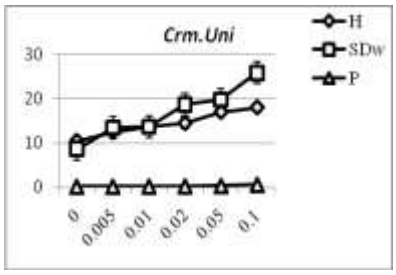

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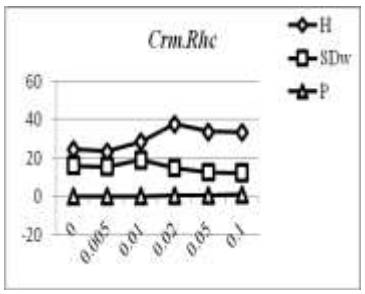

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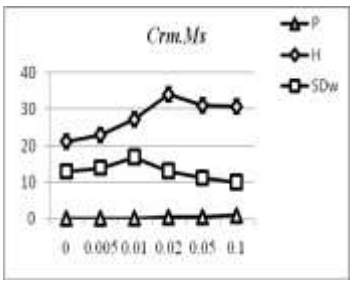

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Figure 1. Plant height, shoot dry weight and P uptake at different P concentrations (mg/g) in AMF inoculated and un-inoculated treatments. Key: H, height; SDW, Shoot dry weight; P, Phosphorus; Ev, *Ensete ventricosum*; Cae, *Catha edulis*; Coa, *Cordia africana*; Erb, *Erythrina brucei*; Mif, *Millettia ferruginea*; Crm, *Croton macrostachyus*; Rhc, *Rhizophagus clarus*; Rhi, *Rhizophagus intraradices*; Ms, Mixed species; Uni, un-inoculated.

3.2. Mycorrhizal Dependence (MD) of Seedlings of the Selected Crop and Tree Species

Total results on mycorrhizal dependence (MD) of *Catha edulis, Cordia africana, Croton macrostchyus, Ensete ventricosum, Erythrina brucei and Millettia ferruginea* seedlings are presented (Table1). In perennial crops and the agroforestry shade trees, the three AMF inoculants, namely *Rhizophagus clarus, Rhizophagus intraradices* and the two-mixed species significantly ($p \leq 0.05$) increased shoot length. Except for *Catha edulis* inoculated with *Rhizophagus intraradices* and the two-mixed species, the total shoot dry biomass increased in all the treatments. Maximum (41.71%) MD value was recorded for inoculation with *Rhizophagus clarus* in *Catha edulis*, followed by MD value (34.85%) obtained from inoculation with the same *Rhizophagus clarus* in *Millettia ferruginea* and MD (34.45%) value with *Rhizophagus clarus* inoculant in *Croton macrostachyus*. For the two-mixed species, the next highest MD values ranged from 2.57% in *Catha edulis* to 30.67% in *Ensete ventricosum*. The least MD values were recorded in all the inoculation treatments with *Rhizophagus intraradices* in all plant species in the undertaken test (Table 1).

Table 1. Total shoot dry weight and mycorrhizal dependency (MD) of the perennial crops and agroforestry shade trees.

Plant species	SDW and MD%						Un-inoculated
	Rh. Clarus		*Rh. intraradices*		Mixed species		
	Total SDW	MD	Total SDW	MD	Total SDW	MD	SDW
Ensete ventricosum	86.03[c]	27.05a	62.76bc	7.71c	90.53d	30.67d	62.76bc
Catha edulis	0.97a	41.71d	0.57a	3.51a	0.76a	2.57a	0.55a
Cordia africana	143.37[e]	31.34b	117.83f	16.46f	130.6e	24.62c	98.44c
Erythrina brucei	79.7b	26.19a	61.5b	4.34ab	70.6b	16.67b	58.83b
Millettia ferruginea	146.03e	34.87c	111e	14.32e	128.52e	26.0cd	95.11c
Croton macrostachyus	90.53cd	34.45c	66.33d	10.55d	78.43c	24.35c	59.33b

Note: Rh, Rhizophugus; SDW, shoot dry weight; MD, mycorrhizal dependency. For each plant species means in the same column followed by different letter(s) are significantly different by ANOVA and Duncan's multiple range test at P < 0.05 level.

3.3. Effect on AMF Structural Colonization and Spore Density

AM fungi structural colonization (arbuscules and vesicles) of the perennial crops and shade trees after inoculation with *Rhizophagus clarus, Rhizophagus intraracices* and the two-mixed species are presented in Table 2. This finding revealed that formation of arbuscules by the separate *Rhizophagus clarus and Rhizophagus intraradices* inoculations and inoculation with the two mixed species were more favored at lower P concentrations (between 0.05 to 0.02 mg P g^{-1} substrate) than either extremely lower or higher P concentrations. However, there were also some rates of colonization below and above 0.05 and 0.02 mg P g^{-1}

concentration in all inoculated trees and crop species (Table 2). The results also indicated that arbuscule formation occurred at the early stage during the 1st month of inoculation and that of formation of vesicles was intensive during the 2nd and 3rd months after the inoculation.

Table 2. Effects of different phosphorus concentrations (milligrams P per gram substrate) on AMF structural colonization (after 1st, 2nd and 3rd months of growth).

Plants	P mg/g	*Rhizophagus clarus*						*Rhizophagus intraradices*						Mixed					
		AC (%)			VC (%)			AC (%)			VC (%)			AC (%)			VC (%)		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	0	−	-	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
Ensete	0.005	+	+	++	−	+	+	+	+	+	_	+	+	+	+	++	-	+	+
ventricosum	0.01	+	+	++	+	+	+	+	+	++	−	+	++	+	+	+++	-	++	++
	0.02	+	++	++	−	++	++	−	+	+	-	++	++	+	+	++	-	+	++
	0.05	−	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	−	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.005	−	+	+	−	+	+	+	+	+	+	+	+	+	+	++	-	++	++
Catha edulis	0.01	+	+	++	−	+	++	+	+	++	−	+	++	+	+	++	+	++	++
	0.02	+	++	+++	+	+	++	+	+	++	−	++	+++	+	+	++	−	+	+++
	0.05	+	−	+	−	−	+	−	+	+	+	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
Cordia	0	−	−	+	−	−	+	-	+	+	−	+	+	−	+	+	−	+	+
africana	0.005	−	++	++	+	+	+	+	+	+	−	+	++	+	+	++	-	+	+
	0.01	+	+	+++	−	+	++	+	+	++	−	+	++	+	+	++	-	++	++
	0.02	+	++	+++	−	+	++	+	+	+	-	++	+++	+	+	++	-	++	+++
	0.05	+	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	-	+	−	−	+	-	+	+	−	+	+	−	+	+	−	+	+
	0.005	−	+	++	−	+	+	+	+	+	-	+	+	+	+	++	-	+	+
Erythrina	0.01	+	++	++	−	+	++	+	+	++	-	+	++	+	+	++	-	++	++
brucei	0.02	+	++	++	−	++	+++	+	+	+	-	++	++	+	+	++	-	++	+++
	0.05	−	−	++	+	+	+	−	+	+	−	+	+	−	+	+	−	++	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	-	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.005	+	++	++	−	+	++	+	+	+	+	+	++	+	+	++	-	++	++
Millettia	0.01	+	++	++	+	++	+++	+	+	++	−	+	++	+	+	++	-	++	++
ferruginea	0.02	+	+++	+++	−	++	++	+	+	+	+	++	+++	+	+	++	-	++	+++
	0.05	−	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+
	0	−	−	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.005	+	+	++	−	++	+	+	+	++	-	+	+	+	+	++	-	++	+++
Croton	0.01	+	++	++	+	++	+	+	+	++	−	+	+++	+	+	++	-	++	++
macrostachyus	0.02	+	++	+++	−	++	++	+	+	+	+	++	+++	+	+	++	+	++	++
	0.05	−	−	++	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+
	0.1	−	−	+	−	−	+	−	−	+	−	+	+	−	−	+	−	−	+

Note: 1, 2, 3, number of months of plant growth*;* AC, arbuscular colonization; VC, vesicular colonization; absent (-), fair (+), moderate (++), high (+++ and above)

In conclusion, all the plants inoculated with AM fungi showed mycorrhizal colonization that was characterized by the presence of arbuscules and vesicles (Table 2). However, mycorrhizal colonization, arbuscule and vesicle formation decreased significantly with the increase in P concentrations. Also, similar trend was observed with mycorrhizal spore number (Table 3), and positive correlation was recorded between mycorrhizal spore number and percentage root colonization.

Table 3. Effects of different phosphorus concentrations (milligrams P per gram substrate) on root colonization and spore density (after three months of growth).

Plant species	P (mg/g)	*Rhizophagus clarus*		*Rhizophagus intraradices*		Mixed AMF species	
		RLC (%)	SD/50 cm^3 soil	RLC (%)	SD/50 cm^3 soil	RLC (%)	SD/50 cm^3 soil
	0	12.67bc	29.00b	13.87bc	31.67b	20.67c	26.67ab
Ensete	0.005	15.67c	35.00b	20.33cd	34.00bc	24.00c	37.67bc
Ventricosum	0.01	35.00d	58.00c	33.00e	54.67d	29.67d	62.33d
	0.02	17.33c	40.33b	22.33d	42.33c	23.00c	46.33cd
	0.05	9.33ab	7.33a	8.33ab	12.67a	15.33b	9.33a
	0.1	4.90a	5.67a	3.63a	6.67a	4.63a	10.00a
	0	9.33b	22.67b	12.50ab	32.67ab	15.67bc	22.67ab
	0.005	13.73c	28.67b	19.33b	36.67b	22.33bc	43.67c
	0.01	24.33d	58.33c	32.67c	65.33c	35.00d	65.33d
Catha edulis	0.02	16.00c	47.00c	20.00b	56.00c	26.33cd	35.67bc
	0.05	7.67b	6.67a	3.33a	11.67a	13.33ab	10.67a
	0.1	2.00a	2.33a	2.67a	6.67a	3.33a	7.00a
	0	13.83bc	29.00b	14.17b	32.67b	22.67c	28.67b
	0.005	16.83c	35.00b	21.67c	36.00bc	26.00c	39.67bc
	0.01	35.03d	58.00c	33.33d	56.67d	31.67d	56.33d
Cordia africana	0.02	17.87c	40.33b	23.33c	44.33c	25.00c	42.67c
	0.05	8.67ab	8.00a	9.33ab	14.67a	17.33b	11.33a
	0.1	4.93a	6.67a	4.33a	8.67a	6.67a	12.00a
	0	12.33ab	25.33b	16.33bc	32.67b	20.00bc	26.17b
	0.005	17.50bc	30.67b	22.67cd	35.67b	23.33c	36.67cd
	0.01	32.67d	57.33c	33.33e	56.67c	33.33d	39.33d
Erythrina	0.02	21.33c	49.67c	25.67de	42.33b	20.50bc	32.33c
brucei	0.05	10.33ab	6.67a	9.00ab	13.33a	14.67b	9.00a
	0.1	5.17a	3.33a	4.17a	6.67a	3.00a	7.50a
	0	13.33b	26.67b	16.50bc	36.67b	19.33bc	26.67ab
	0.005	17.73c	34.00b	23.33c	40.67b	26.33bc	47.67c
Millettia	0.01	28.67d	62.33c	36.33d	69.33c	39.00d	69.33d
ferruginea	0.02	20.00c	51.00c	24.00c	60.00c	30.33cd	39.67bc
	0.05	11.67b	10.67a	7.67ab	15.67a	17.33b	14.33a
	0.1	6.00a	6.00a	5.33a	10.67a	2.67a	11.00a
	0	11.07b	21.67b	12.00a	35.00c	17.33b	25.00b
Croton	0.005	18.33c	24.67bc	22.33b	34.67c	25.67c	39.00c
macrostachyus	0.01	27.33d	35.67d	28.67c	50.00e	25.43c	59.83d
	0.02	19.00c	33.00cd	20.67b	42.67d	24.00c	35.00bc
	0.05	9.33b	5.67a	9.00a	14.33b	14.33b	11.00a
	0.1	6.00a	3.33a	4.13a	7.00a	2.67a	7.00a

Note: RLC, root length colonization; SD, spore density. For each plant species means in the same column followed by different letter(s) are significantly different by ANOVA and Duncan's Multiple Range Test at $P < 0.05$ level.

Maximum root colonization and spore count per 50 cm^3 sand was observed at P concentrations ranging from 0.005 to 0.02 mg g^{-1} in plants inoculated with AM fungi (Table 3). In this study, results showed that the optimum P concentration for maximum benefit from AMF symbiosis for inoculated agroforestry trees and perennial crops and tree seedlings was in between 0.005 and 0.02 mg g^{-1} and the plant growth decreased with increasing P concentration. Therefore, inoculating plants with a suitable AMF inoculants could result in a benefit comparable to high P input. However, extrapolation of the results to the real conditions of agroforestry systems should be done with precaution because of differences in the substrate used, i.e., sand in the present study. The information on P optimum can form the basis for further pot and/or field experiments involving integration of chemical fertilizers with AM fungi inoculants.

4. Discussion

Previous studies under field conditions have shown that agricultural management practices, such as tillage, fertilization and cropping systems, have a negative impact on the AMF associated with temperate and tropical crop plant species (Douds and Millner, 1999; Cardoso and Kuyper, 2006). Fertilization is an

important abiotic factor influencing growth, colonization, sporulation, composition and distribution of AMF (Wang *et al.*, 2009).

Other studies conducted in the greenhouse conditions have demonstrated that AM fungi usually have their maximum effect on host plant growth when the level of P in the growth medium is optimum (Habte and Manjunath, 1991). According to Habte and Manjunath (1991), when the soil solution P concentration is at or near 0.002 mg per liter, most plant species will respond dramatically to mycorrhizal colonization.

Results of the current study on perennial crops and agroforestry trees revealed the pick for maximum benefit at 0.02 mg P g^{-1} growth medium (sand) and that as P concentration is increased from 0.005 to 0.02 mg g^{-1}, the reliance of plants on AM fungi for P uptake increased and diminished progressively as P concentration increased from 0.05 to 0.1 mg g-1, after which only the very highly mycorrhizal-dependent species responded significantly to mycorrhizal colonization.

The current results also confirmed previous results (Vierheilig and Ocampo, 1991; Ravnskov and Jakobsen, 1995) on functional effectiveness of AMF. The mechanism underlying the reduction in plant growth just above the optimum P is probably due to both effects of P on root growth and direct effects on the fungi (Cardoso *et al.*, 2006). Increase in P supply may decrease the availability of organic substrates from roots to fungi. Azcon *et al.* (2003) reported that low P concentration in lettuce plants allowed the maximum colonization and occurrence of AM fungi. Koide (1991) showed that P levels influenced AMF colonization. Addition of P fertilizers above the optimum delayed and/or inhibited AMF colonization (de Miranda *et al.*, 1989; Baon *et al.*, 1992).

Several other authors have reported that mycorrhizal roots are able to absorb several times more phosphate than non-inoculated roots from soils and from solutions (Dela Cruz *et al.*, 1988). Increased efficiency of phosphorus uptake by mycorrhizal plants could have led to higher concentrations of P in the plant tissues.

The greater phosphate absorption by AMF has been suggested to arise due to superior efficiency of uptake from labile forms of soil phosphate, which is not attributable to a capacity to mobilize phosphate sources unavailable to non mycorrhizal roots (Pearson and Gianinaazzi, 1983). Mycorrhizal roots are known to have not only a considerably greater phosphate inflow rates, but also to possess a pathway of phosphate uptake with a much higher affinity for phosphate than non-mycorrhizal roots.

In this study maximum root colonization and spore count per 50 cm^3 river sand was observed at P concentrations ranging from 0.005 to 0.02 mg P g^{-1} in plants infected by AM fungi and effectiveness decreased with increasing P concentration. The current results are consistent with the findings of Kahiluoto *et al.* (2000), who observed and reported that with increasing P supply, there was a decrease in the colonization and the effectiveness of mycorrhizal colonization. The present results are also in agreement with many reports, which suggest that addition of phosphate fertilizers above optimum levels results in delay in colonization and reduction in chlamydospore production by AM fungi (Koide and Li, 1990; Koide, 1991; Thingstrup *et al.*, 1998).

In general, most of the perennial crops and agroforestry trees are fast-growing plants that require more nutrients during the initial stage of seedling establishment. During this period, the root system was not well developed and the AM fungal symbiosis might play a vital role by supplying the nutrients to the host plant (Muthukumar and Udaiyan, 2006). The results of the present study revealed that mycorrhizal inoculations increased the plant growth and P uptake in different treatments with a few exceptions. This can be due to increase in the sand volume explored for nutrient and water uptake by the mycorrhizal plants from the medium as compared to non-mycorrhizal plants. These results also support results of previous studies and the high rate of P fertilizer application, i.e. 0.05 and 0.1 mg g^{-1} lead to antagonistic inhibition of mycorrhizal colonization, whereas lower P doses with application of the vigorous AM fungus *Rhizophagus intraradices* were able to significantly increase the root colonization and spore density. However, increased P supply increased some growth parameters associated with plant height, shoot and root dry weight. Thus, soil amendment with AM fungi generally have the potential to possibly reduce the application of phosphorus fertilizer for crop improvement, growth, yield and nutritional value of the perennial crops and shade trees in Sidama agroforestry.

The current research results indicated that inoculating plants with a suitable AMF inoculant could result in a benefit comparable to high P input. However, extrapolation of the results to the real conditions of agroforestry systems should be done with precaution because of differences in the substrate used, i.e., sand in the present study. The information on optimum P concentration for better performance of AMF in the agroforestry trees can form the basis for further pot/field experiments involving integration of chemical fertilizers with AM fungi.

5. Conclusion

The present study demonstrated that the inoculation of perennial crops and multipurpose trees with *Rhizophagus intraradices, Rhizophagus clarus* and mixture of both inocula increased all plant growth parameters, but at the same time decreased percentage of mycorrhizal colonization and spore density as the concentration of P increased. Thus, soil amendments with AM fungi have the potential to possibly reduce the application of phosphorus fertilizer for crop and tree growth and improvement in agroforestry. However, to come up with more concrete, accurate and reliable information

on functional efficiency of the AMF species applied, further pot and/or field experiments should be carried out.

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