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Exploring the Role of Mycorrhizal and Rhizobium Inoculation with Organic and Inorganic Fertilizers on the Nutrient Uptake and Growth of *Acacia mangium* Saplings in Acidic Soil

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Abstract: Strong and healthy saplings are a prerequisite to establish a successful forest. Therefore, an attempt has been made to develop the best package for nutrient supplementation to raise healthy *Acacia mangium* saplings, especially in acidic soil. The seeds were sown in pots, receiving different combinations of *Arbuscular mycorrhizal* (AM), *Rhizobium* inoculation with application of lime, and mustard oil cake (MOC). The highest spore count and infection percentage (3220 kg^{-1} soil and 69) were recorded in the AM + MOC + R treated pot, whereas the lowest (2553 kg^{-1} soil and 37) were recorded in the AM + L treated pot. Nitrogen concentration and uptake in the sapling were higher in the *Rhizobium*-inoculated treatments than the uninoculated ones. The sulfur concentration and uptake were higher in the MOC-supplemented treatment. Similarly, the P, K, Ca, and Mg concentrations and uptakes were higher in the limed treatments than the unlimed ones. The micronutrient concentration and uptake were higher in the unlimed treatments compared to the lime practice. The concentration of N in *Rhizobium*-treated pots, P and K in lime-treated pots, and S in MOC-treated pots were increased, whereas the soil pH decreased in all treatments except in the integrated package (AM + MOC + R + L) after 120 days. The Ca and Mg were reduced in all treatments, whereas micronutrients were reduced in all packages except the control. Under different nutrient management practices, plant height and stem girth continuously increased by 9.5 to 12 cm and 3 to 4 times, respectively. The production of robust saplings required integrated application of lime, MOC, AM, and *Rhizobium* in an acid soil that facilitated better root growth with availability of adequate nutrients for saplings.

Keywords: acid soil; arbuscularmycorrhizae; infection; liming; micronutrient; *Rhizobium*

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1. Introduction

With accumulated pressure on forest land to meet the demands of a rapidly growing population, it is essential to plant fast-growing, multipurpose tree species, such as *Acacia mangium*, in agroforestry as well as wastelands to achieve the optimum area of forest and tree cover. In Asia, *A. mangium* is most widely used in forestry programs. The *A.* sp. of 1200–1300 species, which is divided into three sub-genera, viz. *Acacia*, *Aculeiferum*, and *Phyllodinae*, belongs to Mimosaceae family. The *A. mangium* can tolerate a pH between 4.5 and 6.5 [1]. In acidic soil with N and K, phosphorus becomes a limiting plant nutrient for plant growth. phosphorus, in combination with Al and Fe, form insoluble Fe and Al phosphate. The organic acids exuded by the plant's roots compete with inorganic P for the same sorption sites or is solubilized by ligand-promoted mineral dissolution [2]. However, although *A. mangium* can be grown in acidic soil liming is essential for facilitating better root growth and other nutrient availability for the quick establishment of seedlings when in the nursery. Phosphorus is a primary nutrient required for cell multiplication, reproduction, metabolism, storage, and use as energy [3], which are essential for optimum productivity and quality [4]. It plays a vital role in root morphology [5] and root development, and is also helpful for the availability of nutrients [6]. Therefore, plants have developed various strategies, viz. root morphology and architecture, for obtaining optimum phosphorus from soils [7].

The mycorrhizal fungi are soil organisms and create a direct connection between the soils and plant root systems. These fungi belong to the subphylum Glomeromycotina under the phylum Mucoromycota [8] and have a significant effect on the increase of element absorption, growth. Mycorrhizae helps to the host plant in conditions of environmental stress (water stress) by reaching water and nutrients that are unreachable for the plant. Mycorrhizal-fungi inoculation influences the mineral nutrient absorption such as P, K, Zn, Cu, and Fedue to increasing root surface area.

The *A. mangium* seed inoculated with both AM fungi and *Rhizobium* can enhance the dry matter yield as well as thenitrogen content of soil [9]. The introduction of AM fungi is likely to be important in disturbed arid and semi-arid habitats, which have a generally limited naturally-occurring AM [10,11]. The *A. mangium* has been reported to form AM associations with *Gigaspora margarita*, *Scutellospora calospora*, *Glomus mosseae*, *Glomus fasciculatum*, and *Glomus etunicatum*. These fungal species vary in their symbiotic efficiency and their effect on the growth of *A. mangium*. The mycorrhizal associations will be beneficial for the growth of forest plants in such problematic soil conditions, especially acidic soil with a low P content.

The nutrient mineralization from mustard oil cake is slow, which supplies the nutrient to the plant in a timely manner [12]. Ibrahim and Mumtaz [13] reported that fungal inoculation with mustard cake increased plant biomass and yield. Besides that, the mustard oil cake is concentrated, organic manure which can control insect pest, nematodes, weeds, and pathogens due to release of organic substances during its mineralization [14].

Acid soils need amelioration before sowing of any seed or plantation. Lime is an efficient ameliorant for acidic soils [15,16]. The physiology of *A. mangium* indicates that it can grow in any type of soil; however, the seedling stage is a very important phase of its life. A healthy seedling can sustain any stress during its life span. The mustard oil cake helps in crop growth. The AM fungi help the sapling to sustain different stress conditions and give the potential to resist different pest [17] and nematode attacks [18]. The considered findings above clearly indicate that the fungi population will accelerate in the acidic condition, whereas lime will neutralize the acidity and increase the *Rhizobium* efficiency; however, combined approaches are unexplored; especially for forest plants in acidic soil. Therefore, the integrated application of lime, MOC, *Rhizobium*,and AM was considered in this study to produce better-quality saplings of *A. mangium* in acidic soil for successful plantation in problematic soils.

2. Materials and Methods

2.1. Collection and Processing of Soil for Poly Pots

The experimental soil was collected from the central farm of Odisha University of Agriculture and Technology, Bhubaneswar, India, situated at 20°16′50.14″ N 85°47′10.11″ E. The collected soil was dried under shade and sieved through a 2 mm sieve for removal of foreign material and gravels. The sand and processed soil were fumigated with Ethylene-dibromide (EDB) for 72 h [19]. The vermicompost was autoclaved at 121 °C and 1.1 kg cm^{-2} pressure for one hour a day for two consecutive days.

2.2. Physicochemical Properties of Initial Soil

The initial soil was sandy loam, consisting of sand (78.8%), silt (12.6%), and clay (5.6%), and a bulk density of 1.38 Mg m^{-3}, a particle density of 2.52 Mg m^{-3} and a porosity of 44%. The soil was strongly acidic in reaction (pH:5.29) with electrical conductivity of 0.11 dS m^{-1}. The lime requirement was 4.7 t $CaCO_3 ha^{-1}$ to raise the pH to 6.5. The soil's organic carbon (5.2 g kg^{-1}), available P (10 g kg^{-1} of soil), and K (106 g kg^{-1} of soil) were medium in status, whereas the available N (94 g kg^{-1} of soil) and S (9 g kg^{-1} of soil) were low in status. The exchangeable Ca and Mg were deficient in experimental soil (1.32 and 0.42 cmol (p+) kg^{-1} soil, respectively). The DTPA extractable Fe, Mn, Cu and Zn were adequate in soil (64.42 mg kg^{-1}, 2.76 mg kg^{-1}, 1.45 mg kg^{-1} and 2.39 mg kg^{-1} soil, respectively).

2.3. Preparation of Soil Mixture and Poly Pots

The processed soil was mixed thoroughly with sand and vermicompost in the ratio of 2:1:1, respectively. The sterilized soil mixture of 4 kg was filled in each poly pot with a volume of 7065 cm^3 (radius-7.5 cm, height-20 cm). At the time of filling of poly pots 1.0 g of lime (L) and 1 g of mustard oil cake (MOC) per poly pot were mixed thoroughly, as per the treatment combinations. The AM fungi (*Glomus fasciculatum*) were isolated from the soil and multiplied in the cement pit in controlled conditions by planting maize as a host plant. The spores were collected directly from the maintained cement pit and were applied at 510 spores per poly pot.

2.4. Pre-Sowing Treatment, Inoculation, and Sowing of Seed

The seeds of *A. mangium* passed pre-sowing treatment (keeping the seed in 80 °C for 10 min and thereafter soaked in cooled water for 24 h). The seeds were soaked with a liquid *Rhizobium* (MH 661260) broth, and sown at 2–3 cm depth in the pot. One healthy plant in each pot was maintained, and during sunny days, the amount of water was measured and given to each poly pot to maintain80% of field capacity to avoid water stress in the saplings.

2.5. Details of Experiment

The designed experiment followed the Complete Randomized Design (CRD) and each treatment was replicated eight times. The AM fungi were inoculated with different organic and inorganic input combinations with an absolute control, viz.: T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*.

2.6. The Observation during the Growth Period

The plant height and stem diameter were measured at 30th, 60th, 90th, and120th days after sowing, and data were expressed in cm. The poly pot was removed carefully without damaging the roots. The adhering soil of the root was washed by running water and the root length was recorded. The weight of the fresh and oven-dried (dried in the hot air oven at 65–70 °C until constant weight) shoot and root were noted and the data expressed in g $plant^{-1}$. The volume of the roots was measured by water displacement method at 30th, 60th, 90th and120th days.

2.7. Infectivity Test

Root infection by AM was studied by the staining techniques given by Grace and Stribley [20]. The *Chlamydospore* population in soil was recorded by wet sieving and decanting [21]. The colonization % was calculated by a gridline to intersect method [22].

$$\text{Colonization } (\%) = \left(\frac{\text{Total number of infected roots intersecting grid lines}}{\text{Total number of roots intersecting grid lines}}\right)100$$

2.8. Plant and Soil Analysis

The harvested plant samples were processed and then analyzed for the determination of N, P, K, Ca, Mg, S, Fe, Mn, Cu, and Zn concentrations. The total nitrogen of the plant was determined by the Kjeldahl digestion method as described in AOAC [23]. For total P, K, Ca, Mg, S, Fe, Mn, Cu, and Zn, the samples were digested in the di-acid mixture (HNO_3:$HClO_4$::3:2). The P was estimated by vanadomolybdate method and the S was estimated by the turbidimetric method by using a spectrophotometer. Total K was estimated by flame photometer, Ca and Mg by EDTA titration method. The micronutrients (Fe, Mn, Cu, and Zn) were estimated by atomic absorption spectrophotometer [24].

The soil's physical properties, viz. the textural class of soil, were determined by-Bouyoucos Hydrometer method as described by Piper [25]. The Bulk density and particle density were estimated by a core sampler method and pycnometer method, respectively, as described by Black [26]. Porosity was calculated by using the formulae:

$$\text{Porosity } (\%) = 1 - \left(\frac{\text{Bulk density}}{\text{Particle density}}\right)100$$

The lime requirement of initial soil was determined by Woodruff's buffer method [27]. The initial and postharvest soil nutrients were estimated by the following standard methods: the soil's reaction (pH) and electrical conductivity were measured by using a glass electrode pH meter and conductivity meter respectively [28] in a soil water suspension of (1:2.5). The organic carbon in soil was estimated by the wet oxidation method as suggested by Walkley and Black [29]. The available N was estimated by the alkaline permanganate method given by Subbiah and Asija [30].The Bray's-1P was estimated by the method described by Page et al. [24]. The available K was estimated by the neutral normal ammonium acetate extraction method [28] and the available S was estimated by the turbidimetric method [31].

The plant bioavailable fraction of Zn, Cu, Mn, and Fe were extracted by diethylenetriamine pentaacetic acid (DTPA) extractant (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 Mtriethanolamine, adjusted to pH 7.30) [32], and estimated by an atomic absorption spectrophotometer (AAS).

2.9. Statistical Analysis

The ANOVA was prepared by taking replicated data of each parameter, standard error (SE) and Duncan's Multiple Test Range (DMRT), performed by using SPSS software version 25.

3. Results

3.1. Plant Height and Stem Diameter

The height of *A. mangium* saplings at 30 nursery days varied between 5.2 and 10.1 cm. (Table 1). By the stage of 120 nursery days, the height increased significantly and varied between 66.4 to 98.5 cm. The lowest height was recorded in the practice of an absolute control and the highest with AM and *Rhizobium* was inoculation with lime, and MOC was also added. The diameters of the sapling stem or the girth were increased (Table 1). The girth diameter ranged from 0.44 to 0.96 mm at 30th days, increased continuously by 3 to 4 times under different nutrient management practices, and attended a stable and desired girth ranging from 3.8 to 4.6 mm.

Table 1. Influence of AM and *Rhizobium* inoculation and integrated use of lime and MOC application on plant height and stem diameter of *A. mangium* sapling.

Treatments	Sapling Height (cm)				Stem Diameter (mm)			
	Days							
	30	60	90	120	30	60	90	120
T_1	5.2 ± 0.09 ^{g}	16.5 ± 0.03 ^{g}	39.9 ± 0.09 ^{f}	66.4 ± 1.21 ^{f}	0.51 ± 0.02 ^{b}	1.77 ± 0.12 ^{e}	2.97 ± 0.24 ^{d}	4.06 ± 0.34 ^{de}
T_2	6.6 ± 0.09 ^{f}	21.4 ± 0.15 ^{f}	47.4 ± 0.17 ^{e}	76.9 ± 0.61 ^{e}	0.49 ± 0.02 ^{bc}	1.19 ± 0.09 ^{g}	2.98 ± 0.26 ^{d}	3.98 ± 0.29 ^{e}
T_3	7.6 ± 0.06 ^{d}	25.1 ± 0.18 ^{c}	52.5 ± 0.12 ^{c}	84.6 ± 0.60 ^{d}	0.54 ± 0.03 ^{b}	1.65 ± 0.19 ^{ef}	3.19 ± 0.29 ^{c}	3.95 ± 0.36 ^{e}
T_4	7.1 ± 0.15 ^{e}	24.0 ± 0.12 ^{d}	52.1 ± 0.34 ^{c}	85.1 ± 0.59 ^{d}	0.51 ± 0.02 ^{b}	1.83 ± 0.18 ^{de}	2.74 ± 0.27 ^{e}	4.57 ± 0.28 ^{b}
T_5	6.6 ± 0.06 ^{f}	22.8 ± 0.20 ^{e}	49.8 ± 0.21 ^{d}	82.6 ± 0.64 ^{d}	0.48 ± 0.02 ^{bc}	1.86 ± 0.14 ^{de}	2.98 ± 0.26 ^{d}	4.14 ± 0.37 ^{d}
T_6	9.4 ± 0.07 ^{b}	23.7 ± 0.12 ^{d}	56.7 ± 1.01 ^{b}	89.2 ± 0.61 ^{c}	0.44 ± 0.01 ^{c}	2.00 ± 0.21 ^{c}	3.14 ± 0.28 ^{c}	4.27 ± 0.38
T_7	8.6 ± 0.09 ^{c}	26.8 ± 0.23 ^{b}	58.1 ± 0.55 ^{b}	91.6 ± 0.90 ^{b}	0.94 ± 0.05 ^{a}	2.21 ± 0.20 ^{b}	3.32 ± 0.31 ^{b}	4.66 ± 0.32 ^{b}
T_8	10.1 ± 0.21 ^{a}	28.1 ± 0.17 ^{a}	59.9 ± 0.30 ^{a}	98.5 ± 0.91 ^{a}	0.96 ± 0.04 ^{a}	3.00 ± 0.25 ^{a}	3.65 ± 0.34 ^{a}	5.18 ± 0.42 ^{a}

T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5-AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*. All values are represented as mean ± SE of triplicate values and analyzed using SPSS 25.0 software with one-way ANOVA followed by DMRT and their relationship was considered to be statistically significant when $P = 0.05$.

3.2. Effect of Mycorrhiza Inoculation on Root Characteristics

The differential root growth of *A. mangium* saplings is presented in Figures 1–3. The root length at 30th days under the influence of mycorrhizal fungi, lime, mustard oil cake, and *Rhizobium* inoculation influenced positively and attended length ranging from 52 to 90 mm (Figure 1). Thereafter, it increased continuously and attended the length ranging from 164 to 288 mm at 120th days. There was a significant influence of the use of agro-inputs on root growth of the saplings, particularly with their combined uses. The root volume of *A. mangium* saplings (Figure 2) were measured at three stages in the nursery (30th, 60th, 90th, and 120th days), and indicated that there was a continuous increase in volume up to 120th days. The root volume and density (Figure 3) were negatively influenced by the influence of integrated nutrient management INM packages. This relationship was better reflected at the 120th day in the nursery, particularly with the complete INM package.

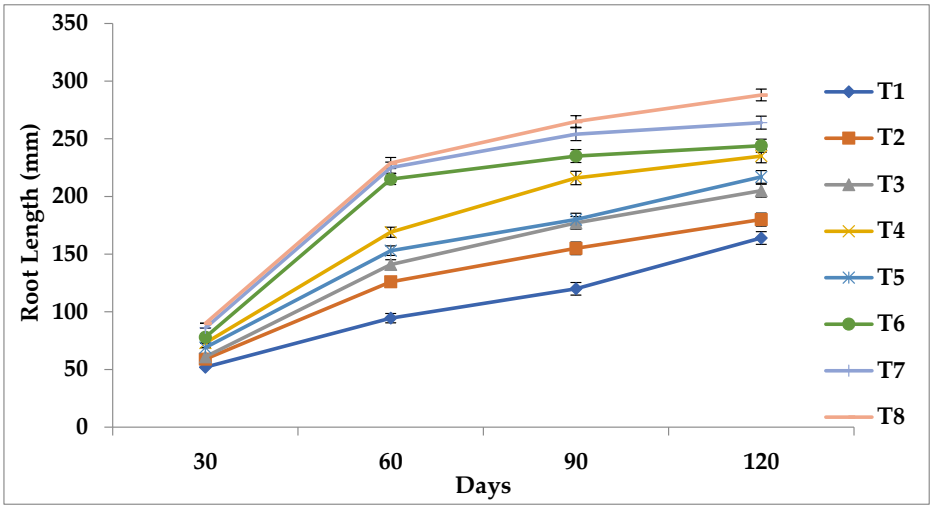

Figure 1. Effect of mycorrhizal inoculation on root length (mm), each bar represents mean ± SE.T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*.

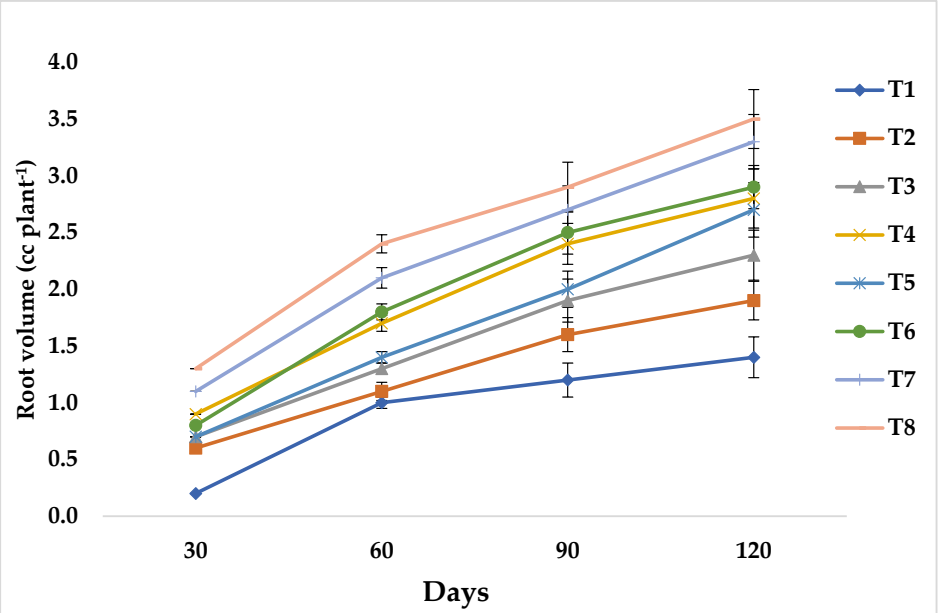

Figure 2. Effect of mycorrhizal inoculation on root volume (cc plant^{-1}), each bar represents mean ± SE.T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*.

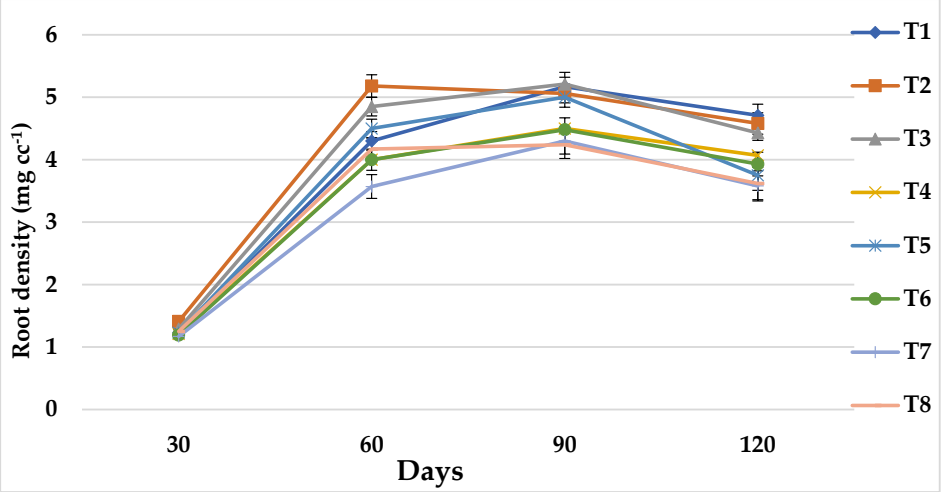

Figure 3. Effect of mycorrhizal inoculation on root density (mg cc^{-1}), each bar represents mean ± SE.T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*.

3.3. Total Biomass

The shoot biomass of *A. mangium* exceeded the root biomass. The biomass of the saplingswas recorded at three stages i.e., 60th, 90th, and 120th days (Figure 4). The influences of agro-inputs were significant. The total biomass of *A. mangium* sapling at 60th days (ranging from 1.83 to 4.50 g plant^{-1}) was almost doubled by 90th days. The complete INM package could double the sapling biomass compared to the control practice. Mycorrhizal inoculation increased the total biomass production by 20.8% over no inoculation. Combining lime and mustard oil cake (MOC) separately in the soil mixture further increased the biomass production by 9.5 and 14%, respectively. Combining *Rhizobium* inoculation with AM inoculation influenced biomass production by 19.6%.The *Rhizobium* seed inoculation

and AM inoculation with lime added to the mixture increased it by 36.6%, similarly *Rhizobium* + MOC package by 47.2%, and lastly, the package of the practice of *Rhizobium* + MOC + lime and AM influenced the biomass by 74%.

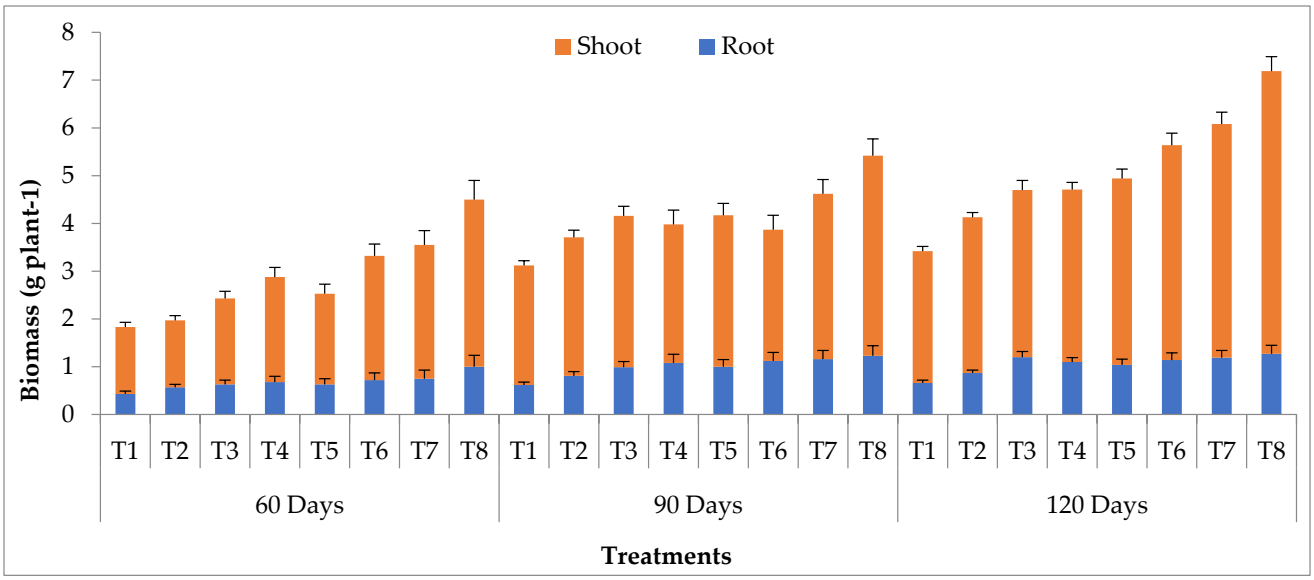

Figure 4. Effect of mycorrhizal inoculation on root biomass, shoot biomass, and total biomass of *Acacia* mangium saplings, each bar represents mean ± SE.T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM +MOC + lime + *Rhizobium*.

3.4. Mycorrhizal Inoculation and Infection

Each seed inthe poly potswas applied with 510 spores per kg of soil mixture below the seed at the time of sowing. The data related to spore counts at 120 nursery days are presented in Figure 5. At 120th days of *A. mangium* sapling, the mycorrhizal spore count was 2875 spores per kg of pot mixture. Combining lime to pot mixture with AM inoculation significantly decreased the spore count (11.2%), whereas adding MOC and *Rhizobium* seed inoculation increased the spore count by 2.8% and 2.0% compared to AM inoculation alone respectively. The seed inoculation of *Rhizobium* with lime improved the spore count by 3.8% compared to lime alone, whereas 7.8% less compared to AM inoculation alone. Seed inoculation with *Rhizobium* and AM together with MOC to pot increased spore count by 12.0% compared to AM inoculation alone. However, the combination of all the inputs to the poly pot maintained a 7.1% higher spore count over only the AM inoculated pot.

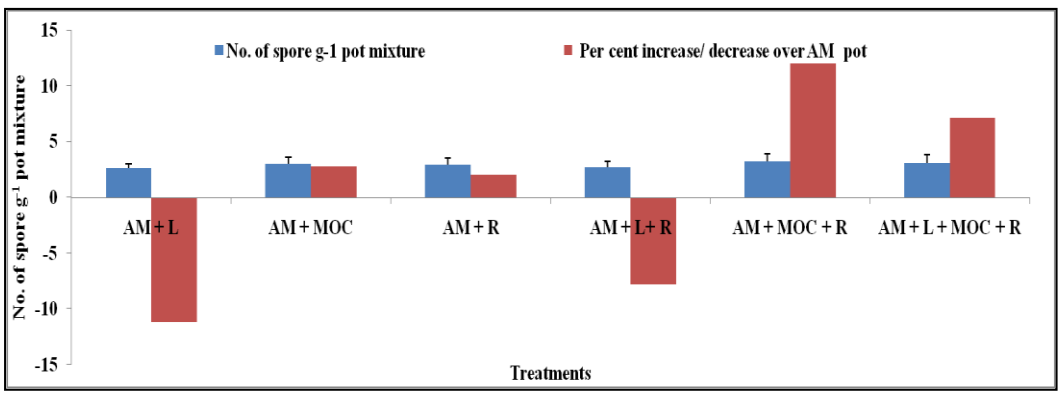

Figure 5. Effect of mycorrhizal inoculation on number of spores per pot at 120 nursery days.

3.5. Mycorrhizal Infection of AcaciamangiumRoots

The data related to root infection of *A. mangium* are presented in Figure 6. The infection % at 30th days ranged from 18 to 31%. The lowest infection percentage was observed in the pot where AM was applied with lime and the highest with AM + lime + MOC + *Rhizobium*. The infection percentage of *A. mangium* saplings continued to increase and maintained the highest infection percentage ranging from 37% to 69% following the same trend in AM inoculated practices.

The AM inoculation exhibited a 45% root infection, whereas the lime added to the same practice decreased infection by 17.8%. The mustard oil cake addition and the rhizobial inoculation of seeds both positively and significantly increased the infection percentage by 14 and 25.1%, respectively. Integrating *Rhizobium* seed inoculation with lime alone, or with MOC and adding altogether, improved the root infection by 2.2%, 53.3%, and 44.4%, respectively. The colonization percentage was reduced with the application of lime, whereas in the integrated application of inputs in the pots the colonization was highest.

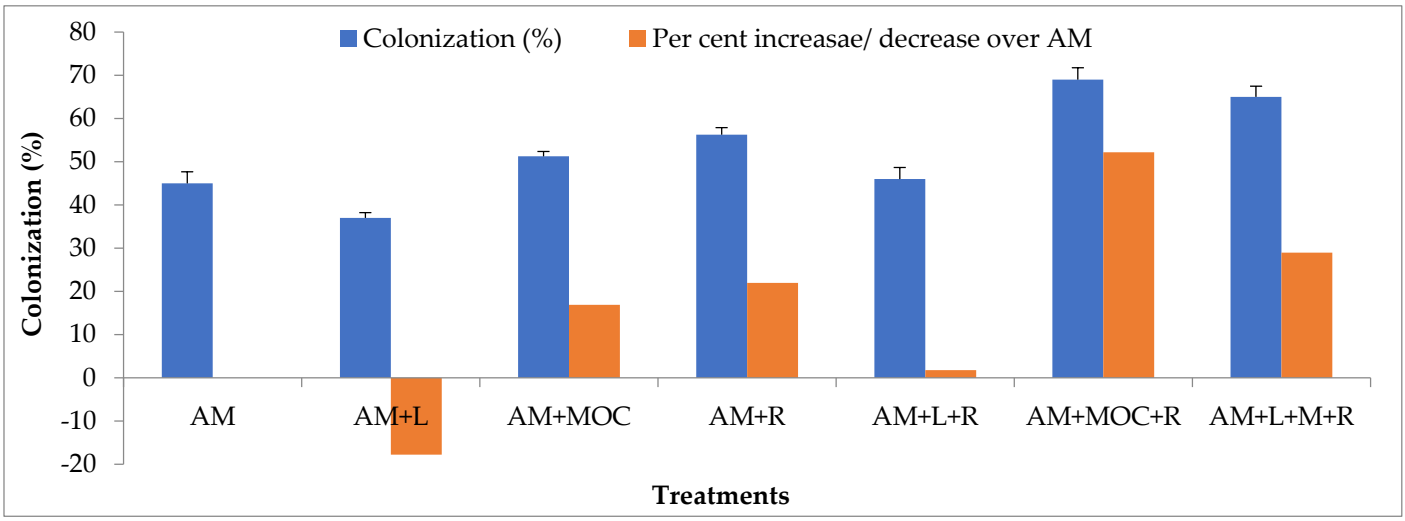

Figure 6. Effect of mycorrhizal inoculation on colonization (%) in root at 120 nursery days.

3.6. Plant Nutrient Concentration and Uptake

3.6.1. Macro-Nutrient

The data related to macronutrient concentration and uptake by 120th days sapling of *A. mangium* are presented in Table 2. The concentration of major nutrient followed the order: N (from 0.48% to 0.99%), K (from 0.39% to 0.54%), P (from 0.154% to 0.308%), Ca (from 0.09% to 0.20%), Mg (from 0.07% to 0.17%), and S (from 0.05% to 0.19%). The AM inoculation influenced the concentration of the nutrients significantly higher than non-inoculation practice. The lime/MOC/*Rhizobium* seed inoculation, when practiced alone or in combinations, had a significant influence on the uptake of all major nutrients. The uptake of major nutrients by *A. mangium* sapling followed the order: N (from 16.4 to 71.0 mg sapling^{-1}) K (from 13.4 to 39.1 mg sapling^{-1}), P (from 5.3 to 22.2 mg sapling^{-1}), Ca (from 3.20 to 14.0 mg sapling^{-1}), Mg (from 2.5 to 12.0 mg sapling^{-1}), and S (from 1.85 to 13.3 mg sapling^{-1}).

Table 2. Effect of AM and *Rhizobium* inoculation and integrated use of lime and MOC application on nutrient concentration (%) and uptake (mg plant^{-1}) by *Acacia mangium* at 120th days.

Treatments	Concentration (%)			Uptake (mg plant^{-1})		
	N	P	K	N	P	K
T_1	0.48 ± 0.02 ^{h}	0.154 ± 0.03 ^{g}	0.39 ± 0.01 ^{f}	16.4 ± 1.2 ^{e}	5.3 ± 0.04 ^{d}	13.4 ± 1.23 ^{f}
T_2	0.51 ± 0.03 ^{g}	0.191 ± 0.04 ^{f}	0.42 ± 0.02 ^{e}	21.2 ± 1.3 ^{e}	7.9 ± 0.07 ^{c}	17.3 ± 1.41 ^{e}
T_3	0.60 ± 0.05 ^{f}	0.216 ± 0.04 ^{d}	0.42 ± 0.02 ^{e}	27.1 ± 1.5 ^{d}	9.8 ± 0.07 ^{c}	19.1 ± 1.55 ^{e}
T_4	0.63 ± 0.05 ^{e}	0.230 ± 0.05 ^{bc}	0.46 ± 0.05 ^{c}	29.7 ± 1.7 ^{cd}	10.8 ± 0.09 ^{bc}	21.8 ± 1.57 ^{d}
T_5	0.69 ± 0.05 ^{d}	0.208 ± 0.04 ^{e}	0.47 ± 0.05 ^{c}	33.9 ± 1.9 ^{c}	10.3 ± 0.09 ^{c}	23.1 ± 1.63 ^{cd}
T_6	0.88 ± 0.09 ^{b}	0.234 ± 0.06 ^{b}	0.44 ± 0.03 ^{d}	49.6 ± 2.4 ^{b}	13.2 ± 0.15 $_{b}$	24.7 ± 1.65 ^{c}
T_7	0.87 ± 0.09 ^{c}	0.227 ± 0.05 ^{c}	0.53 ± 0.06 ^{b}	52.8 ± 2.8 ^{b}	13.8 ± 0.17 ^{b}	32.0 ± 1.65 ^{b}
T_8	0.99 ± 0.12 ^{a}	0.308 ± 0.08 ^{a}	0.54 ± 0.04 ^{a}	71.0 ± 3.2 ^{a}	22.2 ± 0.29 ^{a}	39.1 ± 1.74 ^{a}
Treatments	**Concentration (%)**			**Uptake (mg plant^{-1})**		
	Ca	**Mg**	**S**	**Ca**	**Mg**	**S**
T_1	0.09 ± 0.006 ^{f}	0.073 ± 0.006 ^{f}	0.054 ± 0.004 ^{f}	3.2 ± 0.41 ^{c}	2.5 ± 0.12 ^{d}	1.9 ± 0.03 ^{f}
T_2	0.10 ± 0.009 ^{e}	0.087 ± 0.009 ^{e}	0.069 ± 0.009 ^{e}	4.3 ± 0.44 ^{c}	3.6 ± 0.11 ^{d}	2.9 ± 0.09 ^{f}
T_3	0.19 ± 0.013 ^{b}	0.134 ± 0.016 ^{b}	0.099 ± 0.010 ^{c}	8.4 ± 0.67 ^{b}	6.1 ± 0.29 ^{c}	4.5 ± 0.11 ^{e}
T_4	0.17 ± 0.010 ^{d}	0.121 ± 0.014 ^{c}	0.156 ± 0.017 ^{b}	7.8 ± 0.55 ^{b}	5.7 ± 0.15 ^{c}	7.4 ± 0.14 ^{c}
T_5	0.17 ± 0.011 ^{c}	0.103 ± 0.009 ^{d}	0.087 ± 0.008 ^{d}	8.5 ± 0.69 ^{b}	5.1 ± 0.12 ^{c}	4.3 ± 0.09 ^{e}
T_6	0.18 ± 0.012 ^{b}	0.157 ± 0.023 ^{a}	0.104 ± 0.009 ^{c}	10.3 ± 0.73 ^{b}	8.9 ± 0.42 ^{b}	5.9 ± 0.11 ^{d}
T_7	0.17 ± 0.010 ^{cd}	0.137 ± 0.019 ^{b}	0.178 ± 0.031 ^{a}	10.2 ± 0.72 ^{b}	8.3 ± 0.34 ^{b}	10.8 ± 0.21 ^{b}
T_8	0.20 ± 0.013 ^{a}	0.166 ± 0.045 ^{a}	0.185 ± 0.037 ^{a}	14.0 ± 0.81 ^{a}	12.0 ± 0.44 ^{a}	13.3 ± 0.27 ^{a}
Treatments	**Concentration (μg g^{-1} of the plant)**				**Uptake (μg plant^{-1})**			
	Fe	**Mn**	**Cu**	**Zn**	**Fe**	**Mn**	**Cu**	**Zn**
T_1	34.3 ± 1.25 ^{b}	34.2 ± 1.11 ^{c}	3.6 ± 0.05 ^{b}	15.6 ± 0.43 ^{c}	117.1 ± 2.81 ^{f}	117.0 ± 2.01 ^{e}	12.4 ± 0.33 ^{e}	53.4 ± 1.28 ^{e}
T_2	42.6 ± 1.34 ^{a}	39.4 ± 1.13 ^{a}	4.6 ± 0.07 ^{a}	16.4 ± 0.61 ^{c}	176.1 ± 2.99 ^{c}	162.7 ± 2.38 ^{c}	18.8 ± 0.39 ^{c}	67.6 ± 2.29 ^{d}
T_3	27.3 ± 1.11 ^{f}	25.3 ± 0.93 ^{e}	2.3 ± 0.02 ^{cd}	14.5 ± 0.40 ^{d}	123.4 ± 1.16 ^{f}	114.4 ± 1.93 ^{e}	10.4 ± 0.27 ^{f}	65.6 ± 2.27 ^{d}
T_4	28.8 ± 1.14 ^{e}	40.2 ± 1.26 ^{a}	3.8 ± 0.03 ^{b}	16.7 ± 0.66 ^{ab}	135.7 ± 1.19 ^{e}	189.4 ± 2.99 ^{d}	18.1 ± 0.35 ^{c}	78.6 ± 2.45 ^{c}
T_5	26.8 ± 0.79 ^{f}	39.6 ± 1.13 ^{a}	2.7 ± 0.02 ^{c}	16.5 ± 0.60 ^{bc}	132.2 ± 1.99 ^{e}	195.4 ± 3.01 ^{d}	13.4 ± 0.33 ^{d}	81.3 ± 2.99 ^{c}
T_6	28.1 ± 0.89 ^{e}	27.9 ± 1.10 ^{d}	2.1 ± 0.03 ^{d}	13.4 ± 0.44 ^{e}	158.2 ± 2.15 ^{d}	157.4 ± 2.50 ^{c}	12.00.32 ± ^{e}	75.8 ± 2.76 ^{c}
T_7	30.1 ± 0.13 ^{d}	40.4 ± 1.29 ^{a}	3.9 ± 0.04 ^{b}	17.0 ± 0.49 ^{a}	182.7 ± 3.45 ^{b}	245.6 ± 4.19 ^{b}	23.8 ± 0.42 ^{b}	103.3 ± 3.36 ^{b}
T_8	32.9 ± 0.14 ^{c}	38.7 ± 1.25 ^{b}	3.9 ± 0.05 ^{b}	16.9 ± 0.50 ^{ab}	236.2 ± 3.99 ^{a}	278.3 ± 4.44 ^{a}	27.9 ± 0.48 ^{a}	121.4 ± 3.94 ^{a}

T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*. All values are represented as mean ± SE of triplicate values and analyzed using SPSS 25.0 software with one-way ANOVA followed by DMRT and their relationship was considered to be statistically significant when $P = 0.05$.

3.6.2. Micronutrient

The micronutrients concentration and uptake are presented in Table 2. The concentration of micronutrients in the sapling followed the order: Fe (from 26.8 to 42.6 μgg^{-1} of the plant), Mn (from 25.3 to 40.4 μgg^{-1} of the plant), Zn (from 13.4 to 17.0 μgg^{-1} of the plant) and Cu (from 2.1 to 4.6 μg g^{-1} of the plant). The highest concentrations of Fe and Cu were estimated in the tissue of AM inoculated saplings only, which were significantly different from other practices. The Mn concentration was higher in the saplings where the AM was inoculated with lime and MOC, which was statistically at par with the sole application of AM, AM + MOC, and AM + *Rhizobium*. The Zn concentration was highest in the sapling where AM was inoculated with both MOC and *Rhizobium*. Such micronutrient concentration in the 120th growth day in the nursery had resulted in uptake of Fe, ranging from 117.2 to 236.2 μgplant^{-1}, Mn from 117 to 278.3 μgplant^{-1}, Zn from 53.4 to 121.4 μgplant^{-1} and Cu from 10.4 to 27.9 μgplant^{-1}.

3.7. Post-Harvest Soilproperties

The post-harvest properties in poly pot have been presented in Table 3. The lowest (4.9) pH was recorded in the practice where only AM was inoculated and the highest (5.40) with AM + L + MOC + R were used. Compared to the initial pH of the poly pot mixture, the pH of the post-harvest mixture had turned more acidic where only AM was inoculated and in the absolute control. The lowest (0.08 dSm^{-1}) soluble salt content was estimated in the pot where A + MOC + R was inoculated, which was at par with AM + MOC + R inoculated pot, AM + mustard oil cake (MOC), and absolute control practice. The highest EC (0.12 dSm^{-1}) was estimated due to AM + L+ R inoculation.

The organic carbon status was higher than the initial soil (5.29 g kg^{-1}) except in the absolute control. The highest (5.40 g kg^{-1}) was estimated in the practices where AM + L + MOC + R and AM + MOC was applied. The available N was higher than the initial soil in the packages where AM + L, AM + MOC + R and AM + L + MOC + R were applied. In comparison to the initial (10 mg kg^{-1}), the available P and K was higher in all the packages except control. The S was higher in the treatment where mustard oil cake (MOC) was applied than the initial (9 kg ha^{-1}). The decrease was observed in exchangeable Ca and Mg in all the packages. Decreases in available Fe, Cu and Zn were noted in all the packages except the absolute control, whereas the available Mn decreased irrespective of the treatments.

Table 3. Effect of AM and *Rhizobium* inoculation with lime, and MOC application on post-harvest soil properties at 120 nursery days.

Treatments	pH	EC	OC	Available				Exchangeable	
				N	P	K	S	Ca	Mg
	(1:2.5)	(dS m^{-1})	(g kg^{-1})	(mg kg^{-1})				cmol (p+) kg^{-1} soil	
T_1	4.95 ± 0.011 ^{f}	0.10 ± 0.015 ^{bc}	5.1 ± 0.23 ^{c}	93 ± 1.7 ^{b}	10 ± 1.4 ^{e}	48 ± 1.2 ^{e}	6 ± 1.1 ^{e}	1.14 ± 0.02 ^{e}	0.24 ± 0.04 ^{d}
T_2	4.90 ± 0.011 ^{f}	0.11 ± 0.003 ^{b}	5.4 ± 0.25 ^{b}	87 ± 1.1 ^{de}	15 ± 1.5 ^{cd}	150 ± 2.4 ^{a}	6 ± 1.1 ^{e}	1.15 ± 0.02 ^{e}	0.27 ± 0.05 ^{c}
T_3	5.11 ± 0.014 ^{c}	0.11 ± 0.007 ^{b}	5.5 ± 0.23 ^{b}	88 ± 0.9 ^{d}	19 ± 1.1 ^{c}	146 ± 2.0 ^{b}	7 ± 1.1 ^{de}	1.25 ± 0.03 ^{b}	0.37 ± 0.09 ^{a}
T_4	5.07 ± 0.009 ^{d}	0.10 ± 0.005 ^{bc}	5.7 ± 0.25 ^{a}	85 ± 2.0 ^{ef}	16 ± 1.2 ^{d}	146 ± 2.2 ^{b}	11 ± 1.5 ^{b}	1.23 ± 0.03 ^{c}	0.33 ± 0.08 ^{b}
T_5	5.00 ± 0.009 ^{e}	0.11 ± 0.013 ^{b}	5.5 ± 0.22 ^{b}	83 ± 1.6 ^{f}	18 ± 1.1 ^{c}	133 ± 1.6 ^{d}	8 ± 1.0 ^{d}	1.20 ± 0.02 ^{d}	0.29 ± 0.07 ^{c}
T_6	5.03 ± 0.013 ^{de}	0.12 ± 0.008 ^{a}	5.4 ± 0.21 ^{b}	98 ± 1.6 ^{b}	21 ± 1.1 ^{b}	140 ± 1.8 ^{c}	9 ± 1.1 ^{cd}	1.22 ± 0.04 ^{cd}	0.32 ± 0.09 ^{b}
T_7	5.15 ± 0.010 ^{b}	0.09 ± 0.004 ^{c}	5.3 ± 0.20 ^{bc}	105 ± 1.8 ^{a}	21 ± 0.5 ^{b}	138 ± 1.6 ^{c}	12 ± 1.6 ^{b}	1.26 ± 0.05 ^{b}	0.34 ± 0.12 ^{b}
T_8	5.40 ± 0.009 ^{a}	0.08 ± 0.005 ^{c}	5.7 ± 0.23 ^{a}	95 ± 2.2 ^{c}	23 ± 1.5 ^{a}	147 ± 1.5 ^{ab}	13 ± 1.7 ^{a}	1.30 ± 0.05 ^{a}	0.39 ± 0.17 ^{a}
Initial	5.29	0.11	5.2	94	10	106	9	1.32	0.42

Treatments	Available			
	Fe	Mn	Cu	Zn
	(mg kg^{-1})			
T_1	65.21 ± 1.13 ^{a}	2.65 ± 0.09 ^{c}	1.48 ± 0.02 ^{a}	2.42 ± 0.02 ^{a}
T_2	63.99 ± 0.38 ^{b}	2.72 ± 0.18 ^{a}	1.44 ± 0.01 ^{a}	2.39 ± 0.01 ^{a}
T_3	48.24 ± 0.08 ^{e}	2.47 ± 0.08 ^{f}	1.29 ± 0.01 ^{cd}	2.18 ± 0.02 ^{c}
T_4	54.94 ± 0.08 ^{d}	2.67 ± 0.15 ^{b}	1.32 ± 0.01 ^{cd}	2.21 ± 0.01 ^{b}
T_5	60.22 ± 0.24 ^{c}	2.56 ± 0.22 ^{d}	1.30 ± 0.02 ^{c}	2.20 ± 0.01 ^{b}
T_6	47.19 ± 0.11 ^{e}	2.45 ± 0.10 ^{de}	1.27 ± 0.01 ^{cd}	2.16 ± 0.01 ^{c}
T_7	56.73 ± 0.32 ^{d}	2.49 ± 0.08 ^{fe}	1.29 ± 0.01 ^{cd}	2.18 ± 0.01 ^{c}
T_8	45.32 ± 0.20 ^{e}	2.41 ± 0.14 ^{g}	1.26 ± 0.01 ^{d}	2.17 ± 0.01 ^{c}
Initial	64.42	2.76	1.45	2.39

T_1—Absolute control (No inoculation of AM), T_2—AM (only AM inoculation), T_3—AM + Lime, T_4—AM + MOC, T_5—AM + *Rhizobium*, T_6—AM + lime + *Rhizobium*, T_7—AM + MOC + *Rhizobium* and T_8—AM + MOC + lime + *Rhizobium*. All values are represented as mean ± SE of triplicate values and analyzed using SPSS 25.0 software with one-way ANOVA followed by DMRT and their relationship was considered to be statistically significant when $P = 0.05$.

4. Discussion

The plant height was higher with limed than nonlimed practice. It was due to the neutralization of acidity and the enhanced availability of nutrients through better root growth to absorb the nutrients. The seed inoculation with *Rhizobium* increased the colonization of AM because exopolysaccharides produced by *Rhizobium* stimulate the growth of AM by soil bacteria density and producing siderophores, in the mycorrhizosphere. AM also provides a better environment for the growth of the plant root and *Rhizobium* by keeping moisture in the rhizosphere for longer period [33]. Application of AM with MOC increased the colonization compared to the sole application of AM in pot mixture. The colonization percentage was higher in all integrated practices, whereas a reduced colonization percentage was estimated in the pot where AM was applied with lime.

Saplings growing under the control poly pot removed fewer nutrients compared to rest of the practices. The nitrogen concentration and uptake were higher in the saplings where the *Rhizobium* was inoculated. It was due to the fixation of atmospheric N_2 by the *Rhizobium*. The AM has strong mycelia, which increases the surface area of the roots made available for absorption of nutrients [34–36] and then stimulated *Rhizobium* infection, which improved nitrogen-fixation ability and plant growth [37,38]. Among the *Rhizobium* inoculation practice, the highest N concentration and uptake were recorded in the poly pot where *Rhizobium* was inoculated with AM, MOC, and lime, followed by the AM, MOC, and *Rhizobium* inoculated pot, the AM, lime, and *Rhizobium* inoculation practice, and AM + *Rhizobium*, respectively. The N requirement of plant could partially be supplemented by the organic sources [39]. The P concentration and uptake were estimated to be higher in the AM inoculated crops [40] than the uninoculated control pot. Among the AM inoculation practice, the P concentration and uptake were higher in the limed practice than the unlime done, because lime increases the bioavailability of P in acid soil by neutralizing the soil acidity. The application of lime and organic fertilizer also increases the bioavailability of P in soil [41].The AM also increases the P availability to the plant by symbiosis [41–43]. A similar trend was observed in all other macronutrient uptakes by the saplings. The result corroborated with the finding of Adnan et al. [43], who reported that *Rhizobium* inoculation with inorganic fertilizers improved the NPK availability and uptake by wheat crop. The AM inoculation alone had a significant influence on the uptake of major nutrients [44], particularly the combination of all inputs. The combined use of all inputs increased S uptake by 7 folds, Mg by 5 folds, N, P and Ca by 4 folds, and the K by 3 folds compared to the practice using no inputs [45].

The use of lime in the poly pot mixture regulated the uptake of all the micronutrients considerably. The combined use of inputs helped the tree species for the uptake of micronutrients. The use of lime in any combinations of the poly pot mixture had decreased the concentration of micronutrients because of less solubility of Fe, Mn, Cu, and Zn which are pH-dependent [46]. In low pH conditions, the equilibrium shifts toward free protonated anions and metal cations. A high pH favors the presence of hydroxyl complexes and carbonates; therefore, the availability of the micronutrients and toxic ions present in the soil solution (Al^{3+}, Mn^{2+}, and Fe^{2+}) increases with increasing soil acidity [47]. The mycorrhizae can grow in acidic conditions [48], but the total uptake of Fe, Mn, Cu, and Zn was highest in the sapling where the AM was inoculated with *Rhizobium*, MOC, and lime because of the better root growth for its activity. The higher concentration of micronutrients decreases the root growth, but in unlimed saplings the concentration was high due to reduced growth. In the lime-applied poly pot, the sapling root and shoot growth were luxuriant, the concentration of micronutrients was less due to dilution effect, but the total uptake of micronutrients was higher through the sapling in the integrated application of AM, MOC, Lime, and *Rhizobium*. There are reports that the mycorrhizal colonization can also increase the plant's nutrient uptake of Zn [49] and Cu [50] when the supply of these elements in the soil is relatively low. Arboscular mycorrhizae could be used as a potential phytoremadial agent in heavy metal contaminated soil to improve the crop yield [51]. The application of lime neutralized the acidity, hence the pH increased [16,52], and the application of mustard oil

cake increases the soil pH [53]. The soluble salt concentration of after 120th days decreased due to removal of the nutrients by the acacia sapling, in AM, MOC, lime, and *Rhizobium* or AM with MOC and lime treatments. Yanai et al. [54] reported that the removal of cations from soil resulted in a reduction of electrical conductivity.

The organic carbon status had increased invariably under each practice except in the absolute control due to the mycorrhizal fungal inoculation releasing a glycoprotein (glomalin) that show a positive correlation with organic carbon content and consequently contributes to carbon sequestration [55,56]. There was a buildup of available N status, in the practice where the co-inoculation of AM and *Rhizobium* either with MOC or lime or in both. The low pH constraint, symbiotic biological N_2 fixation [57], reduces persistence of Rhizobium in soil, and hence reduces nodulation and causes nutrient imbalance [58]. The lime and MOC application increased the soil pH, which creates a congenial environment for N_2 fixation and nodulation in crops grown in acidic soil. The availability of P and K status had increased in all the treatments except the absolute control. The available P status in the soil increased due to higher organic P mineralization [43,59,60].

The available S status of post-harvest soil also increased where mustard oil cake was used, in comparison to initial status, which was due to the slow mineralization of MOC in soil. The exchangeable Ca and Mg in the post-harvest mixture decreased in all the treatments from the initial concentrations. It was due to uptake of three cations by the plant for growth. The Fe, Cu, and Zn content of the post-harvest soil decreased in all the practices except the absolute control, whereas the Mn content of the soil decreased. It was due to the higher uptake of micronutrients by AM association.

5. Conclusions

Acid soil amelioration with lime provided an environment for root growth and *Rhizobium* to perform better. Application of mustard oil cake increased the infection percentage of AM fungi. Although the acidic environment was suitable for fungal growth integrated application of lime, *Rhizobium*, AM, and MOC had a better effect for sapling establishment in the nursery. The soil pH after 120th days decreased in all the packages except the integrated package. The organic carbon and available P and K status increased in all the packages except the control. Available N increased where *Rhizobium* was inoculated with lime/MOC or both with AM. Application of MOC increased S content. The micronutrient content decreased in all the packages except control, whereas Ca and Mg decreased irrespective of the packages. The study indicated that the combined sources provided a suitable environment for plant growth at an early stage. Hence, these sources can be effectively utilized for the better production of *Acacia mangium* saplings in nursery.

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