Greener Journal of Agricultural Sciences

Vol. 11(4), pp. 187-194, 2021

ISSN: 2276-7770

https://gjournals.org/GJAS

Influence of Arbuscular Mycorrhizal Fungi on the Performance of *Glycine max* (L.) Merr. Grown on Acidified Soil

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ARTICLE INFO	ABSTRACT

Article No.: *100621100*
Type**:** *Research*
Full Text: ***HTML*****,** ***EPUB***

Accepted: *10/10/2021*
Published: *2910/2021*

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Keywords: *Mycorrhiza; biofertilizer; Glycine max; soil properties; buffered soil pH*

Mycorrhiza is an association between the roots of vascular plants and fungus, with a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils. This study evaluated different pH levels with the inoculation of Mycorrhiza (Glomus deserticola) to ascertain the influence of mycorrhiza on performance of Glycine max cultivated on acidified soil. The study consisted of 5 various levels of pH as treatments [L1 (6.00), L2 (5.50), L3 (4.56), L4 (4.00) and the control L0 (4.96)] with 6 replicates laid in a Completely Randomised Design (CRD). TGX 1465- ID Glycine max seeds were cultivated in a pot containing 10 kg sterilized soil. Plant growth parameters were taken bi-weekly after planting while soil physico-chemical parameters were analysed before and after planting using standard procedures. The results obtained for plant growth parameters showed that there was no significant difference among all the treatments as compared with the control. The Phosphorous content (P), Cation Exchange Capacity (CEC) and Organic Carbon (OC) of AMF inoculated soil samples were significantly higher than that of soil before planting (BP) as follows: P – L1(5.40 mg/kg) > BP (1.36 mg/kg), CEC – L1(6.15 Cmol/kg) > BP (4.66 Cmol/kg) and OC – L1 (0.51 %) > BP (0.20 %). The AMF inoculation buffered the soil pH as follows: L1 (reduced from 6.00 to 5.20), L2 (reduced from 5.50 to 5.15), L3 (increased from 4.56 to 5.10) while L4 (increased from 4.00 to 4.87) The results obtained from this study indicated that inoculation of AMF buffered soil pH, enhanced soil structure; increased soil nutrients, growth and yield of Glycine max. Furthermore, soil acidification should be avoided because based on the experiment, pH range for optimal performance of Glycine max is 5.5 – 6.5.

INTRODUCTION

Soybean (*Glycine max*) is a legume of tropical to subtropical origin and an important source of food and income (Bakari *et al.,* 2020). Soybean ranks fifth in the world production of major crops after wheat, rice, potato and maize. The major use of soybean as food is its oil, where as 96 % soybean meal is used as animal feed (Sedaghati and Hokmabadi, 2014). Soybeans are not cereal grains, but a pulse. A pulse is a grain legume. Soybeans in nature need no industrial fertilizers, because they enjoy a natural symbiosis with a Rhizobium bacterium. This relationship provides organic proteins that are made directly from the surrounding atmospheric nitrogen, while eliminating the need for commercial nitrogen-based fertilizers (Beverly, 2014).

Acid soils are formed through human activities, such as construction and mining, which potentially causing the death of the plants. Plants wither due to not only low pH conditions in acid soils but also the dissolution of harmful elements, such as Al, Fe, and Mn, dissolving under acidic conditions (Matsumoto *et al.,* 2019). Soils usually become acidic under heavy rainfall. This is because rainwater is slightly acidic (about 5.7) due to a reaction with CO_2 in the atmosphere that forms carbonic acid. As this rain-water passes through soil pores, it leaches basic cations from the soil as bicarbonates, which increases the percentage of Al^{3+} and H^+ relative to other cations in the soil (Odutola, 2019). Soil acidification occurs naturally very slowly as soil is weathered, but this process is accelerated by productive agriculture. Soil acidification occurs because the concentration of hydrogen ions in the soil increases. Ammonium-based fertilisers are major contributors to soil acidification.

Mycorrhiza is an association between the roots of vascular plants and fungus. This relationship looks like that of root nodules bacteria (Rhizobium) in legumes (Jamal *et al.,* 2020). In this association, the fungi are actually integrated into the physical structure of the root. The fungi colonize the living root tissue during active plant growth. Through mycorrhization, the plant obtains phosphate and other minerals, such as zinc and copper, from the soil. The fungus obtains nutrients, such as sugars, from the plant root. Mycorrhizae help increase the surface area of the plant root system because hyphae, which are narrow, can spread beyond the nutrient depletion zone. Fungi have also been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils.

It is established that high acidity has deleterious effects on plant health, thus, leading to poor yield and performance. Amelioration of high acidity in soil is sine qua non to optimal yield of cultivated Glycine max. Although, some works have been done as regard to reduction of acidity in soil, there is still dearth of information as regards to the performance of acid soil by influence of mycorrhiza. Hence, the objective is to assess of the influence of mycorrhiza on growth performance of Glycine max cultivated on acidic soil.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the Screen House, Department of Crop and Soil Science, University of Port Harcourt at Latitude $4^0$54N and longitude $06^0$55E with an average temperature of 27 ^{0}C, relative humidity of 78 % but decreases slightly in dry season and an average rainfall ranging from 2500 mm – 4000 mm per annum (Atijegbe et al., 2013)

The area has a biomass rainfall pattern with a long rainfall season between March and July and a short rainy season from September to early July after a short spell in August and a longer period from December to February (Akande et al., 2010)

Experimental Design

The experiment consisted of 2 factors namely, one Specie of Mycorrhiza (*Glomus deserticola)* and five levels of acidity 4.96 (Inherent soil pH as control), L1 – 6.00, L2 – 5.50, L3 – 4.56 and L4 – 4.00; laid in a Completely Randomized Design (CRD) with 6 replications.

Source of AMF and Soybean

The Soybean seeds used for the experiment were gotten from the Agricultural Development Program (ADP) Port Harcourt Rivers State. The hybrid variety used for this experiment is TGX 1465- 1D Glycine max.

Soil Sample Collection and Preparation

Soil samples were collected randomly bulked to form composite sample. Sterilization of soil was done at 100 ˚C for 6 hours and allowed to cool for 72 hours. After sterilization, 10 kg of the sterilized soil were weighed and poured into each of the bags. The bags were perforated properly to allow easy flow of water whenever watering was done.

Simulation of Soil pH

Acidification (lowering the pH level): 75 ml of H_2SO_4 dilutions (4 dilution factor) were added to 1500 ml of water to get the various pH levels (4.00 and 4.56) respectively. Liming (increasing the pH level): 10 g of NaOH dilutions (4 dilution factor) were added into 600 ml water to get the various pH levels (5.50 and 6.00) respectively. 1500 ml of water alone was added to soil pH of 4.96 as Control.

Mycorrhizal Application

20 g of *Glomus deserticola* was weighed in a digital weighing balance and applied per bag before planting, stirring thoroughly to mix with the soil.

Planting

Three (3) seeds of *Glycine max* were planted per bag and thinned to 2 after two weeks.

Agronomic Practices

Cultural practices like weeding and watering were observed in the cause of carrying out the experiment. Weeding was done manually by hand picking method. Watering was done either at morning or evening hours; however, all treatment received same amount of 50 cl water.

Data Collection

Data parameters of Plant height, Number of leaves, Leaf area, and Stem girth were collected bi-weekly using a Metre rule (cm) and a Measuring tape (cm).

Data Analysis

The data collected were subjected to ANOVA using GENSTAT (12th edition, 2009) and means were separated with LSD at 5 % probability level.

Soil Analysis

Routine physicochemical analysis of the soil was done before planting and after planting. Soil particles size analysis was done using hydrogen method (Bouyoucos, 1962); Available P by Bray 1 method (Bray and Kurtz,1945); Soil pH was determined in 1:1 (soil: water) ratio using a glass electrode pH meter. Macro kjedahl digestion distillation method was used in measuring Total Nitrogen. Total organic carbon was determined by the wet combustion method of Walkey and Black (1934) as modified by Juo (1979) in selected methods of soil analysis. Organic carbon was oxidized by potassium dichromate in the presence of concentrated sulphuric acid. Ferrous ammonium sulphate was then added and the excess black titrated with standard potassium permanganate. 1.0 g of representative soil sample was shaken in a conical flask with 50 ml of 1N- ammonium acetate for about 2 hours. The mixture was left over night and then filtered into plastic cups. The filtrate was used for determination of sodium, potassium, calcium and magnesium using Atomic Absorption Spectrophotometer (AAS).

RESULTS

Physical and Chemical Properties of Soil samples used for the experiment.

The physiochemical properties of the soil are shown in table 1 below.

The pH: It was observed that L4 recorded the least pH value of 5.20. However, pH value of BP (4.96), L0 (4.90) and L4 are statistically the same but lower than pH value of L1 (5.20), L2 (5.15) and L3 (5.10). It is also worthy to note that L1, L2 and L3 are statistically the same.

Sand: Among the various treatments, L4 (85.97 %) was statistically highest; followed by L3 (85.6 %); then BP and L0, while the least sand was recorded in L1 (84.6 %) L2 (84.5 %). The sand value statistically decreases as follows; L4> L3> L0 and BP> L1 and L2.

Silt: L1 was significantly highest in silt content (3.98 %) among all the treatments; followed by L0 (3.50 %) while BP showed the least silt value. It is noteworthy that L1 (3.98 %) is statistically the same with L2 (3.96 %) likewise, the silt value of BP (2.90 %) is statistically the same with that of L4 (2.96 %).The silt value statistically decreases as follows; L1 and L2 > L0> L3 > BP and L4.

Clay: All the treatments were significantly different from each other. BP had the highest clay content (11.90 %), followed by L2 (11.54 %), followed by L1 (11.42 %), followed by L0 (11.33 %), followed by L3 (11.20 %), followed by L4 (11.07 %). The clay content thus decreases as follows; BP > L2 > L1> L0 > L3 and L4.

Total Organic Carbon (TOC): Among the treatments, L1 was significantly highest in TOC content (0.51 %); followed by L2 (0.43 %), followed by L0 (0.34 %), followed by L4 (0.24 %).It is noteworthy that L0 (0.34 %) and L3 (0.30 %) is statistically the same, likewise BP (0.20 %) and L4 (0.24 %) is statistically the same. The TOC value statistically decreases as follows; L1 > L2 > L0 and L3 > L4 and BP.

Table 1: Physical and Chemical Properties of the Soil

TRT	pH	Sand	Silt	Clay	TOC	TN	Av. P	Ca	Mg	K	Na	CEC
		→	→	%	←	←	(mg/kg)	→	→	(Cmol/kg)	←	←
BP	4.96^{a}	85.2^{b}	2.90^{a}	11.90^{f}	0.20^{a}	0.17^{a}	1.36^{a}	2.23^{a}	2.17^{a}	0.12^{a}	0.14^{a}	4.66^{a}
L0	4.90^{a}	85.17^{b}	3.50^{c}	11.33^{c}	0.34^{b}	0.30^{c}	3.40^{c}	2.65^{c}	2.50^{c}	0.17^{b}	0.18^{b}	5.50^{b}
L1	5.20^{b}	84.6^{a}	3.98^{d}	11.42^{d}	0.51^{d}	0.50^{d}	5.40^{e}	2.90^{e}	2.78^{e}	0.24^{d}	0.23^{c}	6.15^{d}
L2	5.15^{b}	84.5^{a}	3.96^{d}	11.54^{e}	0.43^{c}	0.41^{d}	4.13^{d}	2.70^{d}	2.65^{d}	0.21^{c}	0.22^{c}	5.73^{c}
L3	5.10^{b}	85.6^{c}	3.20^{b}	11.20^{b}	0.30^{b}	0.23^{b}	2.80^{b}	2.43^{b}	2.50^{c}	0.18^{b}	0.17^{b}	5.28^{b}
L4	4.87^{a}	85.97^{d}	2.96^{a}	11.07^{a}	0.24	0.20^{ab}	2.50^{b}	2.39^{b}	2.30^{b}	0.13^{a}	0.15^{a}	4.97^{a}

TRT= Treatments, BP = Before planting, TOC = Total Organic Carbon TN = Total Nitrogen Av. P = Available Phosphorus, L0 = pH 4.96, L1 = pH 6.00, L2 = pH 5.50, L3 = pH 4.56, L4 = pH 4.00, CEC = Cation Exchange Cation, means with the same alphabets show no statistical difference while different alphabets show statistical difference.

Total Nitrogen (TN): L1 was significantly the highest in TN content (0.50 %), followed by L0 (0.30 %), followed by L3 (0.23 %) and then BP (0.17 %). L1 and L2 are statistical statistically the same; L3 and L4 is also statistically the same. The TN content thus decreases as follows; L1 and L2 > L0 > L3 and L4 > BP.

Available Phosphorus (Av.P): Among the treatments; L1 was significantly the highest in Av.P content (5.40 mg/kg) followed by L2 (4.13 mg/kg), followed by L0 (3.40 mg/kg), followed by L3 (2.80 mg/kg) and then BP (1.36 mg/kg). It is noteworthy that L3 (2.80 mg/kg) and L4 (2.50 mg/kg) are statistically the same. The available phosphorus value statistically decreases as follows; L1 > L2 > L0 and L3 and L4 > BP.

Calcium: L1 was significantly the highest in calcium content (2.90 Cmol/kg) among the various treatments. This was followed by L2 (2.70 Cmol/kg), followed by L3 (2.43 Cmol/kg), followed by BP (2.23 Cmol/kg). Result showed that L2 (2.70 Cmol/kg) and L0 (2.65 Cmol/kg) are statistically the same, likewise L3 (2.43 Cmol/kg) and L4 (2.39 Cmol/kg) showed the same. The calcium value statistically decreases as follows; L1 > L2 and L0 >L3 and L4 > BP.

Magnesium: L1 was recorded the highest significant value of magnesium content (2.78 Cmol/kg), followed by L2 (2.65 Cmol/kg), followed by L0 (2.50 Cmol/kg), followed by L4 (2.30 Cmol/kg) and then BP (2.17 Cmol/kg). L0 (2.50 Cmol/kg) and L3 (2.50 Cmol/kg) are statistically the same. The magnesium content thus decreases as follows; L1> L2 > L0 and L3 > L4> BP.

Potassium: Among the treatments, L1 was significantly the highest in potassium content (0.24 Cmol/kg), followed by L2 (0.21 Cmol/kg), followed by L3 (0.18 Cmol/kg) and then L4 (0.13 Cmol/kg). It is noteworthy that L3 (0.18 Cmol/kg) and L0 (0.17 Cmol/kg) are statistically the same, likewise L4 (0.13 Cmol/kg) and BP (0.12 Cmol/kg) are statistically the same. The potassium content thus decreases as follows; L1> L2 > L3 and L0 > L4 and BP.

Sodium: L1 was significantly the highest in sodium content (0.23 Cmol/kg), followed by L0 (0.18 Cmol/kg) and then L4 (0.15 Cmol/kg). It is noteworthy that L1 (0.23 Cmol/kg) and L2 (0.22 Cmol/kg) are statistically the same, L0 (0.18 Cmol/kg) and L3 (0.17 Cmol/kg) are statistically the same, L4 (0.15 Cmol/kg) and BP (0.14 Cmol/kg) are statistically the same. The sodium value statistically decreases as follows; L1 and L2 > L0 and L3> L4 and BP.

Cation Exchange Capacity (CEC): L1 recorded significantly highest value (6.15 Cmol/kg) among the treatments, followed by L2 (5.73 Cmol/kg), followed by L0 (5.50 Cmol/kg), and then L4 (4.97 Cmol/kg). It is noteworthy that L0 (5.50 Cmol/kg) and L3 (5.28 Cmol/kg) are statistically the same, L4 (4.97 Cmol/kg) and BP (4.66 Cmol/kg). The CEC value statistically decreases as follows; L1 > L2 > L0 and L3 > L4 and BP.

Effect of Arbuscular Mycorrhizal Fungi and 5 Levels of Acidity on Selected growth parameters *Glycine max*

The effect of Mycorrhiza and 5 Levels of Acidity on selected growth parameters of *Glycine max* is presented in Table 2.

Plant Height

At 4WAP, the mean value of plant height ranged between 50.8 cm (L0) to 46.1 cm (L4). However, there is no statistical difference between all the treatments. At 8WAP, the mean value of plant height ranged between 93.5 cm (L2) to 74.5 cm (L1). However, there is no statistical difference between all the treatments. At 12WAP, the mean value of plant height ranged between 108.8 cm (L2) and 92.8 cm (L4). However, there is no statistical difference between all the treatments.

Leaf Area

At 4WAP, the mean value of leaf area ranged between 10.69 cm^2 (L3) to 8.45 cm^2 (L4). However, there is no statistical difference between all the treatments. At 8WAP, the mean value of leaf area ranged between 14.27 cm^2 (L2) to 10.52 cm^2 (L1). However, there is no statistical difference between all the treatments. At 12WAP, the mean value of leaf area ranged between 18.0 cm^2 (L4) to 15.3 cm^2 (L1). However, there is no statistical difference between all the treatments.

Number of Leaves

At 4WAP, the mean value of number of leaves ranged between 9.60 (L1) to 7.33 (L2). However, there is no statistical difference between all the treatments for number of leaves. At 8WAP, the mean value of number of leaves ranged between 19.8 (L0) to 16.7 (L3). However, there is no statistical difference between all the treatments for number of leaves. At 12WAP, the mean value of number of leaves ranged between 26.8 (L3) to 22.7(L4). However, there is no statistical difference between all the treatments for number of leaves.

Stem Girth

At 4WAP, the mean value of stem girth ranged between 1.875 cm (L0) to 1.500 cm (L4). However, there is no statistical difference between all the treatments. At the 8th week after planting, the mean value of stem girth ranged between 1.917 cm (L0) to 1.792 cm (L2). However, there is no statistical difference between all the treatments. At 12th week after planting, the mean value of stem girth ranged between 1.975 cm (L2) to 1.958 cm (L2). However, there is no statistical difference between all the treatments.

Table 2: Effect of Arbuscular Mycorrhiza Fungi and 5 levels of Acidity on selected growth parameters.

	4WAP				8WAP				12WAP			
TRT	PH (cm)	SG (cm)	LA (cm^2)	NL	PH (cm)	SG (cm)	LA (cm^2)	NL	PH (cm)	SG (cm)	LA (cm^2)	NL
L0	50.80	1.875	9.75	8.67	84.30	1.917	12.13	19.80	106.20	1.958	15.70	25.80
L1	46.90	1.708	8.58	9.60	74.50	1.917	10.52	17.70	93.30	1.958	15.30	23.80
L2	46.30	1.708	9.62	7.33	93.50	1.792	14.27	18.70	108.80	1.975	17.40	25.30
L3	46.90	1.750	10.62	8.33	76.50	1.917	12.94	16.70	99.80	1.958	16.90	26.80
L4	46.10	1.500	8.45	8.33	80.40	1.917	12.80	19.80	92.80	1.958	18.00	22.70
LSD ($P \leq 0.05$)	11.57	0.305	3.26	2.156	25.16	0.284	4.12	7.68	31.42	0.218	7.33	9.93

TRT= Treatment, PH = Plant Height, SG = Stem Girth, LA = Leaf Area, NL = Number of leaves, L0 = pH 4.96, L1 = pH 5.50. L2 = pH 4.56. L4 = 4.00, WAP = Week after planting.

DISCUSSION

In this study, it was observed that AMF after application reduced the pH of treatment L1 (6.00) to 5.20 and pH of L2 (5.50) to 5.15 but increased the pH of treatment L3 (4.56) to 5.10 and pH of L4 (4.00) to 4.87. The obtained result is in accordance with Ouzounidou *et al*., (2015) who reported that application of the MC10 inoculum resulted in reduced levels under alkaline conditions and in increased levels (by about 30 %) under acidic soil conditions.

Generally, the value for Sand content significantly decreased in AMF inoculated samples (84.6 %) than in BP (85.2 %) and Control (85.17 %) respectively. Similarly, Clay content significantly decreased in AMF inoculated (11.07 %) than in BP value (11.90 %) and Control (11.33 %). On the other hand, the Silt content significantly increased in AMF inoculated samples (3.98 %) compared to BP (2.90 %) and Control (3.50). It is obvious that AMF inoculation positively influenced soil aggregation in the study. This observation is in agreement with the studies of Rilling and Mummey (2006) who reported that Mycorrhizal fungi can potentially influence soil aggregation at different levels, namely plant communities, plant roots (individual host), and effects mediated by the fungal mycelium itself and AMF exhibit a strongly positive response to conditions simulating increasingly 'aggregated' soil. AL-Ghamdi and Jais (2013) also stated that the presence of AMF is vital in aggregating the soil particles together to form structured soil. Therefore, one of the important reasons for inoculating AMF may have to do with the effect it renders on the soil structure to form aggregates and facilitate water and nutrient holding capacity of the soil.

Generally, the value for Total Organic Carbon (TOC) significantly increased in AMF inoculated samples (0.51 %) compared with BP (0.20 %). The obtained result is in agreement with Syibli *et al*., (2013) who stated that the mean value of organic carbon increased with increasing AMF spore number.

The value for Total Nitrogen significantly increased in AMF inoculated samples (0.50 %) compared with BP (0.20 %). This agrees with the findings of Diagne *et al.,* (2020) who reported that AMF improved plant growth parameters and the uptake of several major nutrients such as nitrogen and phosphorus in stressed conditions. Fungal hyphae are much thinner than roots and are able to penetrate smaller pores and uptake more nutrients.

Available phosphorus significantly increased in AMF inoculated samples (5.40 %) compared with BP (1.36 %). Also, this observation agrees with the findings of Diagne *et al.,* (2020) as aforementioned. This growth stimulation is linked to the fact that AMF extends the absorbing network beyond the nutrient depletion zones of the rhizosphere, which allows access to a larger volume of soil. Also Njunge (2018) stated that Mycorrhizal fungi benefit plants by generally enhancing nutrient uptake, especially with regards to phosphorous and other micronutrients like zinc, copper and manganese. They do this through the fungal hyphae which explore the rhizosphere extensively, accessing nutrients that would otherwise be unavailable to the plant roots. In return, the fungi receive plant carbohydrates that are important for completing the fungal life cycle. It has been found that AM fungi have active phosphate transporters which take up organic phosphate from the soil and facilitate its delivery to the plant. Plants also possess mycorrhizal specific phosphate transporters which receive phosphorous from the plant and deliver it to plant cells.

Generally, Calcium content significantly increased in AMF inoculated samples (2.90 Cmol/kg) compared with BP (2.23 Cmol/kg). Magnesium content significantly increased in AMF inoculated samples (2.78 Cmol/kg) compared with BP (2.17 Cmol/kg). Sodium content also significantly increased in AMF inoculated samples (0.23 %) compared with BP (0.14 Cmol/kg). The value for Cation Exchange Capacity significantly increased in AMF inoculated samples (6.15 Cmol/kg) compared with BP (4.66 Cmol/kg). The above results were in concordance with findings of Keske (2020) who reported that AMF have capacity to increase nitrogen, potassium, calcium, and phosphorus contents to enhanced plant growth. Also, Sakamotoa and Kaji, (2017) reported that colonization of plant roots by AM fungi significantly improves plant mineral nutrition, increases plant growth, and enhances tolerance against pathogens, drought, and other environmental stresses. This observation also is in tandem with the findings of Syibli, et al. (2013) who reported that CEC in the soil increased with the increase of AMF spore number.

Generally for plant growth parameters at 12WAP; the number of leaves for least pH value, 4.00 (L4) is not significantly different from that of the highest pH value 6.00 (L1). The Leaf Area for least pH value, 4.00 (L4) is not significantly different from that of the highest pH value 6.00 (L1). The Stem Girth for least pH value, 4.00 (L4) is not significantly different from that of the highest pH value 6.00 (L1). The Plant Height least pH value, 4.00 (L4) is not significantly different from that of the highest pH value 6.00 (L1). This observation agrees with findings of Perdensen (2004) who reported that there were no differences among lime treatments and soil pH on soybean yield, grain moisture, stem girth and plant height.

CONCLUSION

Arbuscular Mycorrhizal Fungi (AMF) buffers soil pH as reported by many earlier authors. AMF buffers the alkaline and acid induced soil thereby reducing their effects and hence, enhancing the growth and yield of the cultivated *Glycine max.* The optimal pH range for Glycine max production is 5.5 – 6.5. Also, acidification of soil should be avoided to enhance optimal output.

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Cite this Article: Anozie, HI; Wokocha CC; Fenibo EI (2021). Influence of Arbuscular Mycorrhiza on the Performance of *Glycine max* (L.) Merr. Grown on Acidified Soil. *Greener Journal of Agricultural Sciences* 11(3): 187-194.