

# Effect of Arbuscular Mycorrhizal Fungi Inoculation on Growth and Mineral Nutrition of Soybean (*Glycine max*) Grown Under Different Salinity Levels

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**Abstract** – *Glycine max* is one of the most common crops produced in nurseries. Application of microorganisms such as arbuscular mycorrhizal fungi to enhance salt tolerance is quite well-known, but the interaction of mycorrhiza and salinity to growth and nutrient uptake of salt-sensitive soybean (*Glycine max*) seedlings has been less studied. Therefore, the aim of this study was to investigate the effect of AMF inoculation on growth and nutrient uptake of soybean grown under different salinity levels. A pot experiment was conducted under green-house conditions in Hawassa University College of Agriculture. The non-colonized seedlings and seedlings colonized by *Gigaspora rosea* and *Rhizophagus clarus* were exposed to salt stress by irrigation with 50 mM, 100mM and 150mM NaCl solutions. There was no mycorrhizal colonization recorded in the control plants. All plants inoculated with AMF showed mycorrhizal colonization (the formation of arbuscules and vesicles) and the formation of spores. Salt stress significantly depressed the colonization and spore formation of both *Gigaspora rosea* and *Rhizophagus clarus*. The maximum root length colonization (RLC) and spore density (SD) were recorded in plants grown under salt free soils (non-salinized soils) while the minimum RLC and SD were observed in plants grown under highly salt stressed (150mM NaCl) used soils. Mycorrhizal association markedly increased both growth performance (length and diameter of stem, length of root, number of leaf, wet and dry weights of shoot and root) and nutrient uptake (concentrations of N, P, K, Ca and Mg) of soybean seedlings exposed to salt stress at all salinity levels. Belsa 95 groups inoculated with *Gigaspora rosea* shown better growth performance and nutrient uptake than those groups inoculated with *Rhizophagus clarus* while Afgat M5 groups inoculated with *R. clarus* shown better growth performance and nutrient uptake than those groups inoculated with *G. rosea*. Generally, it is clear that mycorrhizal inoculation possesses the potential to enhance salt tolerance of *Glycine max*. Therefore, intelligent nursery inoculation with commercial AMF seems to be a viable solution to promote growth and mineral uptake of *Glycine max* that are grown under salinity-stressed soils.

**Keywords** – Arbuscular Mycorrhizal Fungi, Inoculation, Glycine Max, Salinity, Tolerance.

## I. INTRODUCTION

### *Background of the Study*

*Glycine max* is one of the most common crops produced in nurseries. Producing and consuming more soybeans improves the situation of malnutrition as it can provide a nutritious combination of both calorie and protein. It is also cheap and rich source of protein for poor farmers, who have less access to animal source protein, because of their low purchasing capacity. Besides better nutritional status, the crop has a great significance in improving the status of soil nutrients and farming system when grown solely and in combination with cereal crops [1].

Soil salinity is a major abiotic stress adversely affecting plant growth and crop production worldwide. Increased salinization of arable land is expected to have destructive universal effects, resulting in 30 % land loss

within the next 25 years and up to 50 % by the middle of twenty-first century [2; 3; 4]. Sodic soils having a poor soil structure generally are found over arid and semiarid regions, with high concentrations of  $\text{Na}^+$  at the exchangeable site of clay particles in the soil. Accordingly, the soil would have a great pH (greater than 8.5) with a high exchangeable sodium percentage (ESP) [5].

Saline soils can be generally found in arid regions, estuaries, and coastal fringes. Such kind of soils are dominated by  $\text{Na}^+$  ions with electrical conductivity (EC) more than 4 dS/m corresponding to the approximate concentration of 40mM NaCl. Moreover, saline soils exhibit ESP of <15 and much lower pH (<8.5) values than the sodic soils (pH>8.5) [5]. According to the potential of plants to grow on high salt medium, plants are grouped as glycophytes or halophytes. Most plants are glycophytes and are not tolerant to salt stress.

The early signs of salinity in plants are: (i) Poor germination and establishment; (ii) Leaf scorching or mottling and shedding; (iii) Cuticle fragmentation and cell membrane injury increases solutes leakage; (iv) Inhibited vegetative growth of glycophytes as shoot growth decreases more than root growth; (v) Salt-induced inhibition of plant growth is accompanied by metabolic dysfunction including decreased photosynthetic rate and changes in enzymatic activity in glycophytes; (vi) In halophytes, Physiological processes may not be altered by salt concentrations that are inhibitory to glycophytes; (vii) Salinity decreases the production of carbohydrate or growth hormones thereby inhibiting growth; (viii) High salt concentrations may adversely affect the activity of enzymes by influencing the protein structure; (ix) Salinity negatively affects plant nutritional balance [6].

One of the natural ways that aids plants to adapt to saline soils is symbiotic association with arbuscular mycorrhizal (AM) fungi (7). Arbuscular mycorrhizal fungi are the type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of vascular plants. They are able to colonize plants, in their natural environment. Arbuscular mycorrhizal fungi are associated with the roots of over 80 % terrestrial plant species [8]. Arbuscular mycorrhizal fungi promote plant growth and salinity tolerance by utilizing various mechanisms, such as: (i) enhancing nutrient uptake [9]; (ii) producing plant growth hormones [9]; (iii) improving rhizospheric and soil conditions [10]; (iv) improvement in photosynthetic activity or water use efficiency (Hajiboland *et al.*, 2010); (v) accumulation of compatible solutes [11]; and (vi) production of higher antioxidant enzymes (Manchanda and Garg, 2011). As a result, AM fungi are considered suitable for bio-amelioration of saline soils.

Horticulture is the most promising area for practical use of AMF for nursery. There are two main benefits from introducing mycorrhizal fungi to horticultural crops: stronger growth in the nursery and improved performance after planting in the field [12].

It is observed that mycorrhizae can improve P absorption in Soybean and increase shoot and root dry weight [13]. Increased growth and yield of soybean in the presence of AM fungi (AMF) has been attributed mainly to the enhanced uptake of P [14]. According to the research report by [15] in Sidama agroforestry practices, inoculating agroforestry trees and perennial crops with AMF enhanced plant growth performance, thus plant height growth of inoculated plants was highly increased when compared to non-inoculated plants as well as the inoculated plants showed increased shoot and root biomass. Similarly, all inoculated plants showed increased tissue nutrients than non-inoculated plants. The aim of this study therefore, is to investigate the effect of AMF (*Gigaspora rosea* and *Rhizophagus clarus*) on plant growth and mineral nutrient acquisition in different varieties of Soybean grown at different salinity level treatments of the soil.

## II. MATERIALS AND METHODS

### Description of Arbuscular Mycorrhizal Fungi Isolation Area

Arbuscular mycorrhizal fungi species were isolated from soil samples collected from Sidamo Zone Wensho woreda huletanya (2<sup>nd</sup> ferro kebele which is found in SNNP regional state. The geographic location of this Kebele lies between 6° 14' and 7° 18' North latitude and 37° 92' and 39° 14' East longitude altitudes ranging from 1170 to 3500 masl (Figure 1). The area receives an annual rainfall of 1000-1800 mm distributed in 8 to 10 months with mean monthly temperature of 10 to 25°C [16].

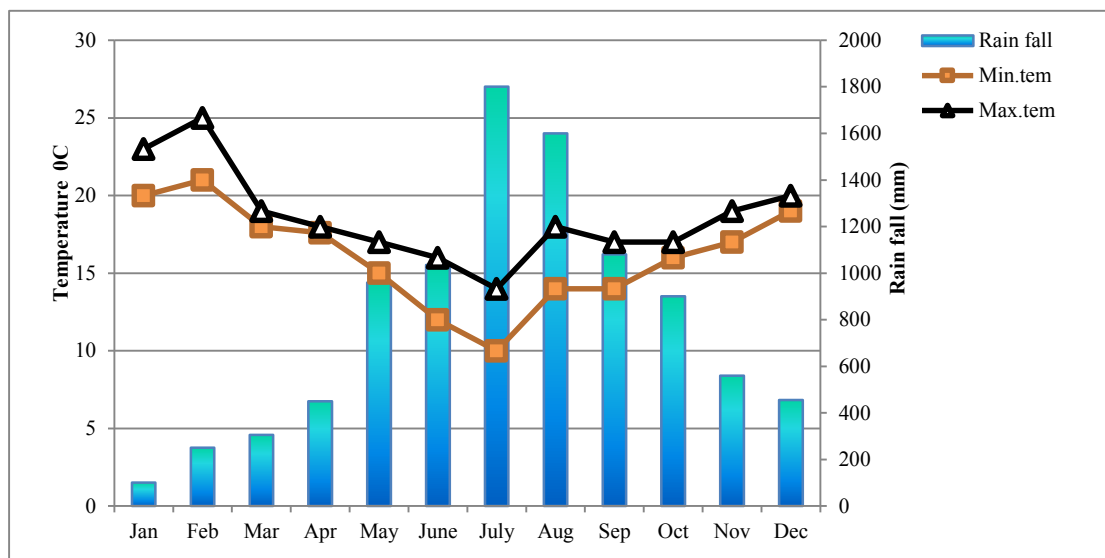


Fig. 1. Climate of soil sampling area for AMF species screening.

The types of natural vegetation in Wensho woreda of Sidama include montane evergreen thickets and scrubs, montane dry evergreen forests, montane moist evergreen forests and various types of grasslands (Abebe Tesfaye, 2005). In areas where agroforestry is dominant, remnant trees of the evergreen forests are observed among which Wanza (*Cordia africana*), Birbira (*Millettia ferruginea*), *Bersama abyssinica* (Teberako, Sidama), Bisana (*Croton macrostachyus*), Korch (*Erythrina brucie*), are very common. Besides these agroforestry trees the farmers grow crops such as coffee (*Coffea arabica*), sugar cane (*Saccharum officinarum*), Enset (*Ensete ventricosum*), Khat (*Catha edulis*), Haricot bean (*Phaseolus vulgaris*), Maize (*Zea mays*) and fruit trees such as Avocado (*Persea americana*) in the research sites [17].

### Isolation and Identification of AM Fungi Spores

Soil samples for isolation and identification of AMF species was collected from the rhizospheres of agroforestry trees and perennial crops such as Birbira (*Millettia ferruginea*) and Coffee (*Coffea arabica*) from Wensho Woreda huletanya ferro kebele. Triplicate soil samples were dugout and collected with spades from depth of about 0-15cm, bulked together, air dried and a composite sample of 1kg was collected in sterile plastic bags that were fumigated with absolute ethanol. The collected soil samples were transported at ambient temperature to soil microbiology laboratory of Hawassa college of Agriculture, HU, Hawassasa. About 500 g of the soil samples were ground, homogenized and passed through a 2mm sieve for soil analysis. The remaining portions of each of the samples were used for AMF spore count and identification. Spore count was determined according to [18].

Briefly, 100 g of each soil sample was suspended into 1.5 liter of water in a 2 liter capacity container (conical flask) and mixed vigorously manually until mixed well to free spores from the soil and roots. The supernatant was subsequently decanted simultaneously through standard sieves (250, 106 and 50  $\mu\text{m}$ ) arranged together in order of decreasing sieve diameter after having been intermittently centrifuged at 2000 rpm for 5 minutes in automatic centrifuge. The last pellet was suspended in 60% sucrose solution and thoroughly mixed and centrifuged at 2000 rpm for 1 minute. After pouring the supernatant, through the smallest mesh sieve, the spores in the sieve were rinsed carefully with tap water and transferred into plastic Petri-dishes. They were counted according to [19] with stereomicroscope (Olympus) under the objective magnification of 4x and spore densities were expressed as the number of spores and sporocarps per 100  $\text{g}^{-1}$  of dry soil.

For identification spores were mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) to identify them into the representative morphospecies based on the descriptions of the International Culture Collection of Vesicular/Arbuscular Mycorrhizal Fungi (<http://invam.caf.wvu.edu>; 2006), and following descriptions by [20] using a compound light microscope (Olympus-bx51) at total of 200-fold magnification. Then, spores were grouped to morphotypes using INVAM (2006) morphological descriptions such as color, wall layers, germination shield, etc. Two most commonly isolated morphotypes *Gigaspora rosea* and *Rhizophagus clarus* were selected as candidate morphospecies for screening in this study.

#### *Production of AMF Inoculums by Trap Culturing*

The trap culture protocol described by [21] was used in the current study to propagate the mycorrhizal isolates. The isolated AMF morphotypes were grown in a sterile nutrient poor soil medium using *Sorghum bicolor* as a host plant for the first time and then evaluated for the purity of the morphospecies in two consecutive culturing.

#### *Preparation of Sterile Nutrient Poor Soil (Soil and Sand Mixture in the Pot)*

Thirty (30) kg of top surface layer soil (25 cm below the surface) was taken from Hawassa University Agricultural College backyard field plots and the sand was brought from around Halaba town north east of Hawassa city. The soil and sand were collected in plastic bags that were sterilized by rinsing with absolute ethanol. The site from which the soil sample was taken is dominated with tomato plant at the time of soil collection. The soil was sundried, ground and then sieved through a 2 mm sieve and autoclaved for 4 h at 120°C to eliminate native microorganisms. Soil and sand that was autoclaved for 4hr at 120°C were mixed in 2:1 ratio (i.e. 1 kg of soil and 0.5 kg of sand) and filled into each of the twenty four pots.

#### *Preparation of the Candidate AMF Morphospecies for Inoculation*

10kg of each of the two most commonly isolated morphospecies of AMF *Gigaspora rosea* and *Rhizophagus clarus* that were screened by using the procedures described above under 3.2 were prepared for inoculation experiment in trap culturing.

#### *Germination of Sorghum Bicolor Seeds as Trap Plant and Transplantation into the Potted Soil*

*Sorghum bicolor* seeds were obtained from Holata agricultural research center. Seeds of *Sorghum bicolor* were surface-sterilized by immersion in 1% sodium hypochlorite for 1 minute, followed by 3 min in 70% ethanol and were rinsed five times with sterile, distilled water [10]. Germination tests were carried out in Petri dishes

(85 × 15 mm) containing 1% water agar. Many surface-sterilized seeds were placed on Petri dish and were arranged in a randomized complete block design. Eventually, the Petri dishes were covered with a polyethylene sheet to avoid the loss of the moisture through evaporation and kept in the plant growth chamber at 28°C. The seeds were checked daily, and the percent germination was recorded after 3 days of incubation. Seeds were considered to have germinated when the emerging radicles were greater than 0.5 cm long. Then the pots that filled with the mixed soil were inoculated with *Gigaspora rosea* and *Rhizophagus clarus* before transplanting by placing 0.25kg of inocula per pot 5cm below the surface of the soil. Then, the germinated seeds were transplanted into the pots.

Finally, the two morphspecies (*Gigaspora rasea* and *Rhizophagus clarus*) were grown as a pure culture using the same *Sorghum bicolor* as inoculums. Mixed AMF inoculum containing spores, sporocarps and roots was applied at the bottom of the transplant hole. *Gigaspora rosea* and *Rhizophagus clarus* are selected as inoculants to investigate their effects on growth and mineral nutrition in soybean grown under different salinity conditions, because they are available in tropical areas as well as are available in Sidama agro ecological zones from where they were isolated.

Each pot was fertilized with half strength of Hoagland's nutrient solution [15] (0.51 g/L KNO<sub>3</sub>, 0.246 g/L Ca(NO<sub>3</sub>)<sub>2</sub>, 0.24 g/L MgSO<sub>4</sub>.7H<sub>2</sub>O, 1.43 g/L H<sub>3</sub>BO<sub>3</sub>, 0.91 g/L MnCl<sub>2</sub>.7H<sub>2</sub>O, 0.11 g/L ZnSO<sub>4</sub>.5H<sub>2</sub>O, 0.04 g/L CuSO<sub>4</sub>.5H<sub>2</sub>O and 0.04 g/L H<sub>2</sub>MoO<sub>4</sub>.H<sub>2</sub>O.) twice in a month and watered on alternate days. After five months of vegetative growth of the host plant, root and soil samples were collected to examine the percent root colonization and number of spores produced. After harvesting, the infected root and soil mass was used as inoculum for further studies (for pot experiments). To obtain a mixed inoculum, the portion of soil at the bottom of the pot and roots were dried and chopped into fine pieces while spores were obtained by wet sieving and decanting which was described under 3.8.2 in detail and the spores were preserved at 4°C.

#### *Preparation of Soil in Which Glycine max Were Grown*

The substrate used in the experiment was a mixture of soil and sand. Eighty kg of soil was collected in plastic bags that were sterilized with ethanol. The site from which the soil and sand were obtained and the procedures of sterilizing as well as mixing of soil and sand was done as in 3.3.1 above. A portion of the soil was used for physicochemical analysis in the DZARC soil chemistry laboratory.

#### *Experimental Design*

The experiment was consisted of a randomized complete block design with three factors:

1. Soybean variety difference (AFGAT M5 and BELSA 95).
- 2) AMF treatments (with AMF (M) and without AMF (NM)) and
- 3) four salinity levels (S0: 0mMNaCl; S1: 50mMNaCl, S2: 100mMNaCl and S3: 150mMNaCl). Each treatment was replicated three times with 3 plants per pot (i.e. comprises 72 pots in total treatment) (table 1).

Table 1. Layout of the three factor ANOVA experiment.

Glycine max variety	Salinity Treatment	AMF Inoculation			Replication Summary
		NAMF	GR	RC	
AFGAT M5	0	3 replications	3 replications	3 replications	9 replications
	50mMNaCl	3 replications	3 replications	3 replications	9 replications
	100mMNaCl	3 replications	3 replications	3 replications	9 replications
	150mMNaCl	3 replications	3 replications	3 replications	9 replications
BELSA 95	0	3 replications	3 replications	3 replications	9 replications
	50mMNaCl	3 replications	3 replications	3 replications	9 replications
	100mMNaCl	3 replications	3 replication	3 replications	9 replications
	150mMNaCl	3 replications	3 replications	3 replications	9 replications
Total replication		24replications	24 replications	24 replications	72 replications

Key: GR-*Gigaspora rosea*; RC-*Rhizophagus clarus*; NAMF-Non-arbuscular mycorrhizal fungi; mM-millimol.

### Germination and Transplantation of Glycine Max Seeds

*Glycine max* seeds of “Belsa 95 and Afgat M5” variety were obtained from Gambella agricultural research center. The whole germination procedures used for the germination of *Sorghum bicolor* seeds in 3.3.2 above was similarly used for the germination of *Glycine max* seeds. One hundred fifty surface-sterilized seeds of each *Glycine max* variety were allowed to be germinated. The germinated soybean seedlings were transplanted into the pots filled with mixture of soil and sand and allowed to grow for 75 days.

### Plant Material and Growth Conditions

The germinated *Glycine max* seeds were transplanted into plastic pots (16 cm in depth and 20 cm in mouth diameter) on 5 December 2010. The potted substrate was inoculated with *Gigaspora rosea* and *Rhizophagus clarus* before sowing, by placing 0.5kg of inocula (soil, spores, hyphae and infected roots) 5 cm below the surface of the soil. Non-inoculated pots were supplied with 0.5kg sterilized substrate as control.

Plants were established for 5 weeks before being subjected to four NaCl levels (0, 50 and 100 and 150mM NaCl) to prevent plants not to be subjected to an early stage salinity stress. The plants were salinized by addition of a salt solution to soil with the irrigation water. After 15 days of inoculation (Weaver and Fredrick, 1982), plants were supplemented once a week with half strength of Hoagland’s solution whose compositions were as described in 3.3.3 above and the seedlings were watered every two days with 200ml tap water. The salinity was induced by adding 100ml of 50mMNaCl, 100mMNaCl and 150mMNaCl solutions twice a week and the control (0mMNaCl) seedlings were irrigated with 100ml of tap water. The soil was salinized step-wisely to avoid subjecting plants to an osmotic shock [22].

### Measurements (Data collection)

#### Growth Parameters

The seedlings in the control and salt treatment pots were allowed to grow for 10 weeks, at which time the experiment was terminated for evaluation of growth parameters. The number of leaves per plant was counted.

Length and diameter of stems and length of roots were measured. The fresh weight of shoots and roots were measured immediately after removing the plants from the pots and the roots were cleaned. Also dry weight (DW) of shoots and roots were measured after oven-drying at 80°C until constant weight was obtained.

#### *AMF Colonization*

AMF colonization was assessed according to Phillips and Hyman (1970). The fine root samples (0.5mg) were cut into 1cm pieces, washed with tap water, preserved in 50% ethanol and stored at 4°C. Preserved root samples were cleared in 10% (w/v) KOH by heating in a water bath at 90°C for 1-2 hours and cooled at room temperature. After cooling, the root samples were washed 3-5 times with tap water, acidified in 1% HCl for 1hr and stained with 0.05% trypan blue and finally destained in acidic glycerol. The AM fungal structures were observed under a compound-light microscope (Olympus-bx51) at 200X magnification. Fungal colonization were estimated using the magnified intersection method of [23], as total root length colonization  $RLC = 100 [(G-N)/G]$ , the percentage of root length colonized by arbuscules, arbuscular colonization  $AC = 100(A/G)$  and the percentage of root length colonized by mycorrhizal vesicles, vesicular colonization  $VC = 100(V/G)$ . RLC, N, A, V and G designated as: RLC (total root length colonization), N (no fungal structure), A (arbuscules), V (vesicles) and G (total intersection) respectively. All were quantified by examining 100-150 intersections per sample. Mycorrhizal dependency was calculated according to [24]:  $[(M-NM)/M] \times 100$ , where: M is the total dry biomass of mycorrhizal plant; NM is the total dry biomass of non-mycorrhizal plant.

#### *Spore Density*

Arbuscular mycorrhizal fungi spore density associated with the two varieties of *Glycine max* under four levels of salinity treatments was determined by using similar procedures that was done in 3.2 above.

#### *Mineral Nutrition*

The mineral nutrient contents in *Glycine max* tissue were analyzed in the DZARC soil chemistry laboratory by the help of laboratory experts. The dried shoots (stems + leaves) were passed through a 0.5 mm sieve in a cyclone laboratory mill and were stored for determination of mineral nutrients.

Molybdovanado phosphate method was applied to measure Phosphorus content [25]. Briefly, 1g of dried and ground plant tissue was weighed into 150-mL beakers and samples were digested using the dry ashing procedure. Then samples were transferred quantitatively into 100-mL volumetric flasks and were diluted with distilled water. Using a dilutor-dispenser, the samples were diluted and the 20, 40, 60 and 80 mg P L<sup>-1</sup> standards 1:100 with the working solution. Colors were allowed to develop for at least 30 minutes before reading concentrations by spectrophotometer at 660 nm wave length. Flame photometry as described by [26] was used to measure contents of potassium (K), calcium (Ca) and magnesium procedurally as follows:

Dry ashing procedure was performed on a known dry weight of tissue. The resulting product was wetted with a small amount of deionized water and was brought into solution using 2 mL of concentrated HCl. It was diluted to 100-mL final volume with deionized water. After bringing to final volume, the solution was mixed by inversion of the volumetric flask several times. Potassium was determined by flame photometry. Serial dilutions were made until the K concentration reading was become within the standardized range of the instrument using 0.3 M HCl diluents. For determinations of Ca and Mg by flame photometry, an aliquot of the above solution was

diluted with the La standard using 1mL of unknown + 9mL of 1000 mg La L<sup>-1</sup>. La is the solution prepared from La<sub>2</sub>O<sub>3</sub>). The linear working range for Ca is from 0 to 10 mg Ca L<sup>-1</sup>, while that of Mg is 0 to 0.5 mg Mg L<sup>-1</sup>.

Finally, total N was measured in samples of 0.1 g dry mass using Kjeldahl method [27]. Firstly, plant tissue sample was placed in Kjeldahl digestion tube and 1.1 g of salt/catalyst mixture was added. Blanks containing only reagents with each set of samples were digested and 3mL of concentrated H<sub>2</sub>SO<sub>4</sub> was added. Then samples were heated slowly to 200<sup>o</sup>C. Once the frothing has subsided, the temperature was brought up to 350 to 375<sup>o</sup>C and heated until the digest clears. It was digested at 350 to 375<sup>o</sup>C for an additional 35 minutes to 1 hour past clearing. The digest was cooled and 20 mL of deionized water was added. Then 5 mL of H<sub>3</sub>BO<sub>3</sub> indicator solution was added to a 50-mL flask and the flask was placed under the condenser with the condenser tube below the surface of the indicator solution. 20mL of 10 MNaOH was added to the digested sample and immediately the tube was transferred to the Kjeldahl distillation apparatus in which the distillation was begun. The distillate was collected until the level in the H<sub>3</sub>BO<sub>3</sub> flask has reached approximately 35mL (usually 12 minutes). Finally, the distilled NH<sub>3</sub> was titrated into the H<sub>3</sub>BO<sub>3</sub> solution using standard 0.01 MHCl or 0.005 MH<sub>2</sub>SO<sub>4</sub> and as the solution shown a pink color the end point was reached.

#### *Data Analysis*

All the data on plant growth and mineral nutrition were subjected to a one way analysis of variance (ANOVA) for testing the effects of AMF inoculation and salinity level, 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) was carried out with the SPSS software package (version 20.0).

### **III. RESULT AND DISCUSSION**

#### *Soil Physicochemical Parameters*

The soil physicochemical characteristics were determined in DZARC soil chemistry laboratory by the help of laboratory experts. According to soil analysis result, some chemical and physical characteristics of the soil were shown in table 2 below.

Table 2. Physicochemical parameters of the soil.

<b>Parameters determined</b>	<b>Results obtained</b>
Ph	6.6
Total nitrogen%	0.6
Available phosphorus (mg kg <sup>-1</sup> )	5.82
Exchangeable K (cmol(+))kg <sup>-1</sup> )	1.15
Organic carbon (%)	0.19
Exchangeable calcium (cmol(+))kg <sup>-1</sup>	2.5
Exchangeable Magnesium (cmol(+))kg <sup>-1</sup>	1.5
Exchangeable Sodium (cmol (+) kg <sup>-</sup>	0.8

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*Growth Parameters*

Table 3 shows the effect of inoculation with two species of AMF and treatment with different salinity level on the growth parameters of the two *Glycine max* varieties. At harvest, all growth parameters (shoot height, stem diameter, leaf number and root length) per plant were recorded. At the end of two and half months (75 days), all growth parameters of inoculated plants was highly significant at  $P < 0.05$  level as compared to non-inoculated plants as salinity increased. As it was shown from the result, mycorrhization increased significantly all growth parameters of all plants, implying that the plants with AMF had higher growth parameters than non-mycorrhizal plants regardless of salinity levels, which means that AM-inoculated plants under saline conditions grew better than non-mycorrhizal plants. This is in agreement with results reported on Green pepper by [28].

Growth parameters increment of the two *Glycine max* variety was different based difference of inoculated AMF species and salinity level, implying that the extent of AM effect on *Glycine max* development varied with *Glycine max* variety and salinity level. Similar result was reported on wheat cultivars by [29]. In case of Afgat M5, all growth parameters increment was better in RC inoculated groups while in case of Belsa 95, all plant growth parameters increment was better in GR inoculated groups. For instance, Afgat M5 inoculated with GR had shoot height ranging from 37.67 to 49.33cm, stem diameter ranging from 2.20 to 4.97cm, leaf number ranging from 5.33 to 14.67cm and root length ranging from 13.67 to 25.33cm while RC inoculated Afgat M5 plants had shoot height ranging from 42.30 to 53.17cm, stem diameter ranging from 3.12 to 5.29cm, leaf number ranging from 7.67 to 16.00cm and root length ranging from 17.67 to 27.67cm, thus the maximum growth parameters were recorded in salt-free soils infected by RC, and the minimum growth parameters were occurred in GR- colonized seedlings subjected to salt stress. This indicates that, in Afgat M5, RC is significantly better than GR in improving growth parameters under salinity stressed soils.

In contrast, Belsa 95 inoculated with GR had shoot height ranging from 43.00 to 53.00cm, stem diameter ranging from 2.88 to 5.27cm, leaf number ranging from 7.67 to 16.67 and root length ranging from 18.67 to 28.82cm while RC inoculated Belsa 95 plants had shoot height ranging from 35.50 to 50.17cm, stem diameter ranging from 2.17 to 5.17cm, leaf number ranging from 4.67 to 15 and root length ranging from 14.33 to 27.53cm, thus the maximum growth parameters were recorded in salt-free soils infected by GR, and the minimum growth parameters were occurred in GC- colonized seedlings subjected to salt stress. This indicates that, in Belsa95, GR is significantly better than RC in improving growth parameters under salinity stressed soils. The result showed that, even all growth parameters were affected by salinity, the difference among growth parameters was not significant under the first two (0 and 50mM NaCl) salinity treatments ( $P > 0.05$ ) except shoot height which was increased significantly ( $P < 0.05$ ) in mycorrhizal plants than non-mycorrhizal ones under all salinity level treatments.

In general, as the finding revealed, most of the growth parameters of the inoculated and non-inoculated *Glycine max* plants that grown under the first two salinity level treatments were not significant at  $P < 0.05$  level except shoot height of both *Glycine max* varieties in which the shoot height was significantly higher in mycorrhizal plants than non-mycorrhizal plants under controlled groups and plants treated with all salinity levels (table 3). But the growth parameters were decreased significantly as salinity increased in both mycorrhizal and non-mycorrhizal plants. Salinity hampered all growth parameters of non-mycorrhizal plants in both *Glycine max* varieties. This is in agreement with results observed on *Glycine max* by [30]. By contrast,

mycorrhization was found to increase the fitness of soybean plants by maintaining greater growth at all salinity levels when compared with non-mycorrhizal plants. It is similar with result that was reported on soybean by [31].

According to report of [32], the enhanced growth of AM-inoculated plants in saline conditions is related partly to mycorrhizal-mediated enhancement of mineral uptake, especially phosphorus nutrition in host plants. The result of this study also supported this idea.

Table 3. Effect of AMF inoculation and salinity on growth (Shoot height, Stem diameter, Leaf number and Root length) of *Glycine max* plants

Salinity (NaCl in mM)	MI	Shoot height		Stem diameter		Leaf number		Root length	
		Afgat	Belsa	Afgat	Belsa	Afgat	Belsa	Afgat	Belsa
0	NAMF	45.00 <sup>a</sup>	47.17 <sup>a</sup>	4.63 <sup>ab</sup>	4.83 <sup>ab</sup>	13.67 <sup>a</sup>	13.67 <sup>a</sup>	24.33 <sup>ab</sup>	26.29 <sup>a</sup>
	GR	49.33 <sup>b</sup>	53.00 <sup>b</sup>	4.97 <sup>a</sup>	5.27 <sup>a</sup>	14.67 <sup>ab</sup>	16.67 <sup>ab</sup>	25.33 <sup>a</sup>	28.83 <sup>ab</sup>
	RC	53.17 <sup>cf</sup>	50.17 <sup>c</sup>	5.29 <sup>ab</sup>	5.17 <sup>ab</sup>	16.67 <sup>a</sup>	15.00 <sup>ab</sup>	27.67 <sup>a</sup>	27.53 <sup>a</sup>
50	NAMF	42.50 <sup>d</sup>	47.83 <sup>da</sup>	4.42 <sup>b</sup>	4.47 <sup>b</sup>	10.33 <sup>b</sup>	12.00 <sup>ba</sup>	21.33 <sup>b</sup>	23.20 <sup>ba</sup>
	GR	46.00 <sup>e</sup>	51.00 <sup>eb</sup>	4.61 <sup>ba</sup>	5.00 <sup>ca</sup>	12.67 <sup>b</sup>	14.67 <sup>b</sup>	22.67 <sup>ba</sup>	26.00 <sup>bd</sup>
	RC	52.00 <sup>f</sup>	49.83 <sup>ec</sup>	4.90 <sup>b</sup>	4.72 <sup>b</sup>	15.67 <sup>ca</sup>	13.33 <sup>b</sup>	24.33 <sup>bd</sup>	24.67 <sup>ba</sup>
100	NAMF	37.00 <sup>g</sup>	35.50 <sup>f</sup>	2.13 <sup>c</sup>	2.23 <sup>b</sup>	4.33 <sup>d</sup>	4.33 <sup>ce</sup>	14.00 <sup>c</sup>	14.00 <sup>ce</sup>
	GR	42.50 <sup>h</sup>	46.83 <sup>g</sup>	3.10 <sup>d</sup>	3.83 <sup>c</sup>	7.67 <sup>ch</sup>	10.67 <sup>d</sup>	17.00 <sup>df</sup>	22.00 <sup>df</sup>
	RC	46.83 <sup>i</sup>	41.33 <sup>h</sup>	3.80 <sup>d</sup>	3.12 <sup>d</sup>	11.67 <sup>f</sup>	7.67 <sup>ce</sup>	21.00 <sup>d</sup>	17.67 <sup>c</sup>
150	NAMF	33.00 <sup>j</sup>	30.50 <sup>i</sup>	1.37 <sup>e</sup>	1.27 <sup>e</sup>	2.00 <sup>gd</sup>	2.00 <sup>e</sup>	10.33 <sup>e</sup>	11.07 <sup>e</sup>
	GR	37.67 <sup>k</sup>	43.00 <sup>j</sup>	2.20 <sup>f</sup>	2.88 <sup>f</sup>	5.33 <sup>h</sup>	7.67 <sup>fd</sup>	13.67 <sup>f</sup>	18.67 <sup>f</sup>
	RC	42.30 <sup>l</sup>	35.50 <sup>k</sup>	3.12 <sup>g</sup>	2.17 <sup>g</sup>	8.33 <sup>i</sup>	4.67 <sup>e</sup>	17.67 <sup>fd</sup>	14.33 <sup>e</sup>

Key: MI-mycorrhizal inoculation; mM-millimol; NAMF-non-arbuscular mycorrhizal fungi; GR-*Gigaspora rosea*; RC- *Rhizophagus clarus*. The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at P<0.05 level.

Most of the peaks of shoot and root wet and dry weights of the two varieties of *Glycine max* occurred in the first two salt treatment ranges (0 and 50mM NaCl). Under these two salt level treatments, both shoot and root weight of the two *Glycine max* that were inoculated and non-inoculated were decreased with slight difference. However, as salinity increased, both the wet and dry weights of the two varieties were decreased significantly (p<0.05) regardless of AMF inoculation (table 4).

Although, the weights of all plants were decreased with increasing of salinity, mycorrhizal inoculation increased the fitness of *Glycine max* plant at all salinity levels when compared with non-mycorrhizal plants, implying that AMF promoted the growth of *Glycine max* under saline stress by increasing P concentrations which indicates that AM significantly increased plant wet and dry weight while the P concentration was decreased in that of non-inoculated plants. Similar responses were reported by [31] in *Glomus fasciculatum*-colonized *Glycine max* grown under salt stress and by [33] in *Gigaspora rosea*-colonized *Centchrus ciliaris*. Compared to GR inoculated groups, RC inoculated Afgat M5 were more effective in weight increase

enhancement response as salinity increased while GR inoculated Belsa95 showed higher weight increment response than RC-colonized Belsa 95 groups as salinity increased.

GR inoculated Afgat M5 had shoot and root wet weights ranging from 8.18-21.62g/pot and 4.73-14.25g/pot respectively and dry weights ranging from 5.73-14.62g/pot and 3.10-12.04g/pot respectively while Afgat M5 inoculated with RC had shoot and root wet weights ranged from 13.02-24.30g/pot and 7.20-15.37g/pot respectively and dry weights ranging from 6.98-16.06g/ pot and 5.63-12.51g/pot respectively, thus the maximum increment in weight was recorded in RC-colonized Afgat M5 in salt-free soils were as the minimum weight was occurred in GR inoculated Afgat M5 seedlings subjected to salinity stress. This informs that in Afgat M5, inoculation of RC is significantly better than inoculating with GR in mitigating salinity stress of soil on plant growth (table 4). Reversely, GR inoculated Belsa95 had shoot and root wet weights ranging from 13.02-27.14g/pot and 7.53-16.02g/pot respectively and dry weights ranging from 9.00-17.50g/pot and 5.17-12.37g/pot respectively while Belsa 95 inoculated with RC had shoot and root wet weights ranged from 7.85-25.48g/pot and 5.07-15.77g/pot respectively and dry weights ranging from 5.11-16.45g/pot and 3.00-12.20g/pot respectively which shows that the maximum increment in weight was recorded in GR-colonized Belsa 95 in salt-free soils while the minimum weight was occurred in RC inoculated Belsa 95 seedlings subjected to salinity stress. This indicates that salinity stress in Belsa 95 will be significantly alleviated better by inoculating with GR than inoculating with RC.

Generally, from overall seedlings of the two *Glycine max* varieties grown for this experiment with all types of inoculation and salinity treatments, the minimum weights were found in plants that were not inoculated and subjected to the highest salinity stress (150mM NaCl). Contrastingly, even their weights were decreased as salinity increased; mycorrhizal colonization had significant effect on *Glycine max* shoot and root weights. The results showed that the weights of mycorrhizal inoculated plants were much more than non-mycorrhizal plants. This is in agreement with the earlier study by [31] in *Glomus fasciculatum*-colonized *Glycine max* and by [34] in *Rhizophagus clarus*-colonized *Glycine max*.

As presented in table 6, the two AMF inoculants (GR and RC) species significantly ( $P < 0.05$ ) improved shoot and root dry biomass when compared to non-inoculated plants as salinity increased, which indicates that *Glycine max* plants grown in severe salinity stress relied heavily on mycorrhizal symbiosis were as those grown in low salinity stressed soil were less dependent on symbiosis. The finding showed that the two varieties are different in their dependency on AMF. The average MD of Afgat M5 inoculated with GR was ranged from 13.30-25.90 and that of RC-colonized was 16.08-33.65, thus the maximum average value of MD was recorded in plants with RC treated with 150mM NaCl which indicates that Afgat M5 relied significantly on RC than GR as salinity increased while the average MD of GR-colonized Belsa 95 was ranged from 10.36-26.19 and that of inoculated with RC had 7.99-21.80 from which the Belsa 95 inoculated with GR at 150mM NaCl had maximum average MD value (26.19) implying that Belsa 95 is significantly more dependent on GR than RC at salinity stressed conditions.

Table 4. Effect of AMF inoculation and salinity on shoot and root wet and dry and mycorrhizal dependence (MD) of *Glycine max* plant.

Salinity (NaCl in mM)	MI	SFW (g/pot)		RFW (g/pot)		SDW (g/pot)		RDW (g/pot)		MD	
		Afgat	Belsa	Afgat	Belsa	Afgat	Belsa	Afgat	Belsa	Afgat	Belsa
0	NAMF	19.63 <sup>ad</sup>	24.20 <sup>a</sup>	13.57 <sup>ac</sup>	15.53 <sup>a</sup>	13.35 <sup>ac</sup>	16.32 <sup>a</sup>	10.83 <sup>ac</sup>	12.13 <sup>a</sup>	-	-
	GR	21.62 <sup>b</sup>	27.14 <sup>b</sup>	14.25 <sup>a</sup>	16.02 <sup>ab</sup>	14.62 <sup>a</sup>	17.50 <sup>ab</sup>	12.04 <sup>b</sup>	12.37 <sup>ab</sup>	13.30 <sup>ab</sup>	10.36 <sup>a</sup>
	RC	24.30 <sup>c</sup>	25.48 <sup>c</sup>	15.37 <sup>bd</sup>	15.77 <sup>a</sup>	16.06 <sup>bc</sup>	16.45 <sup>ab</sup>	12.51 <sup>bc</sup>	12.20 <sup>a</sup>	16.08 <sup>a</sup>	7.99 <sup>ab</sup>
50	NAMF	18.65 <sup>d</sup>	19.66 <sup>d</sup>	11.30 <sup>c</sup>	13.50 <sup>ba</sup>	11.85 <sup>c</sup>	15.30 <sup>ba</sup>	10.00 <sup>c</sup>	11.17 <sup>ba</sup>	-	-
	GR	20.11 <sup>eb</sup>	24.30 <sup>c</sup>	12.27 <sup>ac</sup>	14.43 <sup>b</sup>	13.47 <sup>da</sup>	16.16 <sup>b</sup>	11.07 <sup>db</sup>	11.77 <sup>b</sup>	12.56 <sup>a</sup>	10.58 <sup>a</sup>
	RC	21.88 <sup>e</sup>	22.88 <sup>c</sup>	13.55 <sup>d</sup>	13.97 <sup>ba</sup>	15.35 <sup>c</sup>	15.73 <sup>b</sup>	12.10 <sup>c</sup>	11.43 <sup>ba</sup>	15.87 <sup>a</sup>	9.97 <sup>a</sup>
100	NAMF	8.20 <sup>f</sup>	10.41 <sup>f</sup>	4.54 <sup>e</sup>	4.67 <sup>c</sup>	5.12 <sup>f</sup>	5.69 <sup>c</sup>	2.80 <sup>f</sup>	2.90 <sup>c</sup>	-	-
	GR	13.00 <sup>g</sup>	19.13 <sup>g</sup>	7.33 <sup>f</sup>	11.00 <sup>d</sup>	8.69 <sup>g</sup>	12.15 <sup>d</sup>	5.63 <sup>g</sup>	8.57 <sup>d</sup>	20.89 <sup>b</sup>	19.50 <sup>b</sup>
	RC	17.59 <sup>h</sup>	14.25 <sup>h</sup>	10.22 <sup>g</sup>	7.43 <sup>e</sup>	10.29 <sup>h</sup>	8.82 <sup>c</sup>	8.33 <sup>h</sup>	5.27 <sup>e</sup>	27.57 <sup>c</sup>	15.34 <sup>c</sup>
150	NAMF	3.89 <sup>i</sup>	3.50 <sup>i</sup>	2.50 <sup>h</sup>	2.60 <sup>f</sup>	2.70 <sup>i</sup>	2.01 <sup>f</sup>	0.83 <sup>i</sup>	0.90 <sup>f</sup>	-	-
	GR	8.18 <sup>j</sup>	13.02 <sup>j</sup>	4.73 <sup>i</sup>	7.53 <sup>g</sup>	5.73 <sup>j</sup>	9.00 <sup>g</sup>	3.10 <sup>j</sup>	5.17 <sup>g</sup>	25.90 <sup>d</sup>	26.19 <sup>d</sup>
	RC	13.02 <sup>k</sup>	7.85 <sup>k</sup>	7.20 <sup>j</sup>	5.07 <sup>h</sup>	6.98 <sup>h</sup>	5.11 <sup>h</sup>	5.63 <sup>k</sup>	3.00 <sup>h</sup>	33.65 <sup>e</sup>	21.80 <sup>e</sup>

Key: MI-mycorrhizal inoculation; mM-millimol; NAMF-non-arbuscular mycorrhizal fungi; GR-*Gigaspora rosea*; RC- *Rhizophagus clarus*; SFW- shoot fresh weight; RFW-root fresh weight; SDW- shoot dry weight; RDW-root dry weight; MD-mycorrhizal dependence. The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at P<0.05 level.

### Root Colonization

Arbuscular mycorrhizal fungi structural colonization (Arbuscular, Vesicular and Root length colonization) of the *Glycine max* seedlings after inoculation with *G. rosea* and *R. clarus* are presented in tables 5 & 6. In this experiment, there was no mycorrhizal colonization recorded in the control plants. *Glycine max* grown in non-saline soil had maximum AM colonization, but the mycorrhizal colonization of plants inoculated with both AMF species showed insignificant difference (P>0.05) under soil that was non-salinized and salinized with 50mM NaCl; whereas as salinity increased, the mycorrhizal colonization and spore number significantly decreased (P<0.05) in both varieties that were inoculated with both GR and RC except that of A formation in Belsa 95 in which the formation was insignificant even in increment of salinity. This indicates that, even though it is sensitive to salinity, Belsa 95 is more tolerant than Afgat M5 to salinity stress.

The colonization rate declined with increased salinity level (Table 5 & 6), implying that salinity suppressed the formation of AM. That means, as revealed from this finding, formation of Arbuscules and Vesicles by the *Gigaspora rosea* and *Rhizophagus clarus* inoculations were more favored at non-salinized soils than higher salt level treatments. Similar results by [31] on *Glycine max* grown under salt stress and [28] on Green pepper grown under Irrigation with saline water were reported.

The colonization percentages of inoculated plants were different between the two varieties of *Glycine max* based on the difference of AMF species used and salinity level. GR inoculated AfgatM5 plants inoculated with GR had colonization percentage ranging from 18.06%-54.44% while RC inoculated groups had colonization percentage ranging from 28.89%-62.78% which tells that, the maximum colonization was recorded in salt-free soils infected by RC (62.78%), and the minimum colonization was occurred in GR-colonized seedlings

subjected to salt-stress (18.06%). This implies that in Afgat M5, RC is significantly ( $P < 0.05$ ) better than GR in salinity stress alleviation (table 5). By contrast, Belsa 95 inoculated with GR had colonization percentage ranging from 38.61%-70.94% while RC inoculated Belsa 95 had root colonization of 30.28%-66.90%, thus the maximum colonization (70.94%) was recorded in GR-colonized non-salinized soils and the minimum colonization (30.28 %) was occurred in RC-infected seedlings subjected to salt-stress. So inoculating Belsa95 with GR is significantly ( $P < 0.05$ ) better than inoculating with RC for soil salinity alleviation. Furthermore, as table 7&8 shows, the plants grown under 0 and 50mMNaCl salinity level had a greater number of AM spores as compared to the plants that grown under the next two higher salinity levels (100 and 150mMNaCl).

AfgatM5 inoculated with GR had the spore number ranging from 120.88-360.00/100g dry soil and in RC-colonized Afgat M5 groups the spore number ranged from 180.02-400.46/100g dry soil, implying that the maximum spore density (400.46/100g soil) was found in RC inoculated plants that were grown in non-salinized soils were as the minimum density (120.88/g soil) was recorded in GR-infected seedlings subjected to salt-stress (table 5). Dissimilarly, in Belsa 95 the spore number of GR-inoculated groups ranged from 210.06-390.20/100g dry soil and the seedlings inoculated with RC had spore number ranged from 180.10-350.92/100g dry soil which indicates that the maximum density (390.20/g soil) was obtained in GR-inoculated plants that were grown in salt-free soils were as the minimum density (180.10/g soil) was found in RC-colonized seedlings subjected to salt-stress (table 8). Based on spore density investigated, RC is significantly ( $P < 0.05$ ) better than GR in Afgat M5 were as GR is significantly ( $P < 0.05$ ) better than RC in Belsa 95 to alleviate salinity-induced adverse effects.

In general, all the plants inoculated with AM fungi showed mycorrhizal colonization that was characterized by the presence of arbuscules and vesicles (table 5 & 6). However, mycorrhizal colonization, A and V formation decreased significantly ( $P < 0.05$ ) as salt concentration increased. Also similar trend was observed with mycorrhizal spore density (table 7&8). Similar result was observed by [35] on salinity treated *Phaseolus Mungo* (L.) Hepper.

Table 5. Effect of AMF inoculation and salinity on AMF structural colonization (%AC, %VC, %RLC) and spore density of Afgat M5.

Glycine max Variety	NaCl (mM)	MI	Structural colonization			
			AC%	VC%	RLC%	SD/100g soil
Afgat M5	0	NAMF	-	-	-	-
		GR	17.63 ± 0.32 <sup>ab</sup>	19.61 ± 0.86 <sup>a</sup>	54.44 <sup>a</sup>	360.00 <sup>a</sup>
		RC	18.00 ± 0.54 <sup>a</sup>	20.17 ± 0.32 <sup>ab</sup>	62.78 <sup>b</sup>	400.46 <sup>b</sup>
	50	NAMF	-	-	-	-
		GR	15.90 ± 2.64 <sup>b</sup>	17.40 ± 3.14 <sup>ba</sup>	40.56 <sup>c</sup>	310.32 <sup>c</sup>
		RC	16.80 ± 1.54 <sup>ba</sup>	18.33 ± 2.45 <sup>b</sup>	52.78 <sup>d</sup>	350.10 <sup>d</sup>
	100	NAMF	-	-	-	-
		GR	10.52 ± 4.65 <sup>c</sup>	11.50 ± 4.1 <sup>c</sup>	30.28 <sup>e</sup>	220.28 <sup>e</sup>
		RC	13.50 ± 3.12 <sup>d</sup>	14.80 ± 3.04 <sup>d</sup>	40.83 <sup>f</sup>	270.10 <sup>f</sup>
	150	NAMF	-	-	-	-
		GR	6.20 ± 0.23 <sup>e</sup>	5.80 ± 1.74 <sup>e</sup>	18.06 <sup>e</sup>	120.88 <sup>e</sup>
		RC	9.42 ± 1.34 <sup>f</sup>	11.60 ± 0.56 <sup>f</sup>	28.89 <sup>b</sup>	180.02 <sup>b</sup>

Key: MI- mycorrhizal inoculation; GR-Gigasporarosea; RC-Rhizophagus clarus; AC- arbuscular colonization; VC- vesicular colonization; RLC- Root length colonization; SD- spore density. mM-millimol. The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at  $P < 0.05$  level.

Table 6. Effect of AMF inoculation and salinity on AMF structural colonization (%AC, %VC, %RLC) and spore density of Belsa 95.

Glycine max Variety	NaCl (mM)	MI	Structural colonization			
			AC%	VC%	RLC%	SD/100g soil
Belsa95	0mM	NAMF	-	-	-	-
		GR	19.3 ± 0.86 <sup>ab</sup>	23.10 ± 1.46 <sup>a</sup>	70.94 <sup>a</sup>	390.2 <sup>a</sup>
		RC	18.5 ± 1.2 <sup>a</sup>	21.00 ± 0.4 <sup>ab</sup>	66.9 <sup>b</sup>	350.92 <sup>b</sup>
	50mM	NAMF	-	-	-	-
		GR	18.2 ± 1.37 <sup>b</sup>	22.7 ± 1.22 <sup>ba</sup>	63.89 <sup>c</sup>	340.94 <sup>c</sup>
		RC	16.9 ± 0.6 <sup>ba</sup>	18.57 ± 1.2 <sup>b</sup>	57.78 <sup>d</sup>	320.54 <sup>d</sup>
	100mM	NAMF	-	-	-	-
		GR	16.80 ± 2.22 <sup>cb</sup>	18.50 ± 2.00 <sup>c</sup>	53.06 <sup>e</sup>	280.98 <sup>e</sup>
		RC	14.30 ± 0.3 <sup>cb</sup>	14.20 ± 0.7 <sup>d</sup>	42.22 <sup>f</sup>	260.04 <sup>f</sup>
	150mM	NAMF	-	-	-	-
		GR	13.50 ± 2.18 <sup>d</sup>	14.25 ± 1.25 <sup>c</sup>	38.61 <sup>e</sup>	210.06 <sup>e</sup>
		RC	12.00 ± 2.22 <sup>dc</sup>	12.50 ± 0.12 <sup>ed</sup>	30.28 <sup>h</sup>	180.1 <sup>h</sup>

Key: MI- mycorrhizal inoculation; GR-*Gigaspora rosea*; RC-*Rhizophagus clarus*; AC- arbuscular colonization; VC- vesicular colonization; RLC- Root length colonization; SD- spore density. mM-millimol. The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at P<0.05 level.

### Nutrient Analysis

In the present investigation, the highest shoot nutrient contents were recorded in AM-colonized *Glycine max* plants that were grown under non-salinized soil (Table 7). This is in agreement with the study on salt stressed *Phaseolus mungo* (L.) Hepper [35]. Salt-stress significantly (P<0.05) affected the nutrient contents in the shoots of *Glycine max* plants in both mycorrhizal and non-mycorrhizal seedlings, implying that nutrient concentrations significantly declined with increasing salinity. Similar result was reported by [36] on tomato grown under salt stressed soil. However, under high salinity, shoot nutrient concentrations remained higher in mycorrhizal plants compared to non-inoculated plants which were consistent with results in several previous studies [33; 37; 22; 36; 34].

The respective values of nutrient concentrations of AM-inoculated plants were generally higher when compared to non-inoculated plants, but differences were significant only under high salinity stress (plants treated with 100mM NaCl and 150mM NaCl). This is in agreement with the result of several previous studies [36; 38]. In most of both *Glycine max* varieties that were grown under 0 and 50mMNaCl, the nutrient uptake of inoculated and non-inoculated plants was not statistically significant at P<0.05 level. However, uptake of all nutrients (N, P, K, Ca and Mg) ions was significantly higher in AM-inoculated plants than non-inoculated plants in both varieties except that of P nutrition in Belsa95 in which P concentration did not differ significantly among mycorrhizal and non-mycorrhizal plants under all salt level treatments, which implies that Belsa 95 is less responsive than Afgat M5 for salinity stress, meaning that Belsa 95 is more tolerant than Afgat M5 for salinity stress. Except P uptake in Belsa 95, the relative uptake rate of all nutrients of AM-colonized and non-inoculated plants was gradually and significantly reduced (P<0.05) due to raised salinity, but it always remained higher in AM-inoculated plants than non-inoculated plants.

Table 7. Effect of AMF inoculation and salinity on nutrient (N, P, K, Ca and Mg) uptake of *Glycine max* plants.

Salinity (NaCl in mM)	MI	%N		%P		%K		%Ca		%Mg	
		Afgat	Belsa	Afgat	Belsa	Afgat	Belsa	Afgat	Belsa	Afgat	Belsa
0	NAMF	0.60 <sup>ac</sup>	0.55 <sup>a</sup>	0.53 <sup>a</sup>	0.50 <sup>ab</sup>	1.99 <sup>a</sup>	2.23 <sup>ab</sup>	0.95 <sup>a</sup>	0.94 <sup>ab</sup>	0.58 <sup>ab</sup>	0.54 <sup>a</sup>
	GR	0.79 <sup>b</sup>	0.95 <sup>bc</sup>	0.62 <sup>bd</sup>	0.54 <sup>a</sup>	2.07 <sup>ab</sup>	2.52 <sup>a</sup>	1.11 <sup>ab</sup>	1.01 <sup>a</sup>	0.66 <sup>a</sup>	0.64 <sup>ab</sup>
	RC	0.90 <sup>bd</sup>	0.57 <sup>c</sup>	0.68 <sup>b</sup>	0.52 <sup>ab</sup>	2.11 <sup>a</sup>	2.32 <sup>ab</sup>	1.21 <sup>a</sup>	0.99 <sup>ab</sup>	0.74 <sup>ab</sup>	0.55 <sup>ab</sup>
50	NAMF	0.45 <sup>c</sup>	0.40 <sup>da</sup>	0.48 <sup>cb</sup>	0.31 <sup>b</sup>	1.96 <sup>ba</sup>	1.97 <sup>b</sup>	0.87 <sup>ba</sup>	0.85 <sup>b</sup>	0.55 <sup>b</sup>	0.49 <sup>ba</sup>
	GR	0.75 <sup>db</sup>	0.85 <sup>c</sup>	0.51 <sup>d</sup>	0.34 <sup>ba</sup>	2.06 <sup>b</sup>	2.22 <sup>ba</sup>	0.92 <sup>b</sup>	0.88 <sup>ba</sup>	0.64 <sup>ba</sup>	0.62 <sup>b</sup>
	RC	0.88 <sup>d</sup>	0.54 <sup>fc</sup>	0.57 <sup>db</sup>	0.33 <sup>b</sup>	2.10 <sup>ba</sup>	2.09 <sup>b</sup>	0.93 <sup>b</sup>	0.88 <sup>b</sup>	0.72 <sup>b</sup>	0.53 <sup>b</sup>
100	NAMF	0.31 <sup>e</sup>	0.33 <sup>gd</sup>	0.17 <sup>c</sup>	0.05 <sup>cd</sup>	0.60 <sup>c</sup>	0.87 <sup>c</sup>	0.21 <sup>c</sup>	0.41 <sup>c</sup>	0.08 <sup>c</sup>	0.11 <sup>cf</sup>
	GR	0.55 <sup>f</sup>	0.58 <sup>hf</sup>	0.35 <sup>f</sup>	0.17 <sup>c</sup>	1.75 <sup>d</sup>	1.79 <sup>d</sup>	0.47 <sup>d</sup>	0.83 <sup>df</sup>	0.23 <sup>dg</sup>	0.46 <sup>d</sup>
	RC	0.75 <sup>gd</sup>	0.50 <sup>h</sup>	0.44 <sup>f</sup>	0.15 <sup>cd</sup>	1.82 <sup>c</sup>	1.46 <sup>c</sup>	0.75 <sup>ch</sup>	0.79 <sup>d</sup>	0.47 <sup>c</sup>	0.28 <sup>ch</sup>
150	NAMF	0.20 <sup>h</sup>	0.11 <sup>i</sup>	0.09 <sup>e</sup>	0.04 <sup>d</sup>	0.25 <sup>f</sup>	0.17 <sup>f</sup>	0.05 <sup>f</sup>	0.16 <sup>c</sup>	0.02 <sup>f</sup>	0.02 <sup>f</sup>
	GR	0.35 <sup>i</sup>	0.42 <sup>f</sup>	0.29 <sup>h</sup>	0.16 <sup>dc</sup>	1.33 <sup>e</sup>	1.22 <sup>e</sup>	0.32 <sup>gd</sup>	0.79 <sup>f</sup>	0.13 <sup>e</sup>	0.36 <sup>gd</sup>
	RC	0.52 <sup>j</sup>	0.38 <sup>h</sup>	0.36 <sup>h</sup>	0.13 <sup>d</sup>	1.58 <sup>e</sup>	0.89 <sup>e</sup>	0.74 <sup>h</sup>	0.53 <sup>gd</sup>	0.35 <sup>h</sup>	0.23 <sup>h</sup>

Key: MI-mycorrhizal inoculation; mM-millimol; NAMF-non-arbuscular mycorrhizal fungi; GR-*Gigaspora rosea*; RC-*Rhizophagus clarus*. The same letter within each column indicates no significant difference among treatments by ANOVA and Duncan's multiple range test at P<0.05 level.

#### IV. CONCLUSION

In summary, it is apparent that pre-inoculation of *Glycine max* transplants with AM fungi have positive enhancement effects in reducing the effects of salt stress through enhancing growth and nutrient uptake. The results indicate that sensitive *Glycine max* plants (e.g. Afgat M5) to moderately sensitive *Glycine max* plants (e.g. Belsa 95) to salt stress can benefit from mycorrhizal inoculation under ecosystem that is affected by salt stress. Even the difference in growth and nutrient uptake among most of the inoculated and non-inoculated plants grown under the first two salt level (0 and 50mMNaCl) treatments was insignificant, the highest growth and mineral concentrations were recorded in plants that were grown under non-salinized soils.

Although salt stress significantly affected the growth and mineral uptake of both mycorrhizal and non-mycorrhizal seedlings, the growth and nutrient concentrations remained higher in mycorrhizal plants compared to non-mycorrhizal plants under high salinity. There was no mycorrhizal colonization and spore density recorded in non-inoculated plants. The mycorrhizal colonization and spore density of plants inoculated with both AMF species significantly decreased as salinity increased. *Rhizophagus clarus* is significantly better than *Gigaspora rosea* in AfgatM5 were as *Gigaspora rosea* is significantly better than *Rhizophagus clarus* in Belsa95 to alleviate salinity-induced adverse effects. These results may have practical importance, as they emphasize the potential of using mycorrhizal fungi inoculation in *Glycine max* to re-vegetate saline lands, especially where indigenous mycorrhizal populations are low or do not result in colonization of *Glycine max*. Besides, prudent use of AMF should be encouraged to prolong their effectiveness in mitigating a serious salinity problem on growth and mineral uptake of *Glycine max*.

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