

# **RESEARCH ARTICLE**

# **RESPONSE OF SOYBEAN INOCULANT WITH MYCORRHIZAL AND ALLEVIATES SALT STRESS** IN THE PLANT IRRIGATION WITH SALINE WATER.

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#### Abstract

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High salinity of the supply water has detrimental effects on soil fertility and plant nutrition and reduces plant growth and yield .This research has been conducted to determine the effect of mycorrhiza on soybean (Glycine max L.) growth under saline conditions. Soybean seeds were sown in pots filled with 6 kg soil. The soil solution electrical conductivity (ECe) was 2.1 dS/m. Plants were irrigated with non-saline water (ECw =  $0.6 \text{ dS m}^{-1}$ ) or saline water (ECw =  $6.2 \text{ dS m}^{-1}$ ) until harvest. These treatments resulted with soil ECe at harvest 1.2 and 5.3 dS m<sup>-1</sup> for non-saline and saline water treatments, respectively. Root colonization with AM fungi was lower under saline than non-saline conditions. Inoculated sovbean plants with AM fungi irrigated with both saline and non-saline water had greater shoot and root dry matter (DM) than non-AM plants. Shoot contents of P, K, Zn, and Ca were higher in AM compared with non-AM plants grown under non-saline and saline water conditions. Results indicate that inoculation of plants with AM fungi has improved growth and can help to alleviate deleterious effects of salt stress on plant yield. The role of arbuscular mycorrhizal (AM) fungi to alleviate salt effects was determining on growth of soybean when irrigated with saline water.

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# Introduction:-

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semiarid areas (Giri et al., 2003; Al-Karaki, 2006). Saline soils occupy 7% of the earth's land surface (Ruiz-Lozano et al., 2001) and increased salinization of arable land will result in to 50% land loss by the middle of the 21st century (Wang et al., 2003). Improving plant tolerance to saline stress is very significant in agriculture because excessive amounts of salts have adverse effects on the physical and chemical properties of soil microbiological processes and on plant growth. (Shokri and Maadi, 2009) demonstrated that an increase in electrical conductivity has adverse effects on soil structural stability, bulk density and permeability. Many studies have demonstrated that inoculation with arbuscular mycorrhizal (AM) fungi improves growth of plants under a variety of salinity stress conditions (Ruiz-Lozano et al., 1996; Al-Karaki et al., 2001) to some extent, these fungi have been considered as bio-ameliorators of saline soils (Azcón, and Elatrash, 1997; Singh et al., 1997). Although the symbiotic association between AM fungi and their hosts is usually believed to be nonspecific, many studies have confirmed the existence of physiological or morphological differences within the species and even within geographic or ecotype isolates of the glomalean fungi (Bethlenfalvay et al., 1988; Bago et al., 1998; Smith and Read, 1997). Amino acid proline

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Corresponding Author:- Idress A. Al Gehani. Address:- Dept. of Plant Production, Faculty of Agriculture, University of Benghazi, Libya. accumulation is one of the most frequently reported modifications induced by water and salt stresses in plants and is often considered to be involved in stress resistance mechanisms. Accumulation of this amino acid is thought to be involved in osmotic adjustment of stressed tissues (Delauney and Verma, 1993; Ashraf and Foolad, 2006). It has been suggested that salt stress induces proline accumulation in legumes (Ashraf, 1989; Sharma *et al.*, 1990; Rabie and Almadini, 2005). There is evidence that proline concentration is greater in *Vigna radiata* (Jindal *et al.*, 1993) and *Vicia faba* (Rabie and Almadini, 2005) when were inoculated with AM fungi. Differences in fungal behavior or characteristics in interacting with hosts have attracted much attention in recent years, in order to improve selection of efficient isolates or to understand functional diversity or ecological plasticity of the fungi (Camprubi and Calvet, 1996; Johnson *et al.*, 1997; Douds and Millner, 1999). It has been found that under long term heavy metal or saline stress, although the richness of AM fungal species decreased, some species were still able to survive due to differences in adapting to these edaphic stresses (Copeman *et al.*, 1996; Weissenhorn *et al.*, 1993; Camprubi and Calvet, 1996; Stahl and Williams, 1986; del Val *et al.*, 1999). The AM fungi which are able to survive in stressed edaphic environments are considered as tolerant isolates which may have a higher ability to improve the survival and growth of host plants than species or isolates from normal edaphic condition. This study was conducted to determine if inoculation with AM fungi alleviates salt effects on growth of soybean when irrigated with saline water.

# Materials and methods:-

#### Soil

A soil with a loamy clay surface texture was collected from Almarj area (north-east of Libya). Soil was passed through a 2 mm mesh sieve, mixed thoroughly and autoclaved (110 °C, 1 h, twice at 48 h intervals) to remove indigenous AM propagules. Soil properties were 29% sand, 35% silt, 36% clay, 2.1% organic matter, pH 7.6, electrical conductivity (EC<sub>e</sub>) 2.1 dS/m; 3.2 mg/kg P (Olsen *et al.*, 1954). The soil total cation exchange capacity (CEC) was 22.62 Cmol/kg (Mg, Ca,Na and K were 0.5, 2.2, 21.6 and 3.02 Cmol/kg respectively).

# Mycorrhizal inoculation and plant growth conditions

Arbuscular-mycorrhizal (AM) species belonging to the genus *Glomus* was used in this study. Mycorrhizal inoculum (*Glomus intraradiaces*) used was stock culture prepared from Faculty of Agriculture, Ain Shams University of Cairo.

The mycorrhizal treatments were carried out by adding 50 g of inoculum per pot of these treatments which was placed bellow the seeds. Inoculum consisted of external mycelium, spores and colonized roots mixed with soil, soybean (*Glycine max* L.) seeds were sown and thinned to one plant per pot, and grown on a bench with mist irrigation in the greenhouse until plants reach appropriate size for planting (60 days). Each treatment were subjected to a destructive measurement, where the following parameters were estimated: root AM fungi colonization, plant dry matter, proline concentration of the fresh shoots and roots and P ,Na, K, Ca, and Zn concentrations.

Irrigation water management treatments were (i) non-saline water: tap water with  $EC_w = 0.6 \text{ dS m}^{-1}$  and (ii) saline water: irrigation with saline water with  $EC_w = 6.2 \text{ dS m}^{-1}$  (Table 1). Soil  $EC_e$  was measured at the end of experiment and values of  $EC_e$  were 1.2 and 5.3 dS m<sup>-1</sup> for non-saline and saline treatments, respectively. Water was supplied to individual every three days to keep the soil at 70% of its field capacity by regular weighing of pots.

Parameter	Non-saline water	Saline water		
pH	8.1	9.2		
EC $(dS m^{-1})$	0.6	6.2		
TDS <sup>*</sup> (ppm)	485	3842		
K (meq $l^{-1}$ )	1.3	26.1		
Ca	3.8	16.0		
Cl	1.2	43.0		
CO3	0.3	0.68		
HCO3	4.0	2.8		
$SO_4$	0.3	8.21		
Total cation	7.2	58.2		
SAR <sup>**</sup>	0.42	4.32		

Table (1): Salt components and conductivity of saline and non-saline water used in this study

\*TDS- total dissolved salts; \*SAR- sodium adsorption ratio

# Plant growth responses

Plant samples with their roots from each pot were taken by a fork, fitted to excavate the soil volume under the area occupied by the plants. Roots were rinsed free from soil and weighed and subsamples were saved for assessment of AM fungi root colonization. Then shoots and roots were oven-dried (48 h, 60 °C) and weighed. Shoot samples from each replicate were saved for mineral analysis. The proline concentration of the fresh shoots and roots was quantified using ninhydrin acid reagent (Bates *et al.*, 1973).

Mycorrhizal dependency (%) = (total dry weight of mycorrhizal plant - total dry weight of non-mycorrhizal plant)  $\times$  100 / total dry weight of mycorrhizal plant. And nutrient content (NC) change was measurement, Nutrient content (NC) change = ((NC AM – NC non-AM)  $\times$  100) / NC non-AM

#### AM fungi root colonization

Root samples for determination of root colonization with arbuscular mycorrhizal fungi (AMF) were cleared with 10% KOH and stained with 0.05% trypan blue in lactophenol as described by Phillips and Hayman (1970), and microscopically examined for AMF colonization by determining percentage of root segments containing arbuscules + vesicles using a gridline intercept method (Giovannetti and Mosse, 1980).

#### Measurements and analysis

Root and shoot biomass were determined after oven drying at 70°C for 90 h. and prepared for determination of mineral nutrients. Shoot P concentration was determined colormetrically (Watanabe and Olsen, 1965) and Na, K, Ca, and Zn concentrations were assayed using a Perkin Elmer 603 atomic absorption spectrophotometer. Mineral contents were calculated by multiplying of mineral concentration by dry weight of shoots.

#### **Experimental design**

The experiment was conducted in a greenhouse under a temperature of 22-30 °C, 12-14 h day light, and 70-75 % relative humidity. This experiment was arranged in a randomized block design, with two water treatments (non-saline and saline water) and two AM fungi inoculum treatments (AM and non-AM) to give a  $2 \times 2$  factorial experimentation with four replications.

# Statistical analysis

Data were subjected to analysis of variance using the ANOVA procedures according Snedecor and Cochran (1972), Differences among means of treatments were compared by Duncan's multiple range test at the 0.05 confidence level.

# **Results and Discussion:-**

The AM fungi root colonization was noted in roots of both AM and non-AM plants, even though the AM plants had higher root AM fungi colonization than non-AM plants (Table 2). The AM fungi root colonization in soybean was reduced by salinity stress regardless of AM fungi status.

Compared with the -AMF treatments, the +AMF treatments significantly enhanced the shoot and root biomass of plants under both non-saline and saline conditions (Table 2). However, shoot and root DM decreased in plants grown under saline compared to non-saline conditions. Results of this study indicated that the level of colonization with AM fungi was reached 32% under saline which might be considered adequate for successful establishment of mycorrhizal plants compared to non-saline conditions. Many studies have indicated that AM fungi may produce spores at low root-colonization levels in severe saline conditions (Aliasgharzadeh et al., 2001). For AM and non-AM plants, shoot proline concentration was significantly higher relative to the non-saline control (Table 2). Shoot proline was significantly lower in AM than in non-AM plants at saline water except for control. Root proline concentrations were higher in both AM and non-AM plants grown under saline conditions compared to the nonsaline control (Table 2). In comparison to the non-saline treatment, there was significant difference in root proline concentration of non-AM plants at saline treatments. In the presence of saline conditions, root proline was significantly higher in AM than in non-AM soybean plants. The comparison of AM versus non-AM soybean plants in the present study indicates that AM inoculation is beneficial in increasing the root concentration of proline. Enhanced proline accumulation in the roots of AM plants may play a role in salt tolerance of the plants. This study agrees with findings that AM fungi help some plants to grow better under salinity (Al-Karaki et al., 2001; Ghazi and Al-Karaki, 2006). Higher levels of proline in roots compared with shoots may be due to the fact that the roots are the primary sites of water absorption and must maintain osmotic balance between water absorbing root cells and external media. Therefore, better growth of AM plants compared to non-AM plants when exposed to salinity may be

a result of increased root proline accumulation (Tian *et al.*, 2004). Several researchers have reported that AMFinoculated plants grow better than non-inoculated by contributes to plant growth via enhancement of mineral nutrient uptake especially immobile soil nutrients (P, Cu, Zn) under salt stress (Zuccarini and Okurowska, 2008).

**Table (2):** Root AMF colonization, shoot and root dry matter (DM) ,proline concentration in shoot and root of mycorrhizal (AM) and non-AM soybean grown under non-saline and saline water conditions.

Water	AM Inoculation	Root Colonization	Dry n (g.pl	natter ant <sup>-1</sup> )	$\frac{\mathbf{Proline}}{(\mathrm{mg.g}^{-1} \mathrm{dw})}$		
Treatment		(%)	Shoot	Root	Shoot	Root	
	Non-AM	12.54 <sup>c</sup>	29.30 <sup>b</sup>	3.80 <sup>b</sup>	0.10 <sup>c</sup>	$0.08^{\circ}$	
Non-saline	AM	58.69 <sup>a</sup>	42.94 <sup>a</sup>	5.62 <sup>a</sup>	0.21 <sup>b</sup>	0.03 <sup>c</sup>	
	Non-AM	5.32 <sup>d</sup>	13.97 <sup>c</sup>	1.21 <sup>c</sup>	$0.27^{a}$	0.22 <sup>b</sup>	
Saline	AM	32.16 <sup>b</sup>	28.26 <sup>b</sup>	3.70 <sup>b</sup>	0.15 <sup>c</sup>	0.28 <sup>a</sup>	

Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level.

The mycorrhizal dependency (MD) of soybean was calculated based on the plant dry matter, and revealed that (*Glycine max* L.) depended on *Glomus intraradiaces* to the extent of 32% and 53%. The AM plants had generally higher shoot P contents, but not concentrations, than shoots of nonAM plants grown under both saline and non-saline conditions (Table 3).

Shoot K concentrations of AM and non-AM soybean plants were similar for plants grown under non-saline and saline conditions (Table 3). Shoots of AM plants had generally higher K contents than shoots of non-AM plants. Shoot K contents decreased for plants grown under saline conditions compared to the plants grown under non-saline conditions. Shoot Na concentrations, but not contents, were lower in AM than non-AM plants grown under saline conditions only (Table 3). No significant differences were noted for shoot Na contents regardless of water treatment or AM fungi inoculation. Shoot concentrations of Ca, and Zn were generally higher for AM than non-AM plants regardless of water treatment, although the differences for Ca and Zn concentrations were only significant under non-saline conditions (Table 3). The AM plants had significantly higher shoot contents of Ca and Zn than non-AM plants grown under saline conditions. Shoot saline and non-saline conditions. Shoot contents of Ca and Zn were lower for plants grown under saline and non-saline conditions.

Many studies have indicated that AM fungi contributes to plant growth via enhancement of mineral nutrient uptake especially immobile soil nutrients (P, Zn) (Marschner and Dell, 1994). In this study, AM soybean plants had higher shoot P contents than non-AM plants. Higher shoot Ca and Zn contents in AM compared to non-AM plants were also noted for plants grown under non-saline conditions. The higher mineral nutrient acquisition in AM compared to non-AM plants likely occurred because of increased availabilities or transport (absorption and/or translocation) by AM fungi hyphae. Enhanced acquisition of P and Zn by AM plants has been reported (Al-Karaki, 2000). Cantrell and Linderman (2001) suggested that improved P nutrition by AM fungi in plants grown under saline conditions might reduce the negative effects of Na and Cl by maintaining vacuolar membrane integrity, which prevented these ions from interfering in metabolic pathways of growth.

Table (3): Shoot concentrations and contents of P, K, Na, Ca and Zn by AM and non-AM soybean grown under non-saline and saline water conditions

Water	AM Inoculation	Concentration				Content					
Treatment		$(mg.g^{-1}DM)$				(mg.plant <sup>-1</sup> )					
		Р	K	Na	Ca	Zn	Р	K	Na	Ca	Zn
Non-saline	Non-AM	4.35 <sup>a</sup>	44 <sup>a</sup>	2.03 <sup>c</sup>	16.10 <sup>b</sup>	$0.01^{b}$	127 <sup>b</sup>	1289 <sup>b</sup>	60 <sup>a</sup>	648 <sup>c</sup>	0.32 <sup>c</sup>
	AM	4.95 <sup>a</sup>	47 <sup>a</sup>	1.88 <sup>c</sup>	28.32 <sup>a</sup>	0.03 <sup>a</sup>	213 <sup>a</sup>	2018 <sup>a</sup>	69 <sup>a</sup>	1216 <sup>a</sup>	1.29 <sup>a</sup>
Saline	Non-AM	2.02 <sup>b</sup>	31 <sup>a</sup>	5.20 <sup>a</sup>	22.89 <sup>a</sup>	0.03 <sup>a</sup>	28 <sup>d</sup>	433 <sup>d</sup>	76 <sup>a</sup>	250 <sup>d</sup>	0.28 <sup>d</sup>
	AM	3.22 <sup>b</sup>	36 <sup>a</sup>	2.92 <sup>b</sup>	24.25 <sup>a</sup>	$0.04^{a}$	91 <sup>c</sup>	1017 <sup>c</sup>	82 <sup>a</sup>	685 <sup>b</sup>	1.05 <sup>b</sup>

Means followed by the same letter in each column are not significantly different by Duncan's multiple range test at 5% level.

The overall effects of AM fungi inoculation on mineral contents of plants grown under non-saline and saline conditions (Table 4). Shoot P contents were enhanced by 68 and 225% for plants inoculated with AM fungi and grown under non-saline and saline water conditions, respectively.

Table (4): Percentage change in nutrient contents due to AM and non-AM of soybean grown under non-saline and saline water conditions

Water	Nutrient content* (%)								
Treatment	Р	K	Na	Ca	Zn				
Non-saline	68	57	37	88	303				
Saline	225	135	12	174	275				
*Netrient content (NC) shares (NC AM NC new AM) > 100) / NC new AM									

\*Nutrient content (NC) change = (NC AM – NC non-AM)  $\times$  100) / NC non-AM

# **Conclusion:-**

In conclusion, inoculation with AM fungi *Glomus intraradiaces* reduced the detrimental effects of salt on soybean growth and productivity. The mechanism by which AM fungi alleviate salt stress remains unresolved, but appears to involve several possible metabolic processes that could because mediated by P nutrition or other element balance, and possibly compartmentalization of sodium within some plant tissues. However, several AM fungi isolates should be investigated in order to maximize efficiencies of AM fungi symbiosis under saline conditions.

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