دور الفطور الميكوريزية في الحد من تأثير الإجهاد الملحي في التغذية المعدنية لمحصول البصل

الدكتور علي أمرير
قسم التربة واستصلاح الأراضي - كلية الزراعة- جامعة الفرات

الملخص

تم تنفيذ تجربة أصص تحت ظروف البيت الزجاجي لدراسة دور سلالتين من النوع الفطري الميكوريزي((*Glomus intraradices*)في الحد من تأثير الإجهاد الملحي لمحلول كلوريد الصوديوم على نبات البصل

وعلى هذا فقد تم زرع ثلاث بصيلات في كل أصيص مع التلقيح بالسلالتين (*G3*\ *G4*) تحت تأثير مستويات من التركيز الملحي **(0.4, 2.0, 4.0 and 6.0 dSm^{-1})**, وذلك بعد أسبوع من الإنبات

الدراسة أظهرت زيادة الوزن الجاف لكل من الجموع الخضري والجذري لنبات البصل الملقح بالميكوريزا مقارنة مع النباتات غير الملقحة وكذلك ارتفاع نسبة استعمار الجذور بسلالتي النوع المستخدم في التلقيح

وفي معاملات التجربة (AMF)نجد أن محتوى جذور البصل من حمض الساليسيليك قد ارتفع بشكل معنوي

(*G*3: 67%, 96%) and (*G*4: 17.50%) تحت تأثير مستويات الملوحة(4.0 and 6.0 dSm^{-1})على الترتيب .أما محتوىا لجذور من الأحماض الأمينية الحرة مثل البر ولين والفينيل آلا نين والأرجينين فقد زادت مع زيادة الإجهاد الملحي وكذلك محتوى النبات من الفسفور والنتروجين قد زاد في النباتات الملقحة بالميكوريزا تحت ظروف الإجهاد الملحي بالمقارنة مع تلك غير الملقحة

الكلمات المفتاحية :الفطور الميكوريزية– الإجهاد الملحي– البصل–الأحماض الأمينية الحرة–استعمار الجذور– حمض الساليسيليك

Introduction:

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semi-arid areas (Al-Karaki, 2006). Saline soils occupy 7% of the earth's land surface (Ruiz-lozano *et al.*, 2001) and increased Stalinization of arable land will result in to 50% land loss by the middle of the 21^{st} century (Wui.and Altman 2006)

High levels of salinity (> 4 dSm^{-1} or > 0.1% soil content; Juniper and Abbott, 1993) in soils is mainly due to the soluble salts in irrigation water and fertilizers used in agriculture (Al-Karaki, 2000), low precipitation and high temperature in these regions and over-exploitation of available water resources (e.g. ground water) (Mouk and Ishii, 2006). The direct effects of salt on plant growth may involve : (a) reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought (Jahromi *etal.*, 2008); (b) toxicity of excessive Na^+ and Cl^- ions towards the cell – the toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of photosynthesis, respiration and protein synthesis (Feng *et al.*, 2002); and (c) nutrient imbalance in the plant caused by nutrient uptake and/or transport to the shoot leading to ion deficiencies (Adiku *et al.*, 2001).

Some microorganisms, particularly beneficial bacteria and fungi can improve plant performance under stress environments and, consequently, enhance yield (Creus *et al.*, 1998). Arbuscular mycorrhizal fungi (AMF) are associated with the roots of over 80%

terrestrial plant species (Smith and Read, 1997). AMF have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition (Al-Karaki and Al-Radad, 1997), (Amrir and Aldahmosh 2008), producing plant growth hormones, improving rhizospheric and soil conditions (Lindermann, 1994), altering the physiological and biochemical properties of the host (Smith and Read, 1995) and defending roots against soil-borne pathogens (Dehne, 1982).

Salicylic acid (SA) is widely distributed in monocot and dicot plants. Recent studies show that SA can enhance plant resistance to environmental stresses, such as heat, chilling and drought (Tissa *et al.*, 2000). Salicylic acid is a plant phenol, and today it is in use as internal regular hormone (Shahba *et al.*, 2010).

This research studies the effect of AMF (*Glomus intrardices*) on the growth, salicylic acid and chemical contents of *Allium cepa* plants were grown under salinity stress (Na Cl).

Materials and methods

The experiment was carried out in the greenhouse of the faculty of Agriculture, Saba – Basha, Alexandria University, Egypt, during the autumn of 2010 with natural light at 30/20 degrees centigrade (day / night). Two mycorrhizal strains *Glomus intrardices* were used in this experiment. The first mycorrhizal spores (strain*G3*) was separated by the(wet-sieving)(Amrir et.al.1996) from the soil sample of grass(*Cyndon dactylon*)from Experimental Station of Alexandria University at Abies, and the second (strain*G4*) was produced by "

Amykor Company, Germany. The inoculums consists of expanded clay aggregates (2-4 mm in diameter, leca), containing chlamydospores and fungus mycelium, which had been produced on *Tagetes erecta* L. (Aboul – Nasr, 2004). The inoculums was applied prior to sowing by mixing the carrier material with the substrate (10%, v.), the control that irrigated with tap water (0.4 dSm^{-1}) received the same amount of heat sterilized expanded clay.

A mixture of sterilized sand and peat moss (45%:45%,v.) at 110 °C for 24 hr were used as a substrate (mixture pH = 7.4 and mixture of soil electrical conductivity (EC) = 0.8 (dSm^{-1}). Three grains of. *Allium cepa*, were sown in plastic pots, (11 cm in diameter). Seedlings were irrigated with tap water as needed prior to stress applications. Salinity treatments were started, after one week germination. Four levels of Na Cl solution were applied; irrigated with tap water (0.4 dSm^{-1} control), 2,4 and 6 dSm^{-1} of Na Cl solution Plants were irrigated as needed with70% of field capacity, Liquid soil fertilizer "Super grow (Agrico., Egypt, 100g /200L), N:P:K = 20:20:20:) was used and the plants received 30 ml weekly. After 4 and 8 weeks from the beginning of salt treatments, i.e. the 5^{th} and 8^{th} week of plant age.

The amount of salicylic acid in fresh roots was measured according to Iqbal and Vaid (2009). Shoot and root dry weights g/ plant were also recorded. Three shoot samples per each treatment from the shoot were analyzed for the determination of N by Nessler's method (Chapman and Pratt, 1978), and P (Jackson, 1973). The concentrations of K and Na were determined by flame photometry as described by Worth (1985). Total phenol , amino acids; (proline, phenylalanine) and soluble sugar were measured. Spectrophotometerically in root According to (Umbreit *et al.*, 1972). At the end of the experiment soil's EC from the different treatments was measured.

The experimental design consisted of 12 (3×4) treatments crossing three mycorrhizal inoculation levels (non-AMF, *G.intraradices* (*G*3) and *G. intraradices* (*G*4) with four salt levels Na Cl(0.4, 2.0, 4.0 and 6.0 dSm^{-1}) Pots were arranged as factorial in a completely randomized block design. Six replicates of each treatment were applied with totaling of 24 pots for each AMF strain. The statistical analysis was done according to SAS (1989).

Results and discussion

1- Measurement of soil electrical conductivity (EC)

At the end of the experiment, soil samples from each treatment were randomized collected and the EC (dSm^{-1}) was measured. Figure (1) shows that the soil EC (dSm^{-1}) at the end the experiment ,while the soil EC at the beginning the experiment was 0.8 dSm^{-1}. In case of non-mycorrhizal plants the soil salinity was increased according to the irrigation of Na Cl level. It reached 0.33, 1.54, 2.41 and 3.54 dSm^{-1} in treated plants with 0.4, 2.0, 4.0 and 6 dSm^{-1} Na Cl solution, respectively. *Glomus intraradices* (*G* 3) reduced soil salinity within all salt concentrations by – 42.0, – 25.0 and –24.0% than the non-inoculated and irrigated plants with 2.0, 4.0 and 6.0 dSm^{-1} respectively. *G*.4 had also

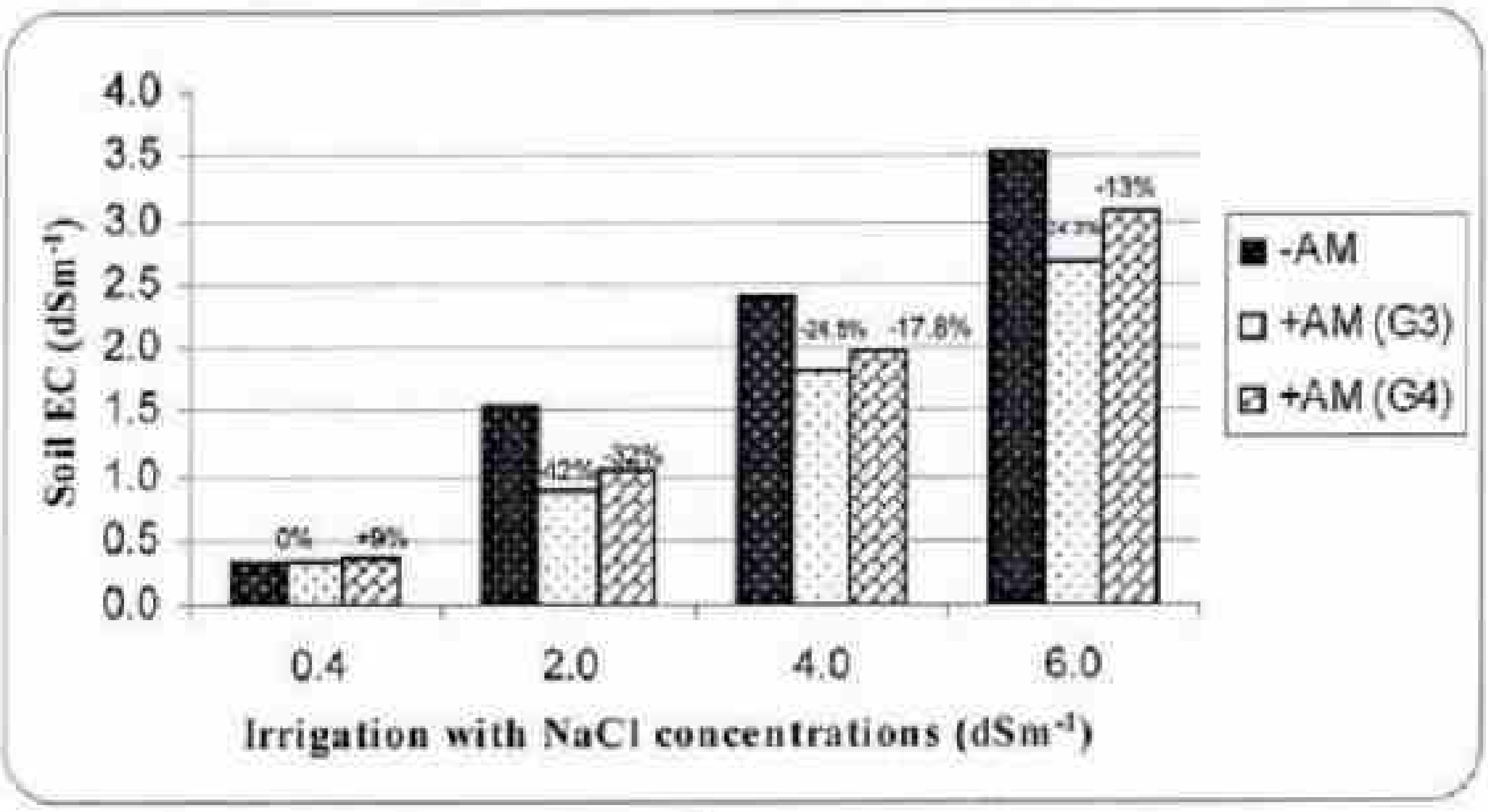


Figure (1). Soil EC (dSm^{-1}) at the end of experiment

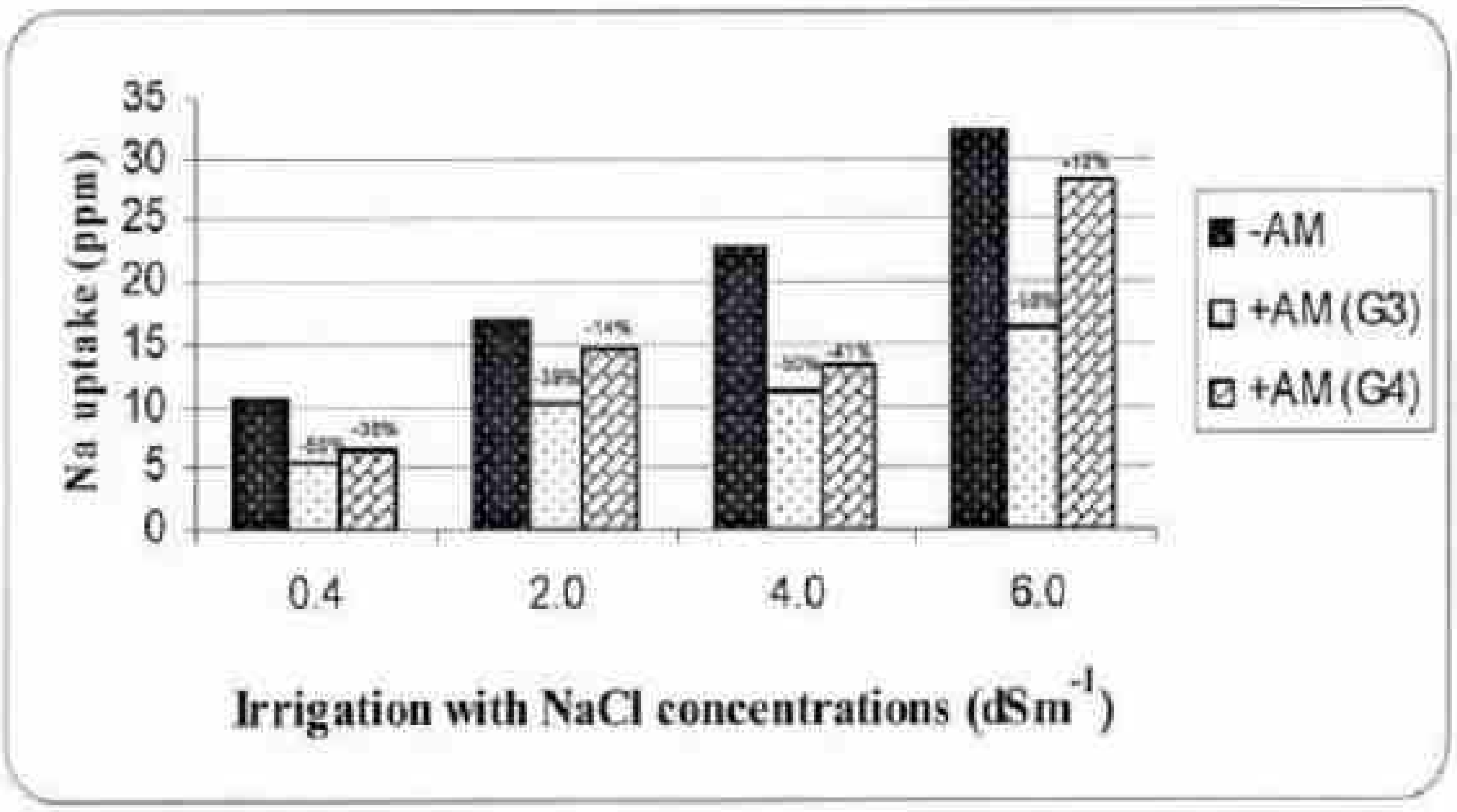


Figure (2). Effect of two strains of *Glomus intraradices* on Na uptake by *Allium cepa* shoots (ppm) under different NaCl level

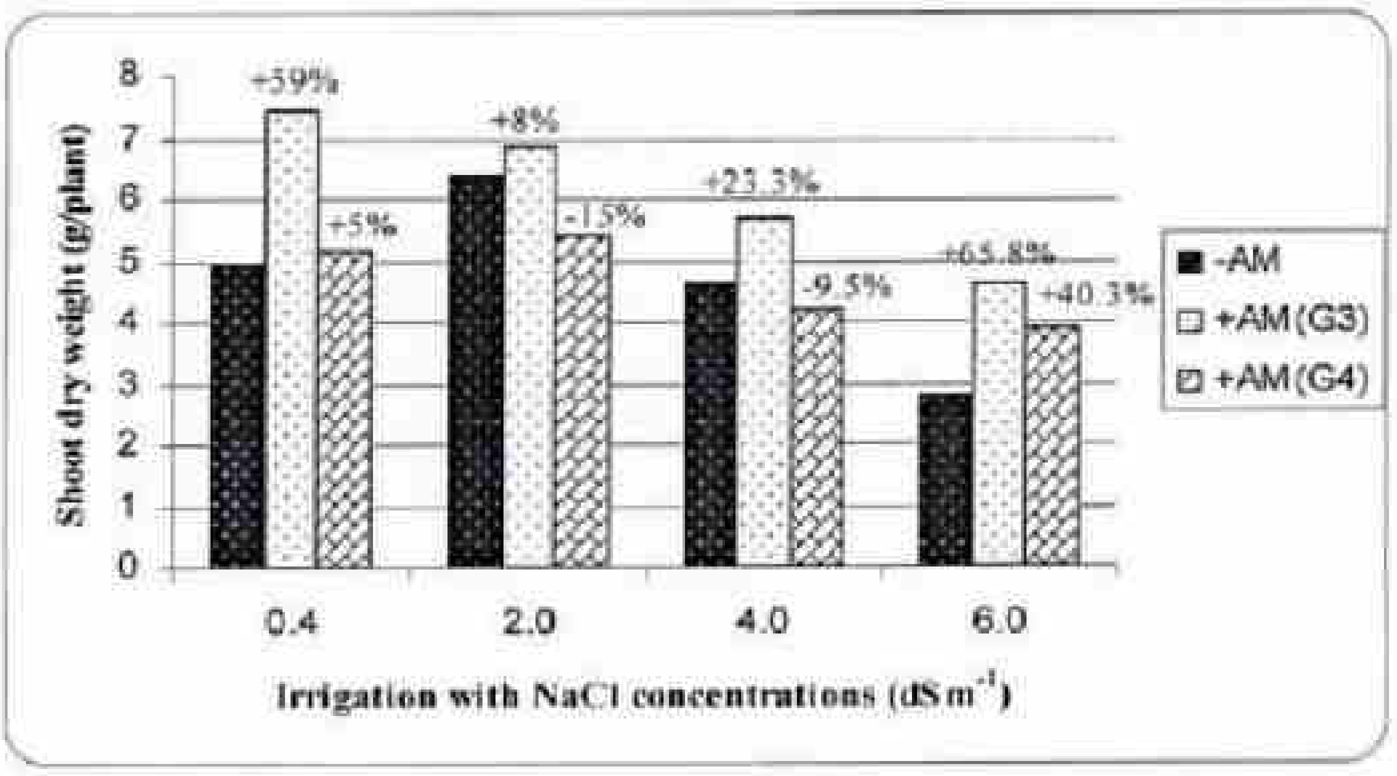


Figure (3). Effect of two strains of *Glomus intraradices* on shoot dry weight (g/plant) under different NaCl level

a positive effect on soil EC and inoculated soil pots with *G*.4 reduced the soil EC in - 32.0, – 18.0 and – 13% reduction than the un-inoculated ones in case of irrigated plants with 2.0, 4.0 and 6.0 dSm^{-1}, respectively.

2- Na uptake in shoots (ppm)

Figure (2) shows the significant effect of both AM strains on the reduction of Na uptake in shoots. Na uptake in shoots of non-AM *Allium cepa* seedlings was 10.6, 17.0, 22.6 and 32.3 ppm in case of irrigated pots with tap water, 2.0, 4.0 and 6.0 dSm^{-1} of Na Cl solution. The percentage decrease of Na uptake in case of *G. intraradices* (*G*3) reached 50% when plants irrigated with moderate and high concentration of Na Cl (4 and 6 dSm^{-1}) compared to non –inoculated plants. *G*4 showed the same trend in Na uptake, but Na uptake was more reduced in *G*3 than *G*4. Similar results were obtained by He *et al*.,(2010). One of the harmful effects of salinity on plant growth involves the excessive accumulation of Na^{+} and Cl^{-} (Ashraf and Hrris, 2004 and Munns *et al.*,2002). More Na^+ accumulation resulted in ionic imbalance, specific ion effects and nutrient deficiency symptoms in plants. They found that the Na^+ concentration in shoots and roots of non – AM tomato seedlings was 1.11 and 1.22 times of AM plants respectively, under salt less condition, 1.57 and 1.26 times under 0.5% salt stress, 1.07 and 1.06 times under 1% salt stress respectively. They also reported that AM colonization seemed to decrease Na^+ under 0.5% salt stress, remarkably. Our results showed that AM helped to sustain ion balance and lower Na^+ accumulation which played a key role in improving salt tolerance of AM *Allium cepa*.

3- Shoot and root dry weights (g / plant)

Na Cl stress significantly reduced plant growth (Figures 3 and 4). Shoot and root dry weights increasement were significantly larger than corresponding non-AM seedlings. The percentage increase in shoot dry

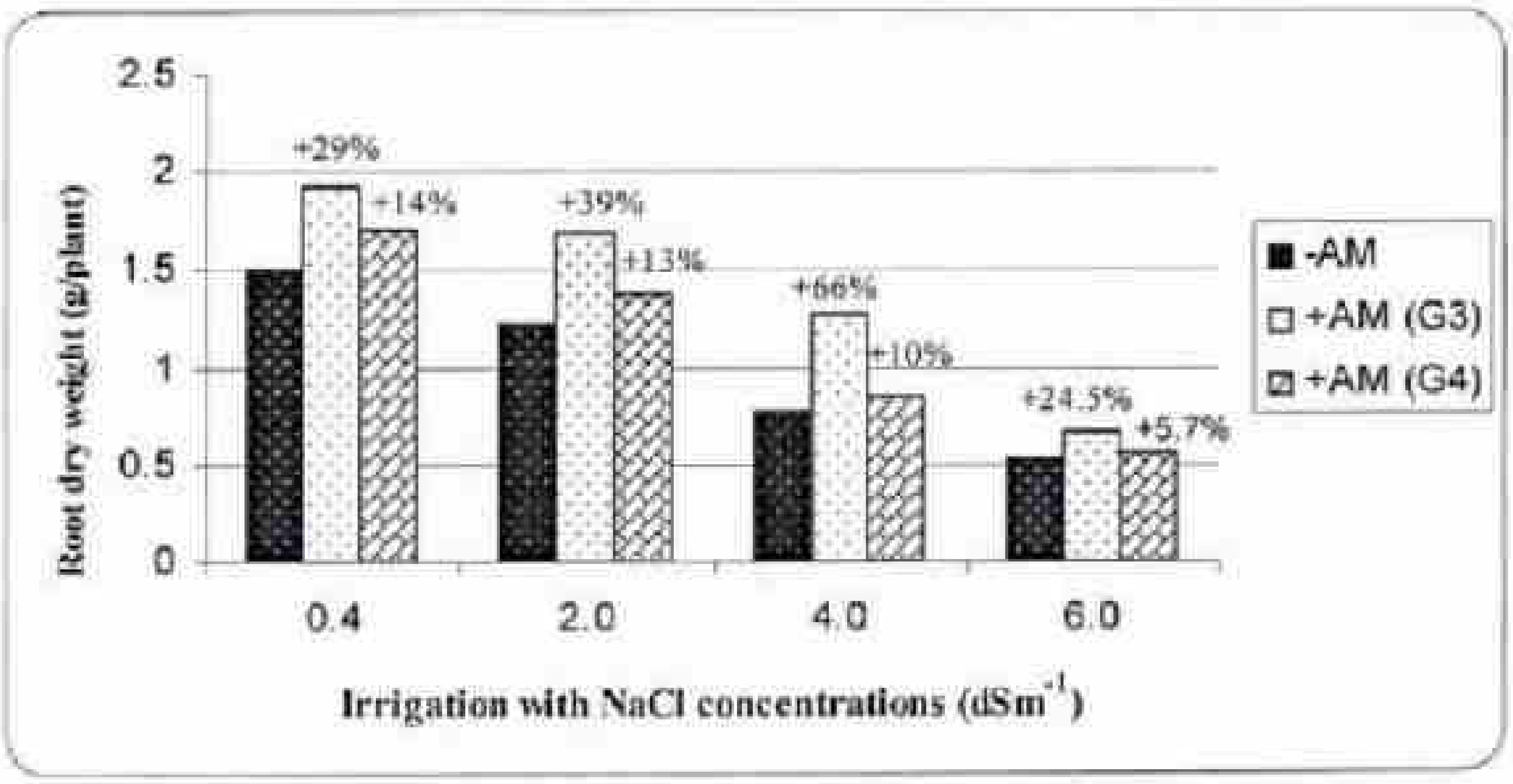
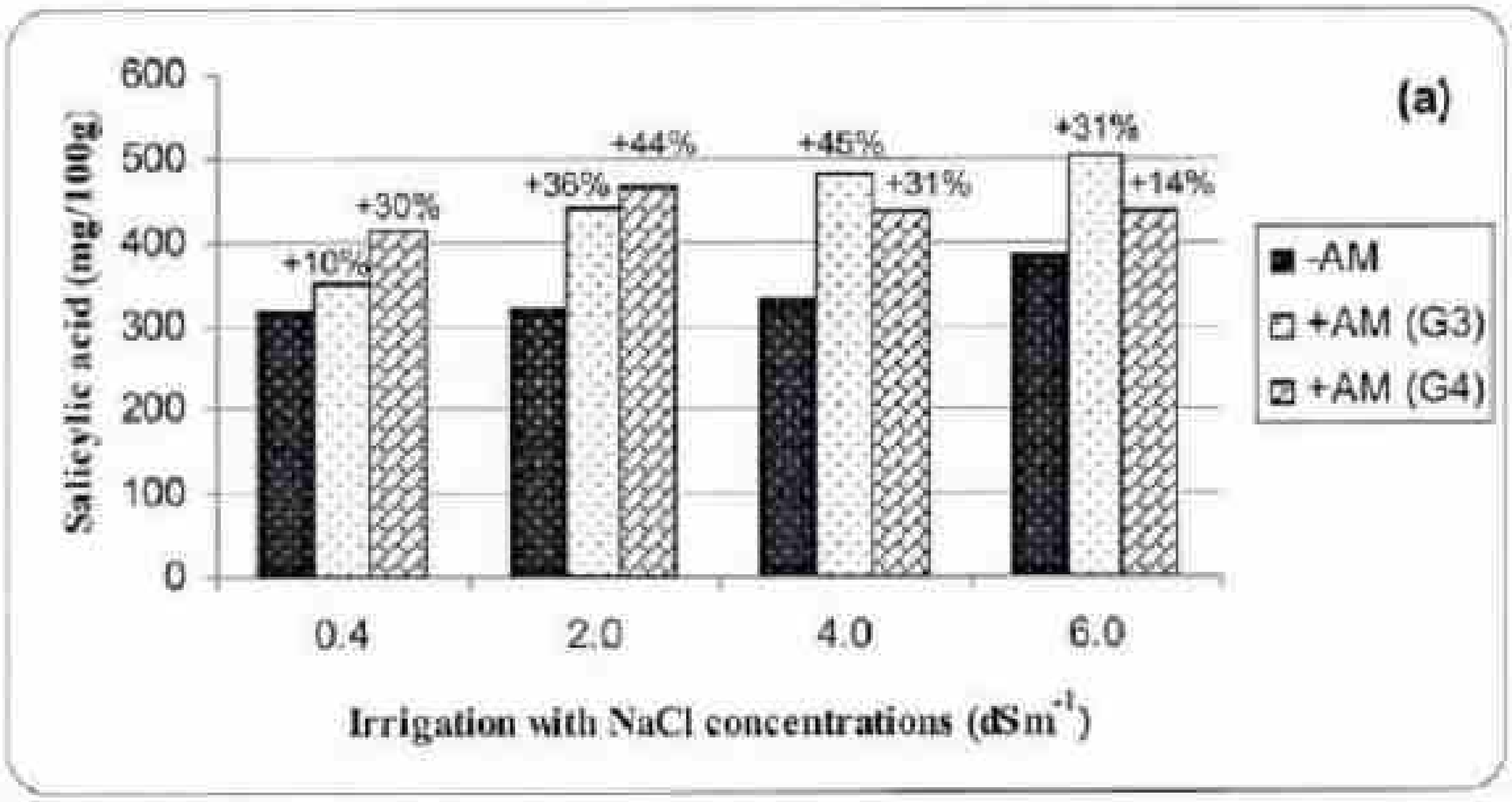


Figure (4). Effect of two strains of *Glomus intraradices* on root dry weight (g/plant) under different NaCl stress

weight in case of inoculated plants with *G3* was 8, 23, 66% more than un-inoculated ones under different salt level stress (2, 4 and 6 dSm^{-1}), respectively. Whereas the percentage increase were 39, 66 and 25%, respectively, in root dry weight compared to non-AM. Inoculated plants with *G4* increased the shoot dry weight in case of the highest concentration of Na Cl (6 dSm^{-1}). The same trend was also found in root dry weight. Similar results were reported by Al-Karaki *et al.*, (2001) and He *et al.*, (2010).

4- Salicylic acid (SA)

Salicylic acid was measured spectrophotometricaly in *Allium cepa* fresh roots (mg /100g fresh root). Data in Figures (5a and 5b) show the significant increase in salicylic acid's amount under the different levels of Na Cl, especially in inoculated plants either with *G3* or *G4* when plants



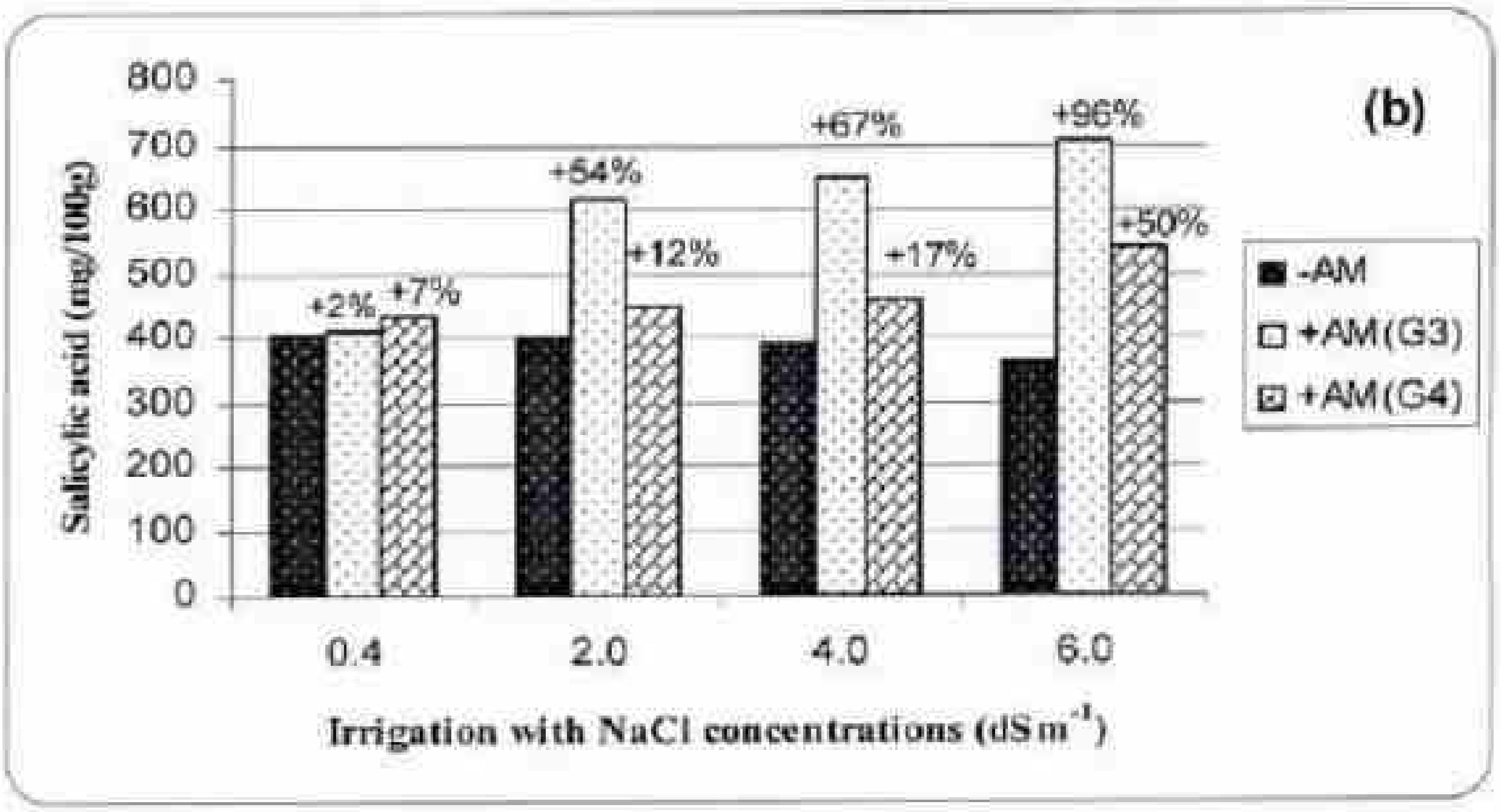


Figure (5). Effect of two strains of *Glomus intraradices* on salicylic acid content in root fresh weight (mg/100g) under different NaCl stress a) after 5 weeks from planting b) after 8 weeks from planting

were 5 and 8 weeks old. The highest percentage increase appeared in *G*3 at the end of the experiment. It reached 54, 67 and 96% in the presence of 2,4 and 6 dSm^{-1}, respectively, compared to non-inoculated plants. The same trend was shown in case of *G*4. Salicylic acid is a plant phenol, and it is in use as internal regulator hormone, because its role in the defensive mechanism against biotic and a biotic stresses (Shahba *et al.*, 2010). Klessig and Malamy (1994) and Clarke *et al.*, (2000) reported that, SA has been firmly established as a natural signal molecule in the induction of systemic acquired resistance (SAR) in tobacco, cucumber and some

other plants. Recent studies show that SA can enhance plant resistance to environmental stresses, such as heat, chilling and drought (Tissa *et al.*, 2000).

5- Free amino acids

Proline, Arginine and phenylalanine were determined in *Allium cepa* roots. Figure (6) shows that with increasing salinity, the root proline increases. Significant increases were obtained in AM roots compared to non-inoculated ones. The high amount of accumulated proline in roots was in case of *G*3 and *G*4 under 4 and 6 d Sm^{-1}.

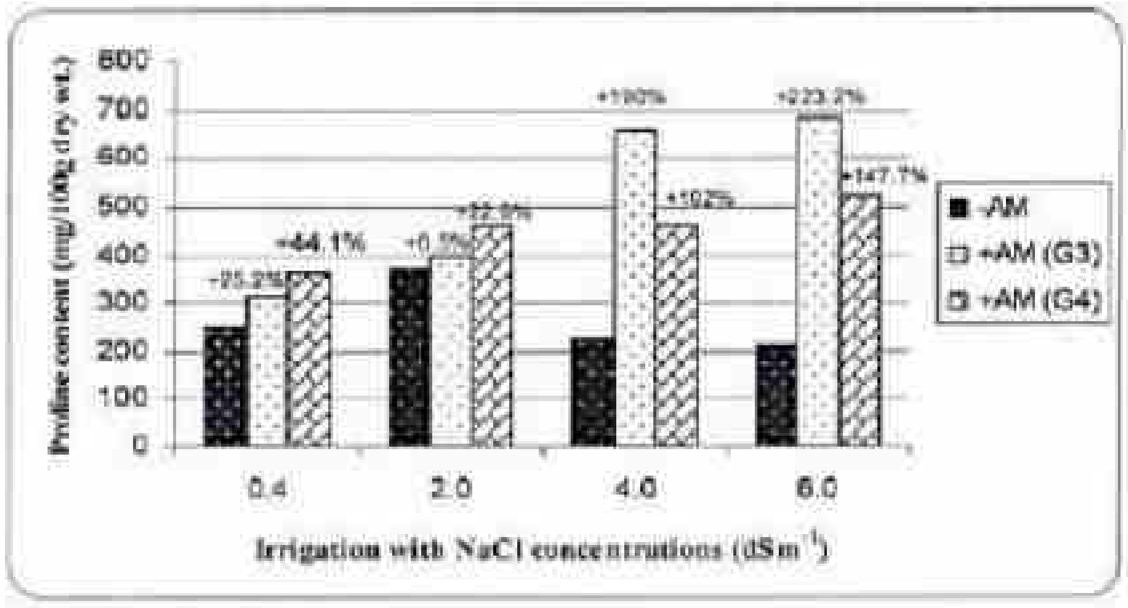


Figure (6). Effect of two strains of *Glomus intraradices* on proline content in root dry weight (mg/100g) under different NaCl stress

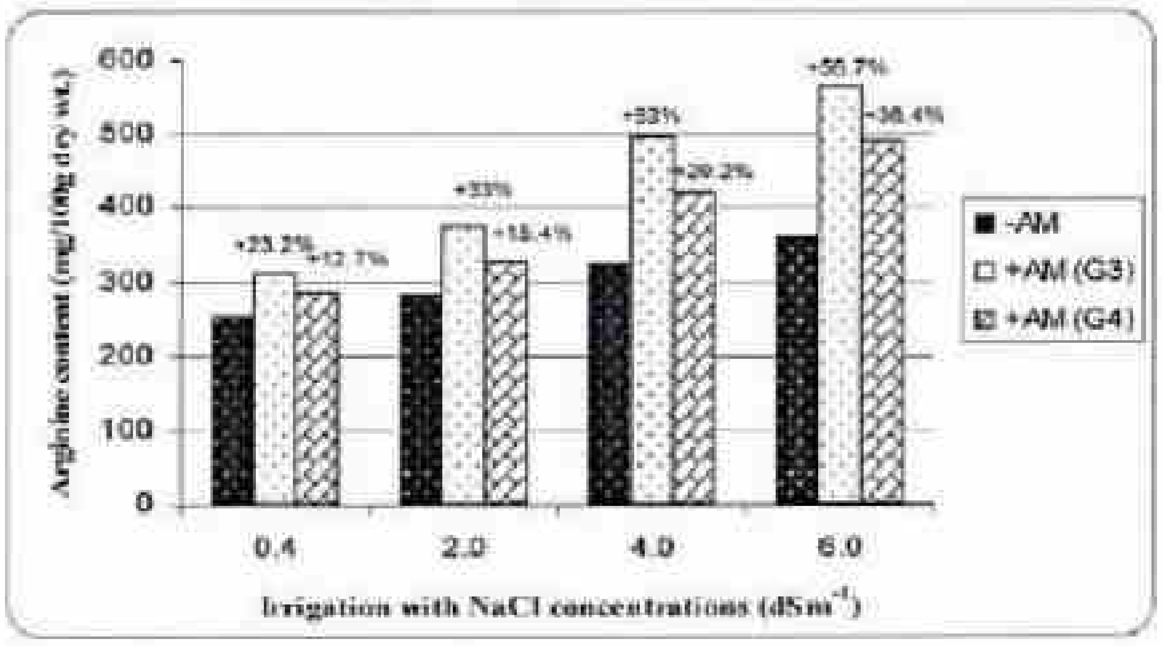


Figure (7). Effect of two strains of *Glomus intraradices* on Arginine content in root dry weight (mg/100g) under different NaCl stress

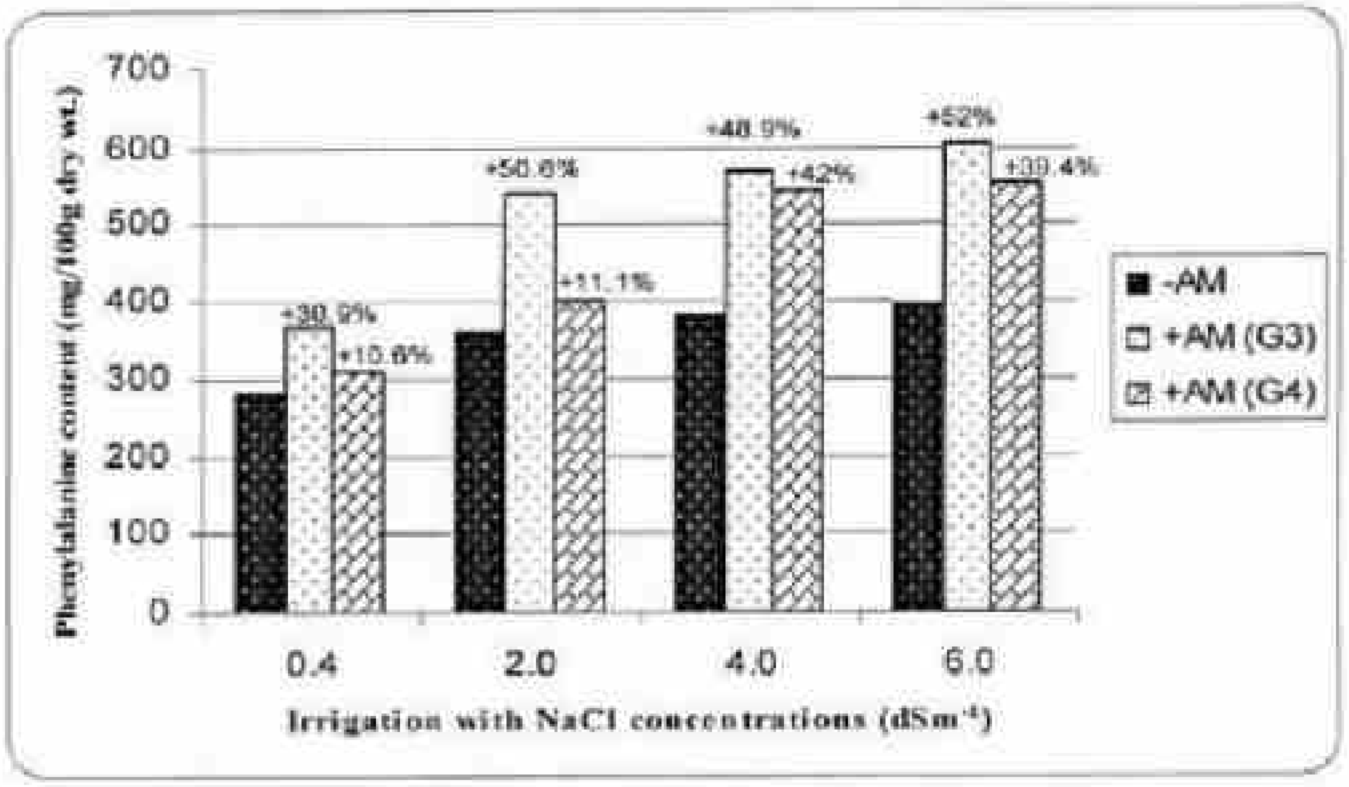


Figure (8). Effect of two strains of *Glomus intraradices* on phenylalanine content in root dry weight (mg/100g) under different NaCl stress

Figures (7) and (8) show the amount of Arginine and phenylalanine as compared to non-inoculated plants. The plants under salinity condition change their metabolism to overcome the changed environmental condition. One mechanisms used by the plants for overcoming the salt stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugar. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm, (Shahba *et al.*, 2010 and Ayala – Astorga and Alcaraz – Melendez, 2010).

6- **Total phenol**

Table (1). shows Total phenol content in *Allium cepa* roots (mg /100g dry weight). Total phenol was increased in AM roots than corresponding non-AM under the different levels of Na Cl stress. Under continuous salt stress, our results showed that the enhanced salt tolerance was closely related to the increase of total phenol accumulation in roots of AM plants. Results obtained by Feng *et al.*, (2002) indicated that improved

Table (1). Effect of two strains of *Glomus intraradices* on total phenol content in root dry weight(mg/100g dry wt.) under different NaCl stress

Parameter	Total phenol content (mg/100g dry wt.)							
NaCl (dSm^{-1})	0.4		2.0		4.0		6.0	
Inoculation	mg/100g	±%	mg/100g	±%	mg/100g	±%	mg/100g	±%
- AM	104,5	--	113.8	--	220.5	--	220.3	--
+ AM (G3)	187.0	+78.9	254.9	+123,9	333.8	+51.3	419.9	+90.3
+ AM (G4)	162.3	+56.9	251.7	+121.1	318.3	+44.3	392.6	+78.1
Interaction L.S.D. $_{0.01}$	41.73***							

± % Increase or decrease compared with non-inoculated plants

tolerance of maize plants to salt stress by arbuscular mycorrhiza was related to higher accumulation of total phenol in roots

7- N P K uptake (mg /100g dry weight)

N P K uptake in *Allium cepa* shoots were determined at the end of the experiment under salt stress.

a- N uptake:

Figure (9) shows that AM inoculation significantly increased N uptake under the different levels of Na Cl compared to non-inoculated plants. Studies have reported that improved N nutrition may help to reduce the toxic effects of Na ions by reducing its uptake and this may indirectly help in maintaining the chlorophyll content of the plant. The form of available N (NO_3^- or NH_4^+) strongly influences Na^+ accumulation (Giri and Mukerji, 2004).However, the exact mechanisms used by AMF to N uptake under salt – stress conditions are not clearly understood.

b- **P uptake (mg /100 dry weigh)**

AMF have been shown to have a positive influence on the composition of mineral nutrients (especially poor mobility nutrients such as phosphorus) of plants grown in salt – stress conditions (Al-Karaki and Clark, 1998).

Figure (10) shows that AM inoculation resulted in highly significant increase of P uptake compared to non-AM plants under all salt stress levels. The percentage was increased in case of *G3* and *G4* by increasing Na Cl level compared to non-inoculated *Allium cepa* plants. At salt

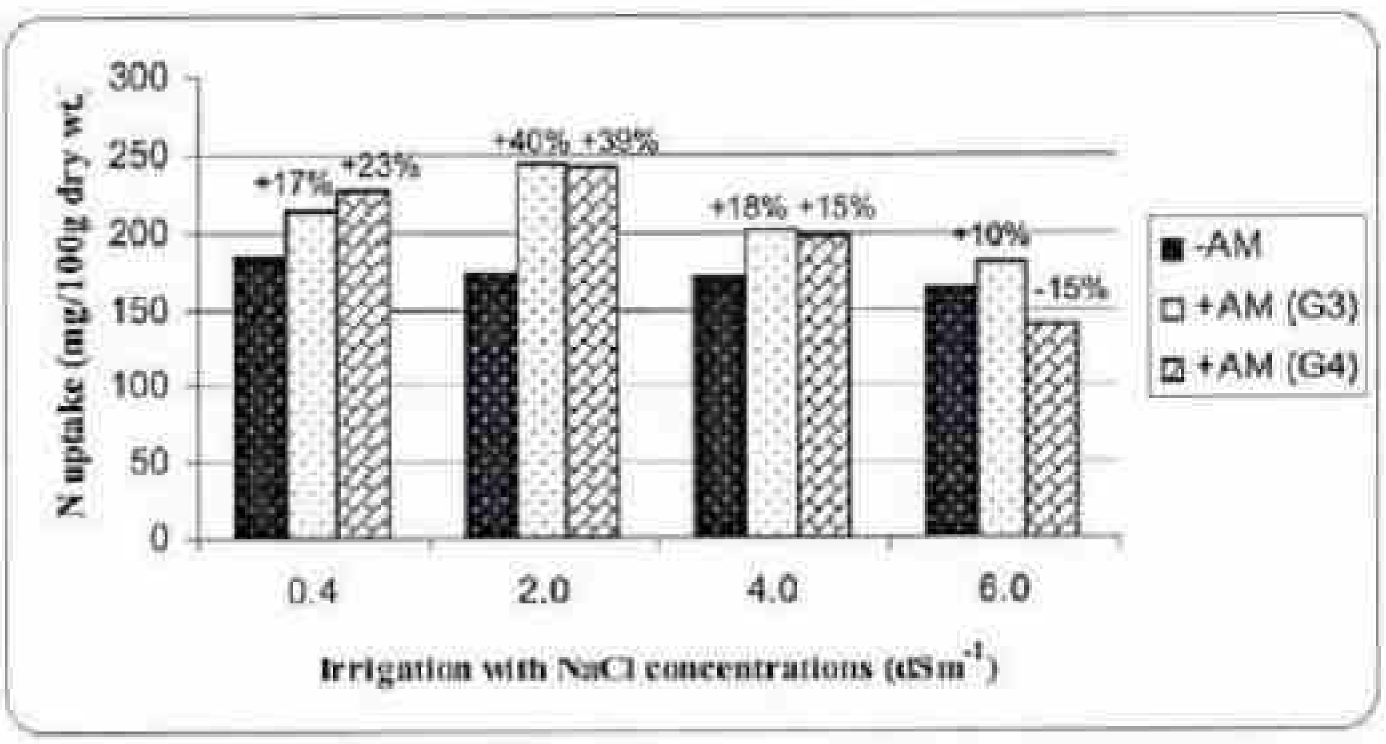


Figure (9). Effect of two strains of *Glomus intraradices* on N uptake (mg/100g dry wt.) in shoot dry weight under different NaCl stress

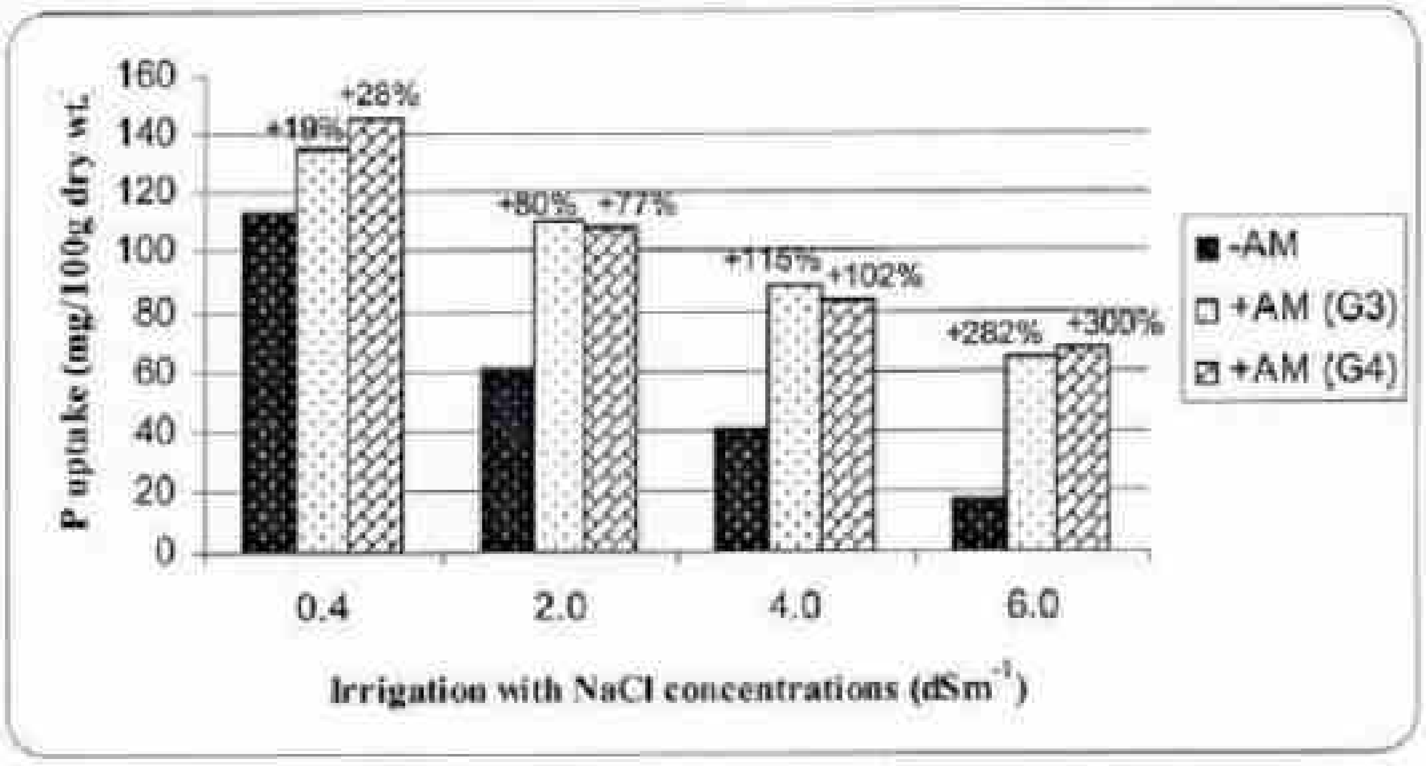


Figure (10). Effect of two strains of *Glomus intraradices* on P uptake (mg/100g dry wt.) in shoot dry weight under different NaCl stress

level 4 dSm^{-1}, P uptake was increased to 115 and 102 % in case of *G3* and *G4*, respectively, more than non-inoculated ones. Whereas the percentage increase of P uptake reached 282 and 300 % more than non-inoculated plants for the same AM strains, respectively. Mycorrhizal inoculation can increase P concentration in plants by enhancing its uptake facilitated by the extensive hyphea of the fungus which allows them to explore more soil volume than the non-mycorrhizal plants (Ruiz – Lozano and Azcon, 2000). It is estimated that external hyphae deliver up to 80% of a plant's P requirements (Matamores *et al*., 1999). Studies have shown higher P content in mycorrhizal than non-mycorrhizal *Acacia nilotica* plants in saline soils at varied levels of soil salinity (Giri *et al*., 2007).

c- **K uptake (mg / 100g dry wt)**

Data in Table (2) shows the effect of *G. intraradices* on K uptake by onion shoots under Na Cl stress. No significant differences were found due to AM inoculation. When Na^+ or salt concentration in the soil is high, plants tend to take up more Na^+ resulting in decreased K^+ uptake. Na^+ ions compete with K^+ for binding sites essential for various cellular functions. Potassium plays a key role in plant metabolism. It activates a range of enzymes, and plays an important role in stomatal movements and protein synthesis (Blaha *et al.*, 2000).

Table (2). Effect of two strains of *Glomus intraradices* on K uptake (mg/100g dry wt.) by shoot dry weight under different NaCl level

Parameter	K uptake (mg/100g shoot dry weight)							
NaCl (dSm^{-1}) / Inoculation	0.4		2.0		4.0		6.0	
	mg/100g	±%	mg/100g	±%	mg/100g	±%	mg/100g	±%
- AM	1263 a	--	1730 a	--	1516 a	--	1050a	--
+ AM (G3)	1494 a	+18	1544 a	-11	1501 a	-1	1281 a	+22
+ AM (G4)	1389 a	+9	1670 a	-4	1419 a	-6	1105 a	+5
L.S.D. $_{0.01}$	317.137 (ns)							

Giri *et al.*, 2007 reported that mycorrhizal colonization can enhance K^+ absorption under saline conditions, while preventing Na^+ translocation to shoot tissues. There are contrasting reports that the uptake of K^+ increased in shoot tissues of mycorrhizal plants even at a high salinity level (9.5 dSm^{-1}). This increases the K^+ : Na^- ratio in roots and shoots of AM plants.

8- the Percentage(%) of the root Colonization

Na Cl stress significantly reduced the Percentage(%) of the root Colonization Table (3). Viscular and arbuscular increasement were significantly larger than corresponding non-AM seedlings. The percentage increase in Colonization in case of inoculated plants with *G3* was 51.10- 43.22-and36.72%) in the presence of 2,4 and 6 dSm^{-1}, respectively more than non Am-plants under the same salt level stress , compared to non-inoculated plants, and the same trend was shown in case of *G4*.

Table (3). Effect of two strains of *Glomus intraradices* on the Percentage(%) of the Root colonization under different NaCl level

Parameter	the Percentage(%) of the root Colonization			
NaCl (dSm^{-1}) / Inoculation	0.4	2.0	4.0	6.0
	8-week	8-week	8-week	8-week
- AM	**0.0**	**0.0**	**0.0**	**0.0**
+ AM (G3)	**55,85**	**51,10**	**43,22**	**36.72**
+ AM (G4)	**57,00**	**49,00**	**38,42**	**31.77**
Interaction L.S.D. $_{0.01}$	**11,513**			

Conclusion:

The role of arbuscular mycorrhizal fungi (AMF) in alleviation salt stress is well documented. Our study explained some mechanisms of AMF employ to enhance the salt tolerance of *Allium cepa* plants such as enhanced nutrient acquisition (P, N), reduced Na^+, biochemical changes (accumulation of free amino acids and total phenol) and enhanced plants resistance to salt stress by increasing the amount of salicylic acid which has been proposed as an internal regular plant hormone.

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Department of Agricultural Microbiology, Faculty of Agriculture, Saba – Basha, Alexandria University, Egypt

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The role of arbuscular mycorrhizal fungi in alleviation effect of salt-stress on the mineral nutrition of Onion *crop(Allium cepa)*

Ali Amrir

Department of soil and soil reclamation, Faculty of Agriculture, Al Furat University-Syria

Abstract:

A pot experiment was conducted under green house condition to study the role of two strains (*Glomus intraradices*) in alleviation effect of salt-stress (Nacl – solution).

Seeds of (*Allium cepa*) were planted in soil inoculated with two AMF *G3* and *G4* (*Glomus intraradices*) under NaCl stress (0.4, 2.0, 4.0 and 6.0 dSm^{-1}), one week after germination.

The study observed a higher shoot and root dry weight and the parentage of colonization of (AMF) in mycorrhizal *Allium cepa* plant than a non mycorrhizal plant.

AMF treatments significantly increased salicylic acid content in roots (*G3*: 67 and 96%) and (*G4*: 17.50%) under 4.0 and 6.0 dSm^{-1}, respectively, compared to non-inoculated ones at the end of the experiment. Proline, Arginine and phenylalanine contents in roots showed pronounced increases. N and P uptake in AM shoots significantly increased under salt stress, compared to non-inoculated plants

Key words: Arbuscular mycorrhizal fungi, salt stress, *Allium cepa*
Free amino acid, salicylic acid – Root-colonization