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AM Fungal Protein's Contribution in Heaving Soil Physique Under Salt Stress

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I. Introduction

Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the middle of the 21st century (Wang *et al.* 2003). Approximately 7% of the global land surface is covered with saline plant habitats (Ruiz-Lozano *et al.* 1996). In semi-arid environments (which comprises of saline and sodic soils), ion toxicity because of high Na^+ and Cl^- concentrations cause destabilisation of soil structure therefore resulting in a considerable reduction in crop yield (Kohler *et al.* 2009). Soil structure is defined as the size and arrangement of particles and pores in soil (Hartge and Stewart, 1995), setting for the activity of soil biota and soil structure is hence important for soil-borne aspects of biogeochemical cycling processes (Paul and Clark, 1989). Sodium is a highly – dispersive agent causing the direct breakup of aggregates and indirectly affecting aggregation though decreased plant productivity (Bronick and Lal, 2005). Soil aggregation is a complex process that is largely dependent upon microorganisms to provide glues that hold soil particles together *via* hyphal enmeshment aggregates (Miller and Jastrow, 2000). These glues are produced by the arbuscular mycorrhizal fungus especially on their hyphae and spores that are abundant in the rhizosphere of their host plants, named glomalin. This is a glycoprotein detected in large amounts in diverse soils as glomalin-related soil protein (GRSP) and acts as the key factor in the contribution of AM fungi to soil aggregation to stabilize aggregates and influence soil carbon storage indirectly by stabilizing soil aggregates (Zhu and Miller, 2003) and therefore bring out soil stability. In alkaline soils, excessive amounts of salts, mainly sodium (Na) salts, in the soil solution cause numerous adverse phenomena such as destabilisation of soil structure, deterioration of soil hydraulic properties and a considerable reduction in crop yield (Lax *et al.* 1994 and Kohler *et al.* 2009), soil microbial biomass carbon and enzyme activities. Recently, the use of arbuscular mycorrhizal (AM) fungi as a practical way to alleviate soil stress on plant growth has received increased attention (Miransari *et al.* 2008) since it represents a living bridge for the translocation of nutrients and in particular, shown to contribute to the stability of soil aggregates, including soils of high salinity such as salt marshes (Caravaca *et al.* 2005). Their contributions to agriculture are well known, but their role in maintenance of soil structure and stability through the enhancement of soil aggregation under saline conditions in addition crop establishment has received less attention which insisted the necessity for this study. Among various preferable host of AM fungi, Onion is an important plant exhibiting excellent symbiotic relation with the fibrous root system (Poss *et al*. 1985, Cantrell and Lindermann, 2001) and hence selected for the present study. This study was undertaken to assess the diversity of AM fungi in sodic soil which basically lagged soil aggregation and soil structure.

II. Materials and Methods

This study was based on the influence of AM fungi in building salt tolerance to Onion crop and to test the effect of glomalin related soil proteins in improving the soil quality through pot culture study. Pots of 12 Kg capacity were filled with sterilized pot mix followed by AM inoculation @ 50 g^{-1} pot. Purified (sodic soil) isolates of AM (TRY 1, TRY 2, TRY 3 and TFS 1) along with two standard cultures (G. *intraradices* and S.

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calospora) were used as inoculants while control was maintained as an absolute control with salt treatment alone (without AM inoculation). The pot mix was first filled upto half the capacity of the pot followed by filling the respective AM inoculum and pot mix in alternate layers upto full capacity except for the head space of the pot. Onion bulbs were planted @ 4-5 bulbs pot^{-1} and then subjected to three levels of salt (1.5, 3.0 and 4.5 dSm^{-1}) by addition of NaCl through irrigation water twice in a week. Salt levels in the soil were maintained by checking the soil EC levels. All the treatments were replicated three times in a completely randomized design.

Inoculants:	*Salt Levels*
T1 - *Glomus intraradices*	L 1 - 1.5 dSm^{-1}
T2 - *Scutellospora calospora*	L 2 - 3.0 dSm^{-1}
T3 - TRY 1 (*Acaulospora* sp.)	L 3 - 4.5 dSm^{-1}
T4 - TRY 2 (*Scutellospora* sp.)	
T5 - TRY 3 (*Glomus* sp.)	
T6 - TFS 1 (*Glomus* sp.)	
T7 - Control (NaCl alone)	

a) Estimation of AM fungal spores in rhizosphere soil

AM fungal spore density was estimated from rhizosphere soil of Onion by wet sieving and decanting technique (Gerdemann and Nicolson, 1963).

b) Soil quality analysis

The post harvest soil was analysed for physical, chemical and biological properties. Standard methodologies (Table 1) were followed for analyzing physical and chemical properties viz., pH, EC, available N, available P and available K.

Table 1 : Standard methods followed for the physico-chemical analysis of soil samples

S. No.	Parameter	Unit	Method	Reference
II.	Physical properties			
1.	Bulk density	Mg m^{-3}	Wet cylinder method	Chopra and Kanwar (1982)
2.	Particle density	Mg m^{-3}	Wet cylinder method	
3.	Porosity	Per cent	Wet cylinder method	
4.	Water holding capacity	per cent	Keen Raczkowski Box	Piper (1966)
5	pH	-	Measured using digital pH meter	Jackson (1973)
6	EC	dS m^{-1}	Measured using conductivity bridge (CM 180 Elico conductivity Bridge)	Jackson (1973)

c) Estimation of microbial biomass carbon

Biomass carbon was determined by the fumigation-incubation technique as per the procedure given by Jenkinson and Powlson (1976). Ten g soil was weighed into 100 mL beaker. The beaker was placed in a 250 mL air tight plastic container into which about 5 mL of water was added. Ethanol free chloroform was prepared, immediately before fumigation by passing 100 mL of chloroform through a glass column containing 75 g of basic aluminium oxide. The fumigation was carried out with ethanol free chloroform for 20 hours at 25°C. After fumigation, chloroform was removed by repeated evacuations. After fumigation and removal of chloroform, the beaker holding the soil was returned to the air tight container together with a scintillation vial holding 5 mL of 0.5 N NaOH. Soil samples were inoculated with a pinch of fresh soil of respective treatments and the soil was incubated for a further period of 10 days at 25°C. Evolved CO_2 was determined by titrating the alkaline traps with 0.5 N HCl after precipitation of CO_3^{2-} with 50% $BaCl_2$ and using phenolphthalein as indicator.

$$Biomass\ C = (C\ fumigated - C\ nonfumigated)\ CO_2 - C\ evolved \times K_C\ factor\ (0.45)$$

d) Total Glomalin content

The total glomalin content in the soil was estimated according to Wright and Upadhyaya, (1996).

e) Percentage water stable aggregates

The aggregate stability percentage in soil was estimated according to Kemper and Koch (1966).

f) Correlation analysis

A simple correlation analysis (p = 0.05) was worked out between soil physicochemical properties and spore density of AM fungi in native soil as well as between soil quality parameters in pot cultured soil.

g) Statistical analysis

The data were subjected to statistical analysis by variance (P=0.05) with mean separation by Least significant difference (LSD) as per the methods detailed by the Panse and Sukhatme (1978). The analysis for microbial population count was based on the log and arcsine transformed values.

III. Results

a) Spore count in rhizosphere of Onion

The spore count was found to increase with increase in salinity level in this study while, maximum was at harvest which proved the nature of AM fungi to

form spores to survive under stress. The results of the present study showed that the rhizosphere of Onion harboured abundance of spores and was found to increase with each level of stress condition where, the maximum was recorded at L3 (4.5 dSm^{-1}). A steady increase in spore load was observed from the initial stage of observation until harvest invariably in all the treatments where, T5 registered the highest of 121.7 spores 100 gm^{-1} soil respectively and interestingly it was on par with T2, the standard isolate (Fig 1). Present results are consistent with Gupta and Rautaray (2005) who recorded highest spore count in the rhizosphere soil treated with 3 per cent NaCl, inoculated with *Glomus* sp. (68.34 ± 12.01 per 100 g soil) and concluded that the presence of spores in the soil reveals the tolerance of AM fungi (*Glomus* sp.) to NaCl induced stress. The high spore content in soil samples and the intense mycorrhizal colonization of the roots does indicate that AM fungal activity plays a role under such harsh conditions in saline and sodic soils. In general, increases in soil pH, nutrient status and salinity in soil are related to a decrease in AM root colonization or in spore density and suffer adverse effects due to the accumulation of some anions and cations. The decrease in spore density in a particular treatment is attributed to the degree of toleration of that particular strain of AM fungi inoculated (Rao and McNelly, 1999). Similar result was reported by Aliasgharzadeh *et al*. (2001) who evaluated AM diversity in tabriz plains and found the number of AM fungal spores was not correlated significantly with soil salinity.

b) Total microbial population

Though there was a decrease in the microbial population with increase in the salt levels (L2 and L3), the population of fungi dominated the rhizosphere than the other microbes (Table 2). The results registered a maximum of 19.30 x 10^5 cfu bacteria g^{-1} soil in T2, 52.40 x 10^4 cfu fungi g^{-1} soil in T1 and 11.12 x 102 cfu actinomycetes g^{-1} soil in T4. Among the total microbial count, fungi were dominating the rhizosphere in T1 (G. *intraradices*) while bacteria and actinomycetes population were enhanced by T2 (S. *calospora*) inoculation. Influence of microbial populations was reported previously by few workers (Boby and Bagyaraj, 2003). Stimulative effect of AM fungi (*Scutellospora* sp. CAM 3) on microbial population was evidenced by Priya and Kumutha, (2009) where inoculation of G. *mosseae* tremendously increased the population of total bacteria (76.08 x 10^6 cfu), fungi (123.40 x 10^4 cfu) and PGPR (103.70 x 10^6 cfu) in the rhizosphere of mycorrhizal plants at all stages of sampling. In case of salinity not only the soil-borne spores of the AM fungi has ability to withstand adverse soil conditions, but also the extraradical hyphae might protect the host plant from toxic levels of deleterious elements in the growth medium (Li and Christie 2001). It has been shown that extramatrical hyphae of AM fungi exude substances and cause soil and organic fractions to aggregate (Sutton and Sheppard, 1976) in which the microorganisms fluorish.

c) Soil Physical parameters

i. *Soil pH and EC*

The soil pH was observed to remain same throughout the experiment with slight variation with respect to each level of salt (8.27 to 8.10) while there was a noticeable reduction in EC levels in inoculated treatments than the control soil (Fig 2). The decreased electrical conductivity of mycorrhizosphere soil demonstrates that AM fungi have a profound effect on the ionic balance as supported by Rosendahl and Rosendahl (1991). This may be the result of increased absorption and translocation by AM fungal hyphae. The reduction in shoot Na uptake and maintaining electrical conductivity of the soil may be significant in helping mycorrhizal plants to survive in saline conditions. Also studies by Cantrell and Lindermann (2001) proved that AM fungal treatments lowered the soil EC while the control did not express much reduction in EC at the end of experiment.

ii. *Bulk density and Particle density (%)*

In the present study, AM fungal inoculations showed notable decrease in bulk density and particle density. The bulk density of the soil was found to be increased with increase in salt levels. Though much significant difference was not observed between the treatments, AM fungal inoculation lowered the bulk density of soils at all the levels of salt where T1 and T2 showed noticeable decrease compared to other treatments. Treatments T1 and T2 reduced the bulk density upto 1.30, 1.34 and 1.40 per cent at L1, L2 and L3 respectively. In contrast to bulk density of the soil, the particle density was found to be increased with increase in salt levels in all the treatments. Only at L1 and L2, the treatments showed a considerable decrease when compared to the control whereas at L3, significant difference was not observed. Both the treatments T1 and T2 showed 2.4 and 2.50 per cent of particle density at L2 and L3 respectively (Table 3) (Fig 3a).

iii. *Water holding capacity and Porosity (%)*

Water Holding Capacity (WHC) of the soil samples were significantly increased in all the treatments but were found to decrease with increase in salt levels. Among the treatments, T1 showed maximum WHC at all the three levels of salt showing 81.11, 78.86 and 76.86 per cent at L1, L2 and L3 respectively. Also the soil porosity was influenced by the AM fungi to some extent, where the increment in salt levels showed a decrease in pore space in all the treatments. Porosity was maximum in T1 and T2 that

showed 53, 51.6 and 50.90 per cent at L1, L2 and L3 respectively (Table 3) (Fig 3b).

Such decreases in bulk and particle density with increase in porosity and water holding capacity at L1 (1.5 dSm-1) than at L2 and L3 (3.0 and 4.5 dSm-1) may be due to that, at high salt levels, the presence of more Na+ ions in the soil cause dispersion of soil aggregates leading to soil compaction leading to hard pan state and therefore a hike in bulk density, particle density and interruption in hydraulic conductivity. Bulk density depends on soil structure and is an indicator of soil compaction, aeration and ease the development of roots. Previous findings confirmed a concurrent decrease in bulk density with an increase in total soil porosity (by 24 per cent) and hydraulic conductivity due to addition of organic materials and a slight increase in soil organic matter due to the AM fungi inoculated treatment (Celik *et al.* 2004; Marinari *et al*. 2000).

d) Soil chemical parameters

i. *Organic carbon (%)*

The organic carbon content is one of the vital parameter indicating soil fertility which was significantly influenced in the AM inoculated treatments than the control (Table 4). Analysis at 45 DAS showed a maximum of 0.53 per cent in T1 and inclined upto 0.6 per cent at harvest which remarked about 16.7 per cent increase over control followed by T5 that registered about 12.8 per cent increase over control. The content of organic carbon decreased with increments in salt level in all the treatments at both the stages of observation. Inoculation of AM fungal treatments enhance organic matter content in soil by increasing the particulate organic matter and glomalin contents which influence soil structure, water holding capacity (WHC), water, oxygen infiltration rates, carbon (C) storage and soil fertility (Nichols, 2003). But with increase in salt levels the organic carbon content was found to decline in all the treatments in this study. The higher level of Na^+ ions could have dispersed the aggregates in the soil leading to loss in organic carbon content and this may be the cause for this decline at high salts inspite of the AM fungal inoculation.

ii. *Microbial biomass carbon*

The microbial biomass carbon represents the available carbon pool in the rhizosphere of the plants that may increase with application of bioinoculants. This analysis determined the capacity of the inoculated cultures in maintaining the carbon pool against various levels of stress imposed and the results showed that the rate of microbial biomass carbon was found to be increased at increasing rate with the days of the crop and was maximum at harvest (Table 5). Though the rate of carbon was found to be decreased at high salt levels, the treatments showed an increase over the control at all the levels. At 45 DAS, remarkable increase in microbial biomass carbon was observed in all the treatments which was found to be augmented still at harvest (Fig 4) registering 327.0 mg kg^{-1} (T1). The performance of treatments with a higher per cent of increase over the control even at L3 than at L1 and L2 indicates the mycorrhizal response at higher stress conditions. Sodic soils are high in exchangeable Na^+ and the changes in biomass inputs or organic matter accumulation will alter soil organic carbon levels in soil. Although soil microbial biomass only comprises 1-5% of SOC (Sparling, 1992) it is critical in organic matter decomposition and can provide an early indicator of SOM dynamics as a whole due to its faster turnover time. Addition of organic material to the scalded soils showed increase in SMB levels and respiration rates than in non degraded soils (Wong *et al*. 2010).

iii. *Total Glomalin (TG)*

Accumulation of glomalin in soil requires a minimum of 8 to 10 weeks and therefore the soil samples were analysed 8 weeks after sowing (at 45 DAS and at harvest). The total extractable glomalin after purification was estimated in the salt imposed soils. The content of glomalin was found to be decreased with increase in levels of salt and maximum accumulation was noticed at harvest than at 45 DAS where the highest was 153 μg glomalin g-1 soil in T1 at L1 (1.5 dSm-1) at harvest. Purified protein was taken for the SDS PAGE analysis which weighed protein bands of 55 kDa indicating the presence of glomalin in all the treatments except the control (Fig 5, 6). With increase in salt levels, the rate of glomalin accumulation decreased at L3 and this is in accordance with experiments by Kohler et al. (2010). They concluded that, Glomalin related soil proteins decreased at high salt stress (66 μg/ g of soil) than at low salt (77 μg/ g of soil) in soils inoculated with *Glomus mosseae*. The higher concentrations of glomalin in the soil aggregates under saline stress may be related to the occurrence of the highest levels of sodium in the soil and the efficiency of glomalin to sequester different toxic elements (Gonzalez-Chavez et al. 2004). The increase in glomalin concentrations in this study at harvest, can be attributed to, multiplication of AM fungi, through high sporulation especially to encounter the stress condition and due to formation of aggregates in soil within which the protein is glued. Since glomalin represents an investment of C by AM fungi, it makes sense that glomalin production increases as C availability rises (Treseder and Turner, 2007).

iv. *Aggregate stability percentage*

Aggregation is a soil quality factor that positively affects water infiltration rates, resistance to erosion and nutrient cycling. The fraction or type of carbon compound influences the persistence and water-stability of aggregates. In this study, formation of aggregates in the soil was very much influenced by the

AM fungal inoculation and the stability of aggregates was maximum at harvest than at 45 DAS showing significant difference with that of control. Aggregate stability percentage was affected by increase in salt levels and the lowest aggregation percentage was observed at L3. At 45 DAS, T1 and T2 marked the highest of 92.3 and 87.4 per cent increase over control respectively and the trend increased at harvest showing maximum of 129.4 and 130.7 per cent increase over control in T1 and T2 (Table 6). Bethlenfalvay et al. (1999) reported that water-stable soil aggregates were positively correlated with root and mycorrhiza infection. Wright and Upadhyaya (1998) showed that there is a strong correlation between aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Mycorrhizal fungi were stated to be a powerful component in soil environments and soil sustainability especially for soil quality (Ortas, 2002).

Mycorrhizal inoculations promoted more soil aggregation to a maximum of 130.7 per cent increase over control in T2 at harvest than at 45 DAS in this study. Hamel *et al*. (1997) found a positive relationship between two species of *Glomus* (G. *caledonium* and G. *macrocarpum*) and the proportion of water stable soil aggregates in the 0.5–2 mm diameter range. With increase in salt levels, aggregate stability was found to decrease (55 53 and 51 per cent at L1, L2 and L3 respectively) and these results were in accordance with Kohler et al. (2010) where, decreased aggregate stability and glomalin-related soil protein (GRSP) concentration were recorded with increasing saline stress in soils inoculated with G. *mosseae*. Also these findings suggested that the use of AM fungi for alleviating salinity stress in lettuce plants would be possible to some extent in bringing out soil structural stability.

v. *Available micronutrient contents*

The soil micronutrient contents were found to be decreased with increase in salt levels as well as with stages of plant growth and the nutrient availability were statistically non significant under the interaction between the treatments and salt levels. Among the three salt levels, soils with L1 (1.5 dSm-1) accumulated higher micronutrients both at 45 DAS and at harvest. Among the micronutrients analysed, iron was higher in soils than others. In L1, highest of 6.17 ppm iron was observed in T2 that was on par with T1 and (Fig 7).

e) *Correlation study between soil aggregation and soil physico-chemical parameters*

A simple correlation analysis was worked out between accumulation of glomalin protein and physicochemical parameters in soil. The analysis indicated that a significant positive correlation existed between glomalin protein with soil organic carbon and aggregate stability with high 'r' value (0.979 and 0.942 respectively) (Fig 8). Hamel *et al*. (1997) reported that, a positive correlation existed between AM fungal inoculations with (G. *intraradices* and G. *versiforme*) and abundance of water stable soil aggregates in the 0.5–2 mm diameter range in leek plants (*Allium porum*). In this study, Iron content in soil correlated highly (r =0.924) with glomalin content and this is line with Nichols and Wright (2005) proved that the changes in Fe percentage were significantly correlated with the changes in glomalin weight and carbon per cent. The glomalin, humin, humic acid, fulvic acid and total carbon weights were related to iron concentration in the aggregates which indicated that these organic matter fractions are stabilized within organo-mineral complexes formed by iron bridging organic matter to clay particles. Another correlation analysis between soil aggregate stability with water holding capacity and porosity also showed a more positive correlation (0.795 and 0.843 respectively) while a negative correlation existed between aggregate stability with bulk density (-0.987) and particle density (-0.963) at 5 % level of significance (Table 7, Fig 8). Celik *et al*. (2004) confirmed a concurrent decrease in bulk density with an increase in total soil porosity (by 24 per cent) due to slight increase in soil organic matter due to the AM fungus inoculated treatment. This proved the effect of soil physical parameters by mycorrhizal inoculations due to the possible stimulating effect on soil aggregation. Marinari *et al*. (2000) also found that bulk density was lowered and total soil porosity increased at the presence of organic matter. Water retention capacity of soils with high porosity was higher than the soils with low porosity. Aggelides and Londra (2000) determined that porosity and water retention capacity of loamy and clay soils increased with application of organic amendments which in turn enhance soil aggregation.

IV. Conclusion

These findings support the correlations that exist between soil organic carbon, bulk density and porosity. The saturated hydraulic conductivity is generally related to soil porosity especially macroporosity since it increases significantly with an increase in porosity. Previous works have also determined that porosity and water retention capacity of loamy and clay soils increased with application of organic amendments. Also the hike in glomalin contents (the compound responsible for sequestering for carbon) in the AM fungal treatments, especially at harvest stage of the crop puts forth to increase in organic carbon contents. The fractions of the soil organic matter is a key attribute of soil quality that impacts soil aggregation and accordingly increases water infiltration and these effects of soil physical parameters by mycorrhizal inoculations overcomes the

hard pan formation and enhances root penetration and proliferation. Thus the effect of AM fungal inoculation in maintenance of soil properties through the influence on glomalin content, soil organic matter content and therefore soil aggregation at various salinity levels in the rhizosphere of Onion is enlightened.

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Table 2 : Effect of AM fungal isolates on microbial count in Onion against various levels of salinity

Treatments	Microbial count (cfu g^{-1} ODS) at 45 DAS														
	Bacteria x 10^5					Fungi x 10^4					Actinomycetes x 10^2				
	L1	L2	L3	Mean	Per cent increase over control	L1	L2	L3	Mean	Per cent increase over control	L1	L2	L3	Mean	Per cent increase over control
G. intraradices	19.17	12.10	10.44	13.90	60.51	52.40	45.17	38.40	45.32	178.89	9.23	6.77	3.86	8.00	422.88
S. calospora	19.30	13.34	10.40	14.35	65.70	39.30	28.00	23.45	30.25	86.15	10.22	9.32	6.82	8.80	474.29
TRY1	11.50	12.82	9.82	11.38	31.41	20.17	36.25	14.48	23.63	45.42	9.61	5.72	4.70	6.68	336.38
TRY 2	11.00	10.95	9.94	10.63	22.75	19.01	24.50	35.90	26.47	62.89	11.12	5.01	3.60	6.58	329.85
TRY 3	15.20	10.34	8.51	11.35	31.06	45.33	41.33	34.40	40.35	148.31	7.21	5.60	3.10	5.30	246.62
TFS 1	12.05	9.34	8.95	10.11	16.74	23.30	33.27	11.33	22.63	39.26	7.20	5.31	3.90	5.47	257.52
Control	10.50	9.12	6.37	8.66	-	10.22	23.33	15.20	16.25	-	2.00	1.48	1.12	1.53	-
Mean	14.10	11.14	9.20	11.48		29.96	33.12	24.74	29.27		8.08	5.60	3.87	5.85	
	SEd		CD (0.05)			SEd		CD (0.05)			SEd		CD (0.05)		
T	0.16		0.33			0.62		1.26			0.15		0.30		
L	0.10		0.21			0.41		0.83			0.09		0.19		
T x L	0.28		0.57			1.08		2.19			0.26		0.52		

L1 – 1.5 dSm^{-1}; L2 - 3.0 dSm^{-1}; L3 – 4.5 dSm^{-1}; DAS – Days after sowing;
Values represent mean of three replicates;
Value in paranthesis indicate per cent increase over control

G. intraradices - *Glomus intraradices* *S. calospora* - *Scutellospora calospora*
TRY 1- *Acaulospora* sp TRY 3- *Glomus mosseae*
TRY 2- *Scutellospora* sp. TFS 1- *Glomus aggregatum*

Table 3 : Effect of AM fungal isolates on percentage of bulk density, particle density, water holding capacity and porosity in rhizosphere of Onion against various levels of salinity

S.No	Treatments	Bulk density (%)			Mean	Particle density (%)			Mean	Water holding capacity (%)			Mean	Porosity (%)			Mean
		L1	L2	L3		L1	L2	L3		L1	L2	L3		L1	L2	L3	
1.	*G. intraradices*	1.30	1.34	1.40	1.34 (-7.4)	2.41	2.50	2.65	2.52 (-3.1)	81.11	78.86	76.86	78.94 (19.7)	53.00	51.60	50.90	51.83 (8.1)
2.	*S. calospora*	1.30	1.34	1.40	1.35 (-7.1)	2.42	2.50	2.65	2.52 (-2.9)	80.90	78.01	76.13	78.35 (18.8)	52.80	51.40	50.00	51.40 (7.2)
3.	TRY 1	1.32	1.35	1.42	1.36 (-6.0)	2.46	2.54	2.66	2.55 (-1.8)	76.86	76.02	72.13	75.00 (13.7)	50.00	49.60	47.40	49.00 (2.2)
4.	TRY 2	1.33	1.36	1.42	1.37 (-5.5)	2.44	2.53	2.65	2.54 (-2.3)	79.24	75.22	73.30	75.92 (15.1)	50.10	49.10	48.00	49.07 (2.4)
5.	TRY 3	1.32	1.36	1.45	1.38 (-5.1)	2.44	2.52	2.65	2.54 (-2.4)	77.75	78.00	75.00	76.92 (16.6)	50.80	49.70	48.10	49.53 (3.3)
6.	TFS 1	1.33	1.36	1.45	1.38 (-4.8)	2.46	2.53	2.66	2.55 (-1.9)	70.48	69.01	68.46	69.32 (5.1)	50.80	49.70	48.10	49.53 (3.3)
7.	Control	1.40	1.43	1.53	1.45	2.53	2.60	2.67	2.60	68.21	65.43	64.22	65.95	49.30	47.50	47.00	47.93
	Mean	1.33	1.36	1.44	1.38	2.45	2.53	2.66	2.55	76.36	74.36	72.30	74.34	50.97	49.80	48.50	49.76
		SEd		CD (0.05)		SEd		CD (0.05)		SEd		CD (0.05)		SEd		CD (0.05)	
	T	0.003		0.006		0.003		0.007		0.25		0.52		0.087		0.177	
	L	0.002		0.004		0.002		0.005		0.16		0.34		0.057		0.116	
	T x L	0.005		0.011		0.006		0.013		0.44		0.90		0.152		0.307	

Table 4 : Effect of AM fungal isolates on organic carbon content in Onion rhizosphere against various levels of salinity

S.No	Treatments	Organic carbon content (%)									
		45 DAS			Mean	Per cent increase over control	At harvest			Mean	Per cent increase over control
		L1	L2	L3			L1	L2	L3		
1.	*G. intraradices*	0.56	0.53	0.50	0.53	8.2	0.63	0.61	0.58	0.61	16.7
2.	*S. calospora* sp.	0.55	0.52	0.50	0.52	6.8	0.62	0.61	0.58	0.60	16.0
3.	TRY 1	0.52	0.51	0.48	0.50	2.7	0.60	0.58	0.55	0.58	10.9
4.	TRY 2	0.52	0.51	0.48	0.50	2.7	0.61	0.58	0.55	0.58	11.5
5.	TRY 3	0.55	0.52	0.48	0.52	5.4	0.61	0.60	0.55	0.59	12.8
6.	TFS 1	0.55	0.50	0.48	0.51	4.1	0.60	0.57	0.55	0.57	10.3
7.	Control	0.52	0.48	0.47	0.49	-	0.54	0.53	0.50	0.52	-
	Mean	0.54	0.51	0.48	0.51		0.60	0.58	0.55	0.58	
		SEd		CD (0.05)			SEd		CD (0.05)		
	T	0.002		0.004			0.002		0.005		
	L	0.001		0.002			0.001		0.003		
	T x L	0.003		0.007			0.004		0.008		

L1 – 1.5 dSm^{-1}; L2 - 3.0 dSm^{-1}; L3 – 4.5 dSm^{-1}; DAS – Days after sowing;
Values represent mean of three replicates;
Value in paranthesis indicate per cent increase over control

G. intraradices - *Glomus intraradices*
TRY 1- *Acaulospora* sp
TRY 2- *Scutellospora* sp.
S. calospora - *Scutellospora calospora*
TRY 3- *Glomus mosseae*
TFS 1- *Glomus aggregatum*

Table 5 : Effect of AM fungal isolates on microbial biomass carbon in Onion rhizosphere against various levels of salinity

S.No	Treatments	Microbial biomass carbon (mg kg $^{-1}$)												
		30 DAS			Mean	45 DAS			Mean	At harvest			Mean	Per cent increase over control
		L1	L2	L3		L1	L2	L3		L1	L2	L3		
1.	*G. intraradices*	183.0 (51.2)	152.0 (32.1)	136.0 (25.9)	157.0	294.0 (50.0)	280.0 (71.7)	269.0 (99.2)	281.0	345.0 (35.8)	325.0 (27.9)	311.0 (42.0)	327.0	43.2
2.	*S. calospora*	176.0 (45.4)	155.0 (34.7)	135.0 (25.0)	155.3	288.0 (46.9)	278.0 (70.5)	244.0 (80.7)	270.0	330.0 (144.4)	317.0 (134.8)	305.0 (39.2)	317.3	39.0
3.	TRY 1	150.0	138.0	125.0	137.7	262.0	248.0	228.0	246.0	289.0	265.0	244.0	266.0	16.5
4.	TRY 2	159.0	138.0	123.0	140.0	269.0 (37.2)	251.0 (54.0)	236.0 (74.8)	252.0	325.0 (140.7)	295.0 (118.5)	268.0 (22.3)	296.0	29.7
5.	TRY 3	162.0 (33.8)	148.0 (28.7)	138.0 (13.9)	149.3	244.0	233.0	221.0	232.7	298.0	272.0	253.0	274.3	20.2
6.	TFS 1	135.0	119.0	111.0	121.7	237.0	218.0	200.0	218.3	277.0	258.0	238.0	257.7	12.9
7.	Control	121.0	115.0	108.0	114.7	196.0	163.0	135.0	164.7	254.0	212.0	219.0	228.3	-
	Mean	155.1	137.9	125.1	139.3	255.7	238.7	219.0	237.8	302.6	277.7	262.6	280.9	
		SEd		**CD (0.05)**		**SEd**		**CD (0.05)**		**SEd**		**CD (0.05)**		
	T	1.06		2.14		2.07		4.19		1.94		3.92		
	L	0.69		1.40		1.35		2.744		1.27		2.57		
	T x L	1.84		3.71		3.59		7.27		3.36		6.80		

L1 – 1.5 dSm^{-1}; L2 - 3.0 dSm^{-1}; L3 – 4.5 dSm^{-1}; DAS – Days after sowing;
Values represent mean of three replicates;
Value in paranthesis indicate per cent increase over control

G. intraradices - *Glomus intraradices* *S. calospora* - *Scutellospora calospora*
TRY 1- *Acaulospora* sp TRY 3- *Glomus mosseae*
TRY 2- *Scutellospora* sp. TFS 1- *Glomus aggregatum*

Table 6 : Effect of AM fungal isolates on percentage of aggregate stability in Onion rhizosphere against various levels of salinity

S.No	Treatments	Aggregate stability (%)									
		45 DAS			Mean	Per cent increase over control	At harvest			Mean	Per cent increase over control
		L1	L2	L3			L1	L2	L3		
1.	*G. intraradices*	48.5 (88.7)	43.2 (24.1)	42.7 (112.4)	44.80	92.3	55.2 (116.5)	53.0 (122.7)	50.8 (154.0)	53.00	129.4
2.	*S. calospora*	46.2 (80.0)	42.8 (78.0)	42.0 (109.0)	43.67	87.4	55.0 (115.7)	53.8 (126.0)	51.1 (155.5)	53.30	130.7
3.	TRY 1	43.2	40.3	38.2	40.57	74.1	45.6	41.6	40.9	42.70	84.8
4.	TRY 2	42.8	40.6	38.9	40.77	75.0	43.7	42.8	41.5 (104.5)	42.67	84.7
5.	TRY 3	45.4 (76.6)	41.6 (72.6)	40.5 (101.5)	42.50	82.4	47.7 (87.06)	43.8 (84.0)	40.9	44.13	91.1
6.	TFS 1	42.7	40.0	38.7	40.47	73.7	47.0	43.5	40.1	43.53	88.5
7.	Control	25.7	24.1	20.1	23.30	-	25.5	23.8	20.0	23.10	-
	Mean	42.07	38.94	37.30	39.43		45.67	43.19	40.76	43.20	
		SEd		CD (0.05)			SEd		CD (0.05)		
	T	0.37		0.75			0.50		1.02		
	L	0.24		0.49			0.33		0.66		
	T x L	0.65		1.31			0.87		1.77		

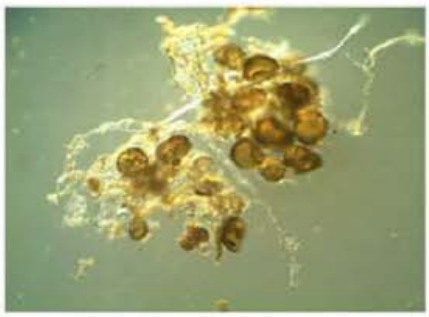
L1 – 1.5 dSm^{-1}; L2 - 3.0 dSm^{-1}; L3 – 4.5 dSm^{-1}; DAS – Days after sowing;
Values represent mean of three replicates;
Value in paranthesis indicate per cent increase over control

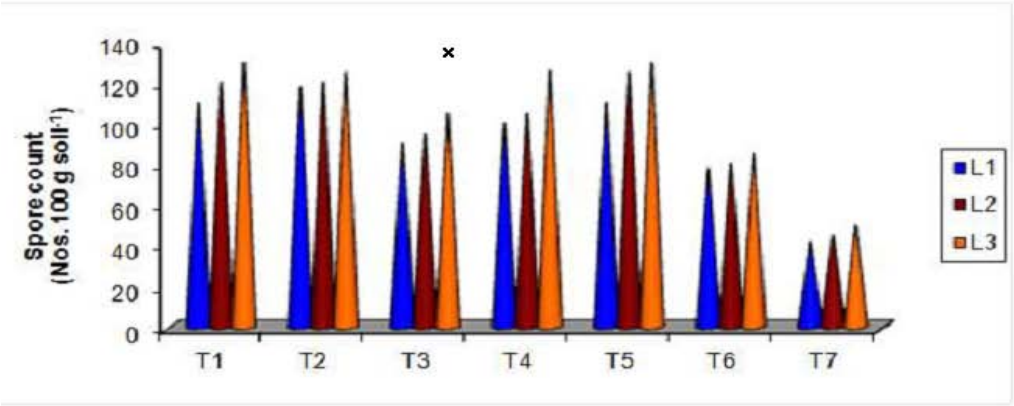
G. intraradices - *Glomus intraradices* *S. calospora* - *Scutellospora calospora*
TRY 1- *Acaulospora* sp TRY 3- *Glomus mosseae*
TRY 2- *Scutellospora* sp. TFS 1- *Glomus aggregatum*

Table 7 : Correlation analysis of Glomalin with soil parameters at L1 (1.5 dSm-1) in post harvest soil of Onion rhizosphere

S. No	Relationship between		Correlation coefficient (r)	Level of significance
	X	Y		
1.	Glomalin	Iron content	0.924	0.05
2.	Glomalin	Organic carbon	0.979	0.05
3.	Glomalin	Aggregate stability	0.942	0.05
4.	Aggregate stability	Water holding capacity	0.795	0.05
5.	Aggregate stability	Porosity	0.843	0.05
6.	Aggregate stability	Bulk density	-0.987	0.05
7.	Aggregate stability	Particle density	-0.963	0.05

Figure 1. Effect of AM fungal isolates on spore count in the rhizosphere of Onion against various levels of salinity at harvest





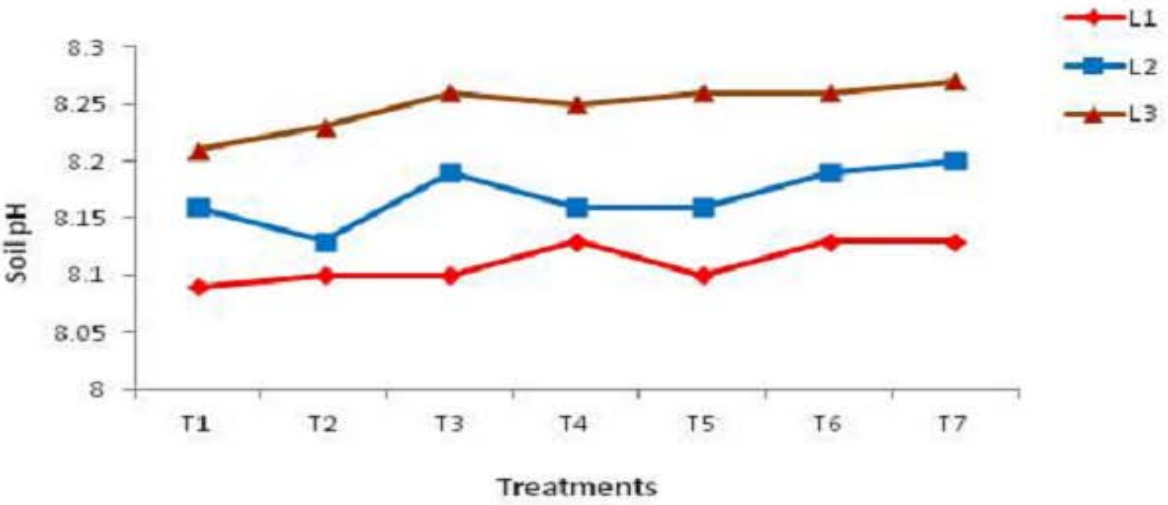
Treatments

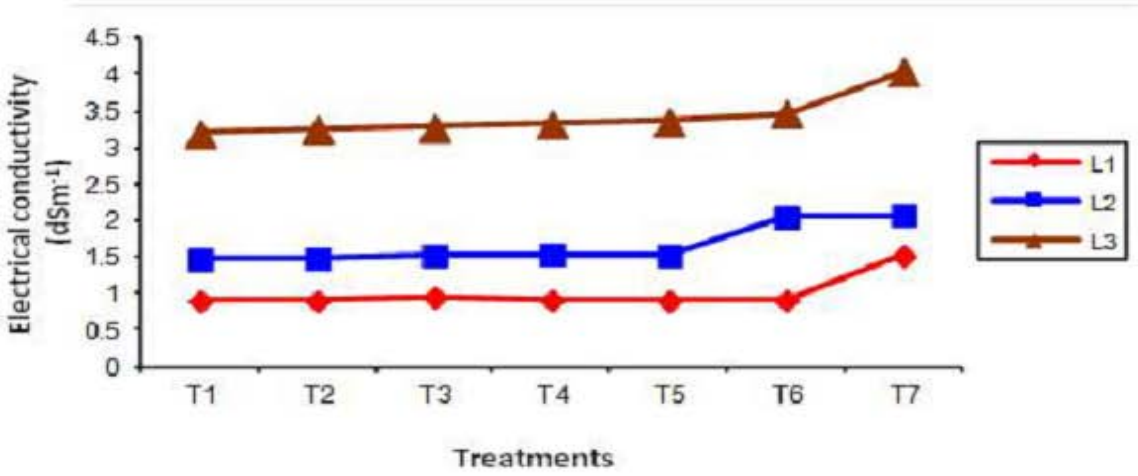
T1 - *Glomus intraradices*
T2 - *Scutellospora calospora*
T3 - TRY 1 (*Acaulospora* sp.)
T4 - TRY 2 (*Scutellospora* sp.)
T5 - TRY 3 (*Glomus mosseae*)
T6 – TFS 1 (*Glomus aggregatum*)
T7 - Control

Levels

L1 - 1.5 dSm^{-1}
L2 - 3.0 dSm^{-1}
L 3 - 4.5 dSm^{-1}

Figure 2. Effect of AM fungal isolates on soil pH and EC in rhizosphere of Onion against various levels of salinity





Treatments

T1 - *Glomus intraradices*
T2 - *Scutellospora calospora*
T3 - TRY 1 (*Acaulospora* sp.)
T4 - TRY 2 (*Scutellospora* sp.)
T5 - TRY 3 (*Glomus mosseae*)
T6 – TFS 1 (*Glomus aggregatum*)
T7 - Control

Levels

L1 - 1.5 dSm^{-1}
L2 - 3.0 dSm^{-1}
L 3 - 4.5 dSm^{-1}

Figure 3a. Effect of AM fungal isolates on bulk density and particle density in Onion rhizosphere at 1.5 dSm^{-1}

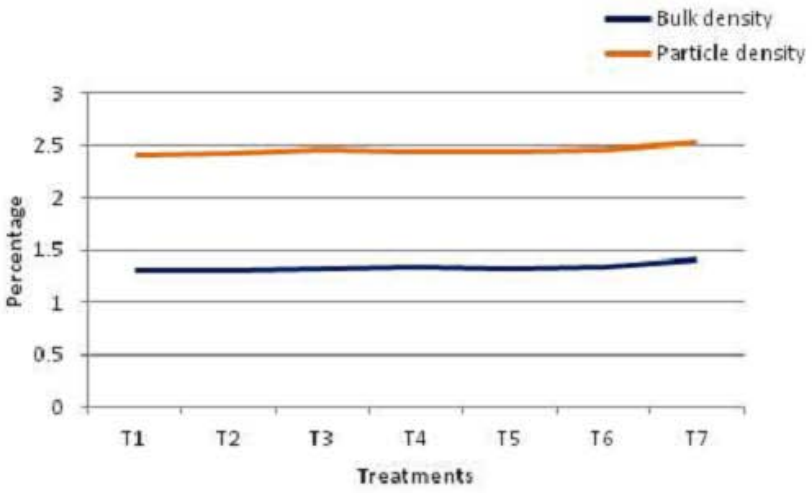
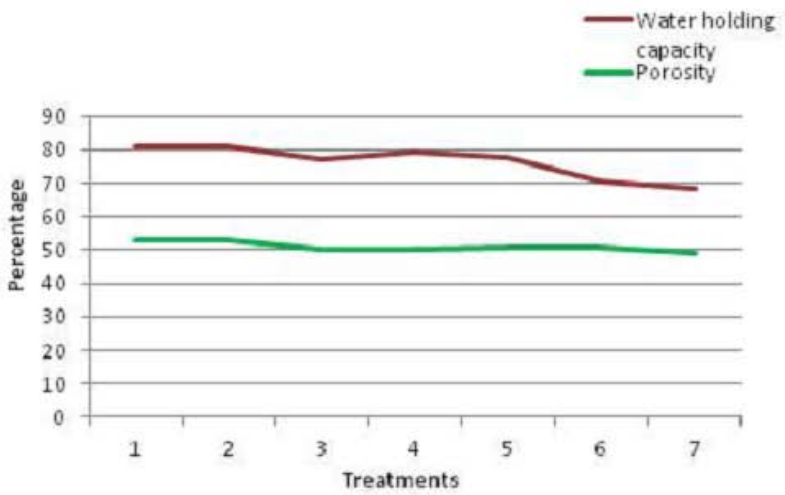


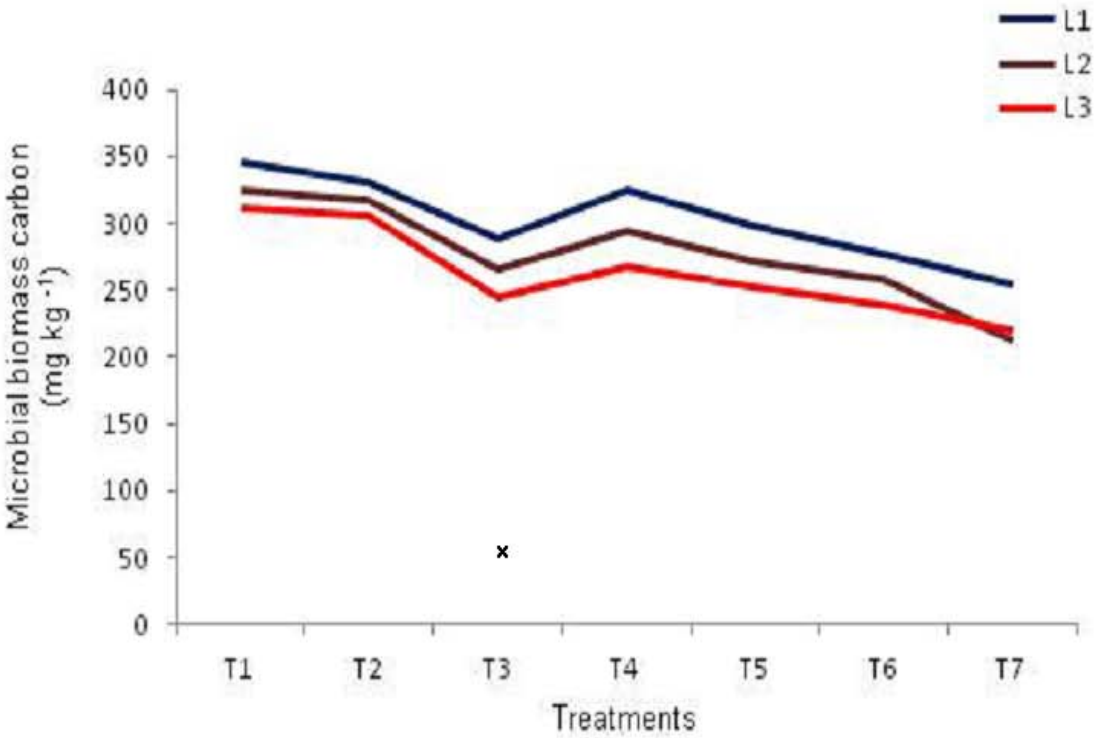
Figure 3b. Effect of AM fungal isolates on water holding capacity and porosity in Onion rhizosphere at 1.5 dSm^{-1}



Treatments

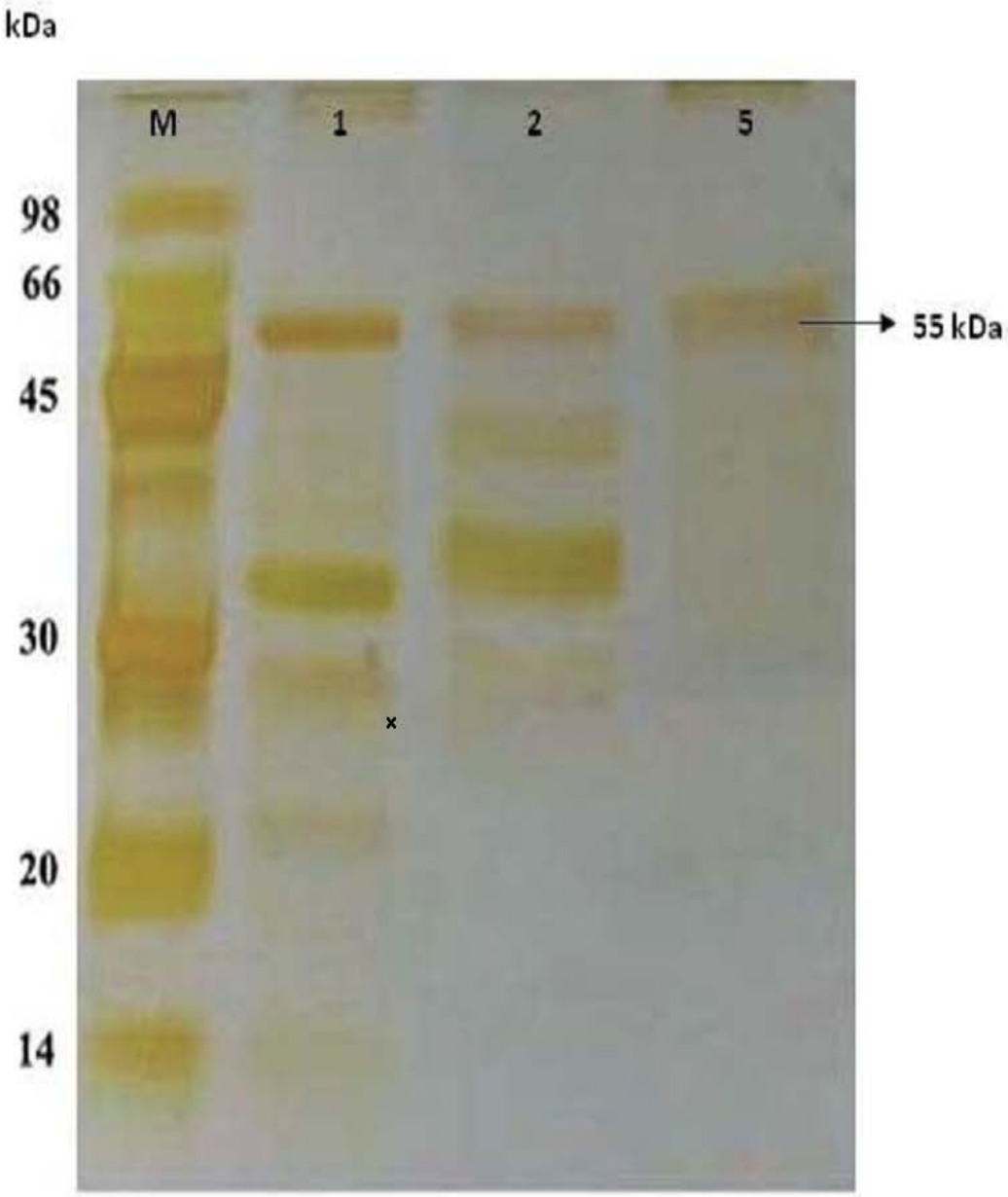
T1 - *Glomus intraradices*
T2 - *Scutellospora calospora*
T3 - TRY 1 (*Acaulospora* sp.)
T4 - TRY 2 (*Scutellospora* sp.)
T5 - TRY 3 (*Glomus mosseae*)
T6 – TFS 1 (*Glomus aggregatum*
T7 - Control

Figure 4. Effect of AM fungal isolates on microbial biomass carbon In the rhizosphere of Onion at harvest



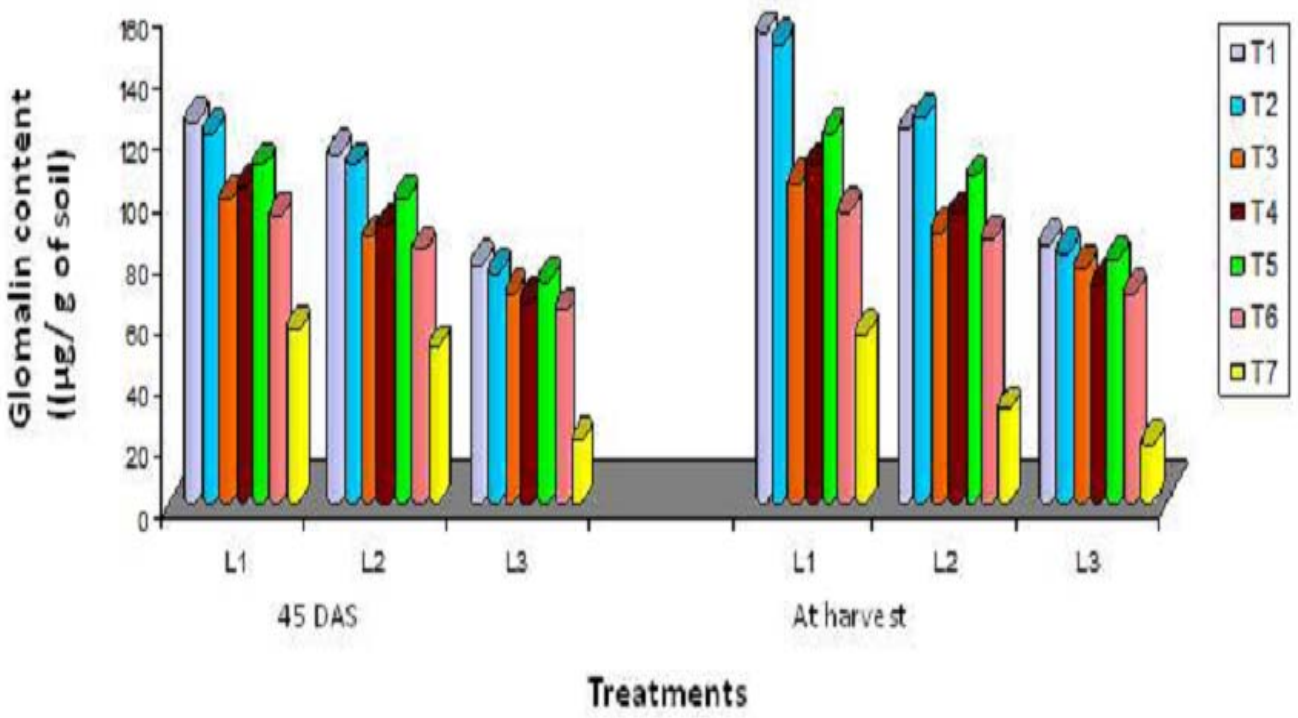
Treatments	Levels
T1 - *Glomus intraradices*	L1 - 1.5 dSm^{-1}
T2 - *Scutellospora calospora*	L2 - 3.0 dSm^{-1}
T3 - TRY 1 (*Acaulospora* sp.)	L 3 - 4.5 dSm^{-1}
T4 - TRY 2 (*Scutellospora* sp.)	
T5 - TRY 3 (*Glomus mosseae*)	
T6 – TFS 1 (*Glomus aggregatum*)	
T7 - Control	

Figure 5. SDS PAGE profile of total glomalin in rhizosphere of onion grown with low level of salinity (L1- 1.5 dSm^{-1})



Lane 1 – T1 (*Glomus intraradices*)
Lane 2 – T2 (*Scutellospora calospora*)
Lane 5 – T5 (TRY 3)

Figure 6. Effect of AM fungal isolates on Glomalin content in the rhizosphere of Onion at various levels of salinity



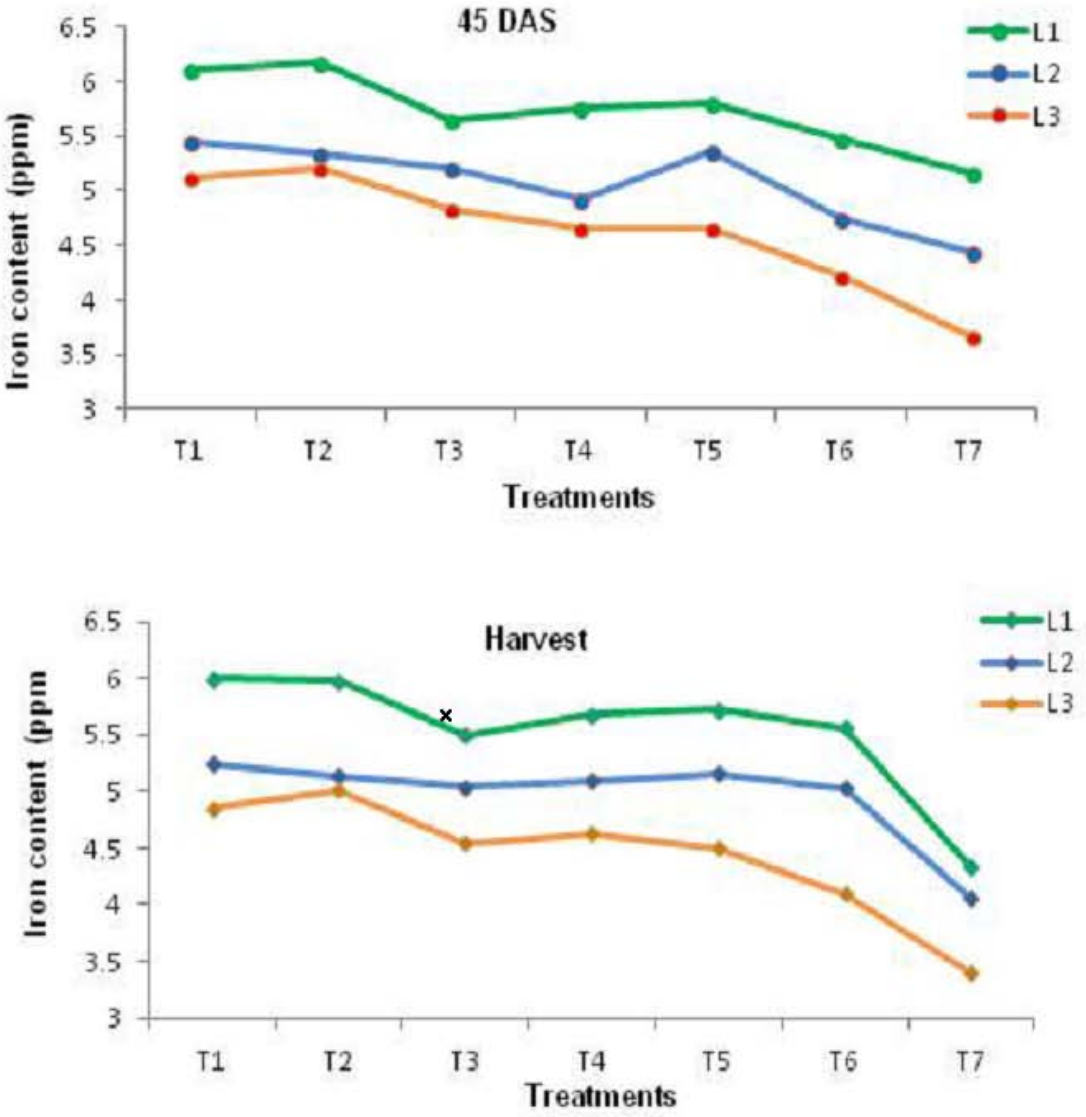
Treatments

T1 - *Glomus Intraradices*
T2 - *Scutellospora calospora*
T3 - TRY 1 (*Acaulospora sp.*)
T4 - TRY 2 (*Scutellospora sp.*)
T5 - TRY 3 (*Glomus mosseae*)
T6 – TFS 1 (*Glomus aggregatum*)
T7 - Control

Levels

L1 - 1.5 dSm^{-1}
L2 - 3.0 dSm^{-1}
L 3 - 4.5 dSm^{-1}

Figure 7. Effect of AM fungal isolates on iron availability in rhizosphere soil of Onion against various levels of salinity



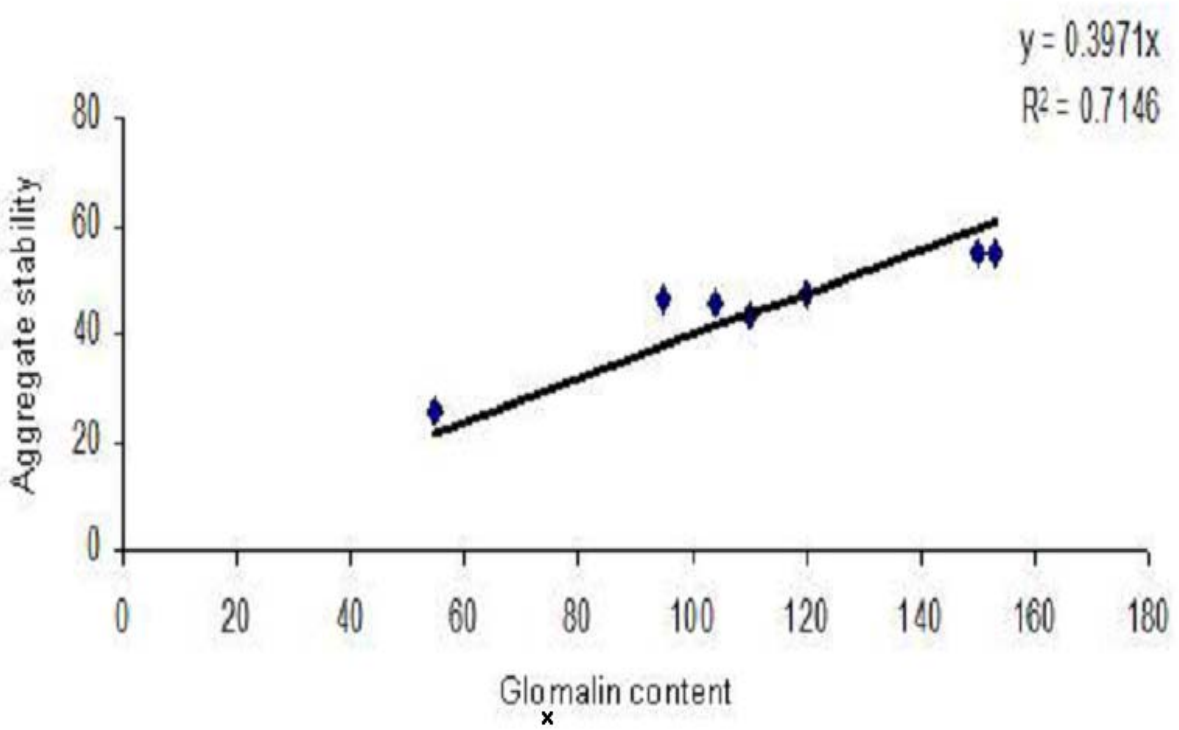
Treatments

T1 - *Glomus intraradices*
T2 - *Scutellospora calospora*
T3 - TRY 1 (*Acaulospora* sp.)
T4 - TRY 2 (*Scutellospora* sp.)
T5 - TRY 3 (*Glomus mosseae*)
T6 – TFS 1 (*Glomus aggregatum*)
T7 - Control

Levels

L1 - 1.5 dSm^{-1}
L2 - 3.0 dSm^{-1}
L 3 - 4.5 dSm^{-1}

Figure 8. Correlation analysis between glomalin content and aggregate stability at L1 (1.5 dSm^{-1})



Treatments

T1 - *Glomus Intraradices*
T2 - *Scutellospora calospora*
T3 - TRY 1 (*Acaulospora* sp.)
T4 - TRY 2 (*Scutellospora* sp.)
T5 - TRY 3 (*Glomus mosseae*)
T6 – TFS 1 (*Glomus aggregatum*
T7 - Control