



Studies on the Role of Arbuscular Mycorrhizal Fungal Enhancement on Soil Aggregate Stability

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Abstract

Arbuscular Mycorrhizal (AM) fungi isolated from various crop rhizosphere of sodic soil sites were purified and selected for inoculation along with two standard strains namely, Glomus intraradices and Scutellospora calospora in a pot culture experiment with maize as host crop to study their influence on soil aggregation. Analysis on soil parameters responsible for improving soil aggregation after a period of 24 weeks showed influence of AM fungal inoculations on root colonization (93 %), soil spore load (620 spores 100 g⁻¹ soil), particulate organic matter (60 mg g⁻¹ soil), microbial count (9.7 x 10⁵ of bacteria, 10.3 x 10⁴ of fungi and 1.4 x 10³ of actinobacteria), micronutrient contents (4.96 ±0.06, 0.83±0.05 and 3.52±0.20 ppm of iron, copper and zinc respectively) soil organic carbon (0.37 %), total glomalin production (62 µg of protein g⁻¹ of soil) as well as the water soluble carbohydrate content (0.67 mg g⁻¹ soil). Therefore the aggregate stability of the soil has been increased to 53 % where, the standard strains ranked the highest followed by the sodic soil isolates, Glomus mosseae (TRY 3) and Scutellospora sp. (TRY 2). Overall results showed the positive influence of AM fungi on soil aggregation.

Keywords: Glomus intraradices, soil aggregation, micronutrient, water soluble carbohydrate, glomalin.

Introduction

Arbuscular Mycorrhizal (AM) fungus exhibit a symbiotic relationship with more than 150 species of all vascular plants occurring worldwide in almost all soil type, forming the dominant type of mycorrhiza. Mycorrhizal fungal hyphae may access to nutrient-rich zones at a distance from the root system¹. AM fungal hyphae can take up both inorganic and organic nitrogen and transport nitrogen to the host plants². About 12 to 30 % of plant photosynthetic carbon is translocated belowground in the form of sugars that support fungal growth and development and the carbon cost to the plant is balanced by access to a greater volume of soil through fungal hyphae which have a much larger surface area to volume ratio than root hairs and fan out up to 8 cm beyond nutrient depletion zones around roots. AM fungi contribute to Soil Organic Matter (SOM) directly by producing extramatrical hyphae and by deposition of organic substances³ indirectly by enhancing plant growth. The extramatrical hyphae of AM fungi support physical protection of SOM by promoting soil aggregation which is especially important in sandy and loamy soils where other binding agents are scarce and these hyphal contribution to physical protection gains a higher relative importance⁴. The soil aggregates are bound by glues that are produced by the AM fungus especially on their hyphae and spores that are abundant in the rhizosphere of their host plants and are named glomalin, a glycoprotein detected in large amounts in diverse soils as Glomalin-Related Soil Protein (GRSP) which acts as the key factor to stabilize aggregates⁵. Hence, the present investigation was taken up to

evaluate the effect of AM fungal isolates of sodic soil on improving soil aggregation through a pot culture study.

Material and methods

Collection of soil samples were done at agricultural college situated at 78°36' East latitude and 10°45' North longitude of Trichy district, Tamil Nadu, India. Sampling sites included seven different rhizosphere soils viz, *Zea mays*, *Occimum sanctum*, *Allium cepa*, *Psidium gujava*, *Oryza sativa*, *Azadiracta indica* and *Bambusa vulgaris* and sampling was done at 15 cm depth with three sub samples each. The laboratory experiments were conducted in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore. The collected soil samples after the analysis of physio-chemical properties were taken for enumeration of AM fungal spore density in the native soil using the method of wet sieving and decantation⁶.

The soil samples showed alkaline pH of 8.5 - 9.5, EC of 0.07 – 0.25 with ESP ranging from 15.5 – 16.8 and available P₂O₅ of 10 – 27 kg ha⁻¹. Based on the density of AM spores, the predominant genus was identified (one from each sample) and taken for purification and multiplication by funnel technique as described⁷. These seven sodic soil isolates of AM fungi (TRY 1, TRY 2, TRY 3, TRY 4, TRY 5, TFS 1 and TFS 2) were used as inoculants for the pot culture experiment along with two authenticated cultures (*Glomus intraradices* and *Scutellospora calospora*) obtained from the Department of Agricultural Microbiology, TNAU, Coimbatore. Using each of

these nine inoculants as individual treatments with inoculum dose of 1 spore g⁻¹ soil, a pot experiment was taken up.

Inoculants: Pots of 10 kg capacity each were filled with sterilized pot mixture (red soil: sand: farm yard manure – 1:1:1) upto half the capacity, while the remaining was filled with two or three alternate layers of inoculum and pot mix and were finally top dressed with a layer of pot mix to cover the inoculum layer. The same trend was followed for all the treatments while the control was maintained uninoculated. Surface sterilized seeds of maize (COH1 MI5 hybrid) were used as host crop for this study and all the treatments were replicated three times in a completely randomized design. The crop was maintained in the pots for a period of 24 weeks through three successive sowing as a continuous culture without disturbing the rhizosphere region, for accumulation of the arbuscular mycorrhizal protein glomalin in the soil figure-1.



Figure-1

Influence of AM isolates in improving soil parameters under 24 weeks continuous culture

Treatment details: T₁ - *Glomus intraradices*; T₂ - *Scutellospora calospora*; T₃ - TRY 1 (*Acaulospora* sp.); T₄ - TRY 2 (*Scutellospora* sp.); T₅ - TRY 3 (*Glomus mosseae*) ; T₆ - TRY 4 (*Sclerocystis* sp.); T₇ -TRY 5 (*Glomus geosporum*); T₈ -TFS 1 (*Glomus aggregatum*); T₉- TFS 2 (*Gigaspora* sp.); T₁₀- Uninoculated control

Sampling and analysis: After 24 weeks of crop growth the soil samples were analysed for their variation in soil

properties due to accumulation of glomalin and the following observations were recorded in the rhizosphere samples of maize. Root colonization and spore density of the AM fungus, total microbial load in the rhizosphere soil, the soil organic carbon fractions that contribute to soil aggregation viz, active carbon pools (soil organic carbon, water soluble carbohydrates, particulate organic matter) and passive carbon pools (humic acid and fulvic acid) along with glomalin were analysed in the post harvest soil.

The root colonization was assessed by the method⁸ and the root colonization percentage was estimated. The spore density was assessed in the rhizosphere soil of maize by wet sieving and decantation technique.

The total microbial population viz, bacteria, fungi and actinobacteria were estimated from the post harvest soil (using soil extract agar medium, Rose Bengal agar medium and Ken-Knight's agar medium respectively and incubated at 28 ± 1°C) on soil dry weight basis and expressed in colony forming units g⁻¹ of dry soil.

Organic carbon in soil sample was analysed by wet chromic acid digestion⁹ and the organic matter fractions were also estimated¹⁰. Particulate Organic Matter (POM) was removed by floatation in a high-density solution. Soil samples (2 g) were covered with a 10 ml of NaCl solution (12 % w/v), vortexed and allowed to settle. After the mineral fraction had settled, the solution was carefully decanted. Floating organic matter (i.e. the POM fraction) was collected on a 0.053 mm sieve. This procedure was repeated four times. The POM fraction collected on the screen was washed with distilled water to remove salt, rinsed from the screen into pre-weighed weigh boats and dried at 70°C whose gravimetric weight was recorded. The settled mineral fraction (i.e. soil minus POM) was washed with distilled water, pelleted by centrifugation, rinsed into pre-weighed weigh boats and dried at 70°C for estimation of humic (HA) and fulvic acid (FA) fractions. After extraction of the POM fraction, HA and FA were co-extracted from the mineral fraction (i.e. soil minus POM) using a multi-step NaOH extraction procedure¹¹. The water soluble carbohydrates were determined by the method outlined¹². Total glomalin content was estimated¹³ and the protein content was estimated using Bradford assay¹⁴ and the protein profiling was done by SDS-PAGE. About 12 % gel was used and size of the identified bands were determined relative to a medium range (12 to 97 KDa) protein molecular weight marker (Bangalore Genei, Bangalore). Aggregate stability is a function of organic matter, clay and free iron oxides. Aggregate stability was determined¹⁵ figure-2. Micronutrient analysis was performed using DTPA extraction method¹⁶ and the value was read in Atomic Absorption Spectroscopy (AAS) for iron, copper and zinc.

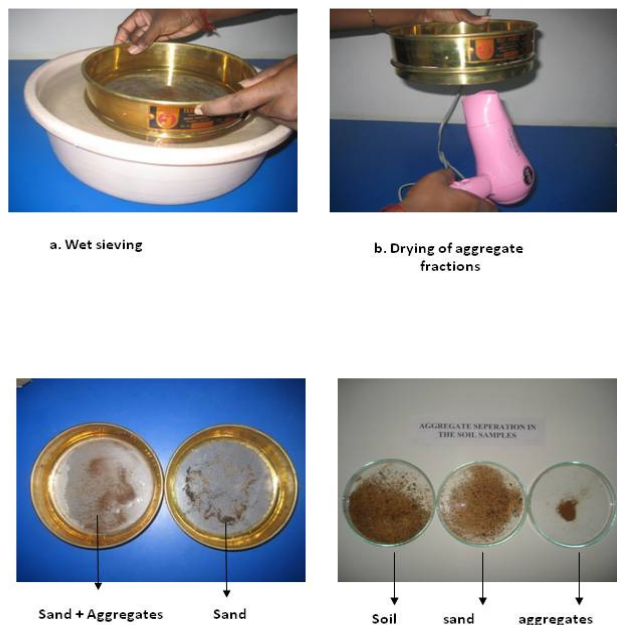


Figure-2

Aggregate separation in soil under 24 weeks continuous

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.

Statistical analysis: The data were subjected to statistical analysis by variance (ANOVA)¹⁷ at $P<0.05$ levels, further the treatment means were statistically differentiated by performing Duncan's Multiple Range Test (DMRT) at $p<0.05$ levels. Statistically differentiated means were denoted by different alphabets. All the above analysis were done using the IRRISTAT (version 92) software developed by the International Rice Research Institute Biometrics unit, Philippines.

Results and Discussion

Assessment of root colonization and spore load: Treatments with AM fungal inoculation showed significant increase in root colonization over control. The root colonization percentage was observed to be highest in T_1 ($93\pm 5.37\%$) with which T_5 was on par and T_4 (88%) performed on par with the standard strain *Scutellospora calospora* table-1. Spore load in the 24 week cultured soil was found to have increased due to the treatments and the standard strains T_2 (620 ± 33.49 spores 100 g^{-1} soil) and T_1 (545 ± 31.47 spores 100 g^{-1} soil) recorded the highest number of spores in the rhizosphere table-1 where, T_4 and T_5 ranked the next best. This ability of the sodic soil isolates to behave on par with that of the standard strains marked their efficiency in colonization and multiplication in the rhizosphere. In most of the treatments, percentage of root colonization was not directly proportional to the spore load. This may be due to that the rhizosphere could have experienced some stress leading to spore formation of the fungi resulting in higher spore load and on

other hand a favourable situation like, the availability of carbon sources could have induced the germination of the existing spores resulting in more colonization percentage figure-3.



Figure-3

Root colonization and spore multiplication observed under 24 weeks continuous culture

Total microbial population: The total microbial population was found to have increased in the treatments than the control Table-2. Among the total microbial count, fungal population was maximum ($10.90\pm 0.31 \times 10^4$ cfu g^{-1} of soil) than that of bacteria and actinobacteria in the T_1 and T_2 treatments followed by T_5 . The bacterial population was varying among the treatments with the highest in T_1 followed by T_2 . On comparison, the actinobacterial population was much lesser than bacteria and fungi in the treatments. Among the treatments, T_1 showed highest of $1.40\pm 0.04 \times 10^3$ cfu g^{-1} of soil. The AM fungi interact with a whole range of microorganisms in soils. Once the mycorrhizal symbiosis has developed, AM hyphae influence the surrounding soil resulting in the development of distinct microbial communities related to rhizosphere¹⁸. When mycorrhiza forms the symbiotic relationship, significant changes were observed in the type of organisms in the rhizosphere before formation of mycorrhiza. But quantitative changes occur as a result of direct metabolic interactions with the mycorrhizal fungal hyphae that makes the key difference between rhizosphere effect of non-mycorrhizal roots and the mycorrhizosphere that extend out some distance from the host tissue into the soil. It has been shown that extramatrical hyphae of AM fungi exude substances that cause soil and organic fractions to aggregate¹⁹ in which the microorganisms such as bacteria, fungi and actinobacteria flourish.

Soil organic carbon: The soil organic carbon content was influenced by the AM fungal treatments compared to control and the highest was observed in T_1 ($0.370\pm 0.021\%$) followed by T_2 ($0.337\pm 0.019\%$) table-3. Such an increase in carbon pool is due to the strong influence of mycorrhizal fungi on the release of compounds from living roots because, these fungi can affect plant carbon metabolism while representing a sizeable sink for plant derived carbon²⁰. In the present study, inoculation with T_1 had significantly increased the soil labile carbon (0.370%) showing 23.3% increase over control and this is supported by a report showing organic carbon content of 0.431% due to *Glomus intraradices* inoculation²¹.

Table-1
Effect of AM fungal isolates on root colonization and spore load in rhizosphere of maize

Treatments	Culture	Root colonization* in Maize roots (%)	Spore load** (No. 100 g ⁻¹ soil)
T ₁	<i>G. intraradices</i>	93±5.37 ^a	545±31.47 ^b
T ₂	<i>S. calospora</i>	89±5.14 ^c	620±33.49 ^a
T ₃	<i>Acaulospora</i> sp	91±5.25 ^{bc}	320±18.48 ^g
T ₄	<i>Scutellospora</i> sp.	88±5.08 ^{cd}	415±23.96 ^c
T ₅	<i>Glomus mosseae</i>	92±5.31 ^{ab}	385±22.23 ^d
T ₆	<i>Sclerocystis</i> sp.	81±4.68 ^c	360±20.79 ^c
T ₇	<i>Glomus geosporum</i>	85±4.91 ^d	350±20.21 ^f
T ₈	<i>Glomus aggregatum</i>	83±4.79 ^d	280±16.17 ^h
T ₉	<i>Gigaspora</i> sp.	72±4.16 ^f	240±13.86 ⁱ
T ₁₀	Control	10±0.58 ^g	56±3.23 ^j
	S.Ed	7.35	34.73
	CD (0.05)	15.33	72.45

* 8 weeks after sowing in 3rd crop of maize; ** post harvest soil after 3 maize crops (24 weeks)

Table-2
Effect of AM fungal isolates on total microbial count in post harvest soil

Treatments	Culture	Microbial count (cfu g ⁻¹ ODS)		
		Bacteria x 10 ⁵	Fungi x 10 ⁴	Actinobacteria x 10 ³
T ₁	<i>G. intraradices</i>	9.72±0.28 ^a	10.27±0.30 ^b	1.40±0.04 ^a
T ₂	<i>S. calospora</i>	9.52±0.27 ^b	10.16±0.29 ^c	0.44±0.01 ^f
T ₃	<i>Acaulospora</i> sp	9.08±0.26 ^d	10.90±0.31 ^a	0.64±0.02 ^d
T ₄	<i>Scutellospora</i> sp.	8.40±0.24 ^c	10.08±0.29 ^c	1.30±0.04 ^b
T ₅	<i>Glomus mosseae</i>	9.44±0.27 ^c	10.12±0.29 ^d	1.04±0.03 ^c
T ₆	<i>Sclerocystis</i> sp.	8.19±0.24 ^f	9.82±0.28 ^f	0.61±0.02 ^d
T ₇	<i>Glomus geosporum</i>	7.95±0.23 ^g	9.51±0.27 ^g	0.47±0.01 ^f
T ₈	<i>Glomus aggregatum</i>	7.82±0.23 ^{gh}	8.51±0.25 ^h	0.64±0.02 ^d
T ₉	<i>Gigaspora</i> sp.	7.40±0.21 ⁱ	8.29±0.24 ⁱ	0.53±0.02 ^c
T ₁₀	Control	5.33±0.15 ^j	2.17±0.06 ^j	0.18±0.01 ^g
	S.Ed	0.752	0.835	0.072
	CD (0.05)	1.570	1.743	0.150

cfu – colony forming units; ODS - Oven Dried Soil

Table-3
Effect of AM fungal isolates on soil organic carbon and water soluble carbohydrate content in post harvest soil

Treatments	Culture	SOC content (%)	Per cent increase over control	Water soluble carbohydrate (mg g ⁻¹)	Per cent increase over control
T ₁	<i>G. intraradices</i>	0.370±0.02 ^a	23.3	0.65±0.040 ^b	109.7
T ₂	<i>S. calospora</i>	0.337±0.02 ^b	12.3	0.67±0.040 ^a	116.1
T ₃	<i>Acaulospora</i> sp	0.310±0.01 ^d	3.3	0.55±0.032 ^d	77.4
T ₄	<i>Scutellospora</i> sp.	0.327±0.02 ^c	9.0	0.59±0.034 ^c	90.3
T ₅	<i>Glomus mosseae</i>	0.320±0.01 ^c	6.7	0.58±0.033 ^c	87.1
T ₆	<i>Sclerocystis</i> sp.	0.325±0.02 ^c	8.3	0.50±0.030 ^e	61.3
T ₇	<i>Glomus geosporum</i>	0.314±0.01 ^d	4.7	0.51±0.030 ^f	64.5
T ₈	<i>Glomus aggregatum</i>	0.312±0.01 ^d	4.0	0.36±0.021 ^{gh}	16.1
T ₉	<i>Gigaspora</i> sp.	0.311±0.01 ^d	3.7	0.37±0.021 ^g	19.4
T ₁₀	Control	0.301±0.01 ^{de}	-	0.31±0.020 ⁱ	-
	S.Ed	0.029	-	0.045	-
	CD (0.05)	0.060	-	0.095	-

Water Soluble Carbohydrate (WSC): Inoculation with AM fungal isolates had remarkable influence on the WSC contents than the control where the inoculation of T₁ and T₂ showed the maximum of 0.67±0.040 and 0.65±0.040 mg g⁻¹ soil respectively with significant increase than the control Table-3. Efficiency of *Scutellospora* sp. in better colonization have been proved by workers earlier²² which supports the present study. Mycorrhizal symbiosis utilizes at least 10 % of the host plant photosynthetic carbon and the transferred carbon enriches microbial activities in the rhizosphere which may have contributed for the enhancement of active carbon pool in the soil. This ability might have put forth to increased utilization of carbon from host roots and therefore deposition of higher water soluble carbohydrates in the soil by this species.

Particulate Organic Matter fraction (POM): The majority of soil organic carbon is comprised of POM, humic substances, the most recalcitrant and stable fractions of soil organic matter and glomalin. Present investigation showed increased humic and fulvic acid contents due to the treatments among which *Glomus* sp. resulted an increase in the passive organic pools in the soil. The total particulate organic matter fraction was noticed to increase in all the AM fungal treatments compared to control. The highest was obtained in T₁ followed by T₃ table-4. The concentration of humic acid fractions analysed from the mineral fraction of soil was found to be 46±2.65 mg g⁻¹ soil in T₁ followed by T₂ and T₅. This study also showed humic acid to be higher than fulvic acid invariably in all the treatments with about 66.7 to 283 % increase over control which is in agreement with a previous study²³. Mycorrhizal products can enter a slower turnover pool of carbon in soil, which would counteract a rapid cycling of carbon in soils, re-entering the atmosphere as carbon dioxide²⁴ figure-4.

Quantitative and qualitative analysis of glomalin: Quantification of glomalin: In this study, the glomalin extracted from the experimented soil appeared wine red to reddish brown in colour which is due to the presence of iron in the soil samples figure-5 and the intensity of the red colour slightly differed between the treatments. Since the quantification of glomalin protein was carried out through Bradford assay, the protein was mentioned as Bradford Reactive Soil Protein (BRSP). The BRSP fraction of Total Extractable Glomalin (TEG) was highest in treatment inoculated with T₁ followed by T₂. The BRSP fraction of Easily Extractable Glomalin (EEG) obtained was again maximum (29±0.33 µg) in T₁ followed by T₂. Among the sodic soil isolates, T₃ and T₅ recorded higher EEG content with an increase of 100 and 127 per cent increase over control respectively. The results of the present study Table-5 is in line with a report²⁵ which showed increase in glomalin concentrations due to inoculation of *Glomus* strains (*G. intraradices*, *G. caledonium*) which may be attributed to the long-term maize monoculture affecting AM fungal populations. Also the TEG contents were comparatively higher than EEG in all the treatments in the present study and this is in line with a finding²⁶ where, TEG content was higher (770 µg) than EEG among the soil organic carbon pools.

Qualitative analysis of purified glomalin through SDS-PAGE: The protein bands detected and resolved using electrophoresis technique illustrated the molecular mass of the protein to be approximately 55 kDa (45 - 64 kDa) in the AM fungal treatments alone Figure-5. Banding patterns also showed T₁, T₂ and T₃ with pronounced expression of glomalin protein and this was in agreement with results of a study²⁷ which showed two prominent bands of glomalin at 55 kDa and 64 kDa.

Table-4
 Effect of AM fungal isolates on Particulate Organic matter (POM) and mineral fractions in post harvest soil

Treatments	Culture	Particulate organic matter (mg g ⁻¹)	Per cent increase over control	Mineral fraction			
				Humic acid (mg g ⁻¹)	Per cent increase over control	Fulvic acid (mg g ⁻¹)	Per cent increase over control
T ₁	<i>G. intraradices</i>	60.0±1.73 ^a	200.0	46±2.65 ^a	283.3	35±2.02 ^a	191.7
T ₂	<i>S. calospora</i>	45.0±1.29 ^c	125.0	40±2.30 ^b	233.3	35±2.00 ^a	191.7
T ₃	<i>Acaulospora</i> sp	47.5±1.37 ^b	137.5	35±2.02 ^c	191.7	28±1.61 ^b	133.3
T ₄	<i>Scutellospora</i> sp.	37.5±1.08 ^d	87.5	20±1.15 ^f	66.7	25±1.44 ^c	108.3
T ₅	<i>Glomus mosseae</i>	35.0±1.01 ^e	75.0	40±2.33 ^b	233.3	24±1.38 ^d	100.0
T ₆	<i>Sclerocystis</i> sp.	25.0±0.72 ^h	25.0	25±1.44 ^e	108.3	18±1.04 ^f	50.0
T ₇	<i>Glomus geosporum</i>	37.0±1.08 ^d	87.0	30±1.73 ^d	150.0	18±1.05 ^f	50.0
T ₈	<i>Glomus aggregatum</i>	27.5±0.79 ^g	37.5	15±0.88 ^g	25.0	18±1.06 ^f	50.0
T ₉	<i>Gigaspora</i> sp.	32.5±0.93 ^f	62.5	25±1.44 ^e	108.3	20±1.15 ^c	66.7
T ₁₀	Control	20.0±0.57 ⁱ		12±1.70 ^h		12±0.70 ^g	
	S.Ed	3.44		2.76		2.19	
	CD (0.05)	7.18		5.76		4.57	

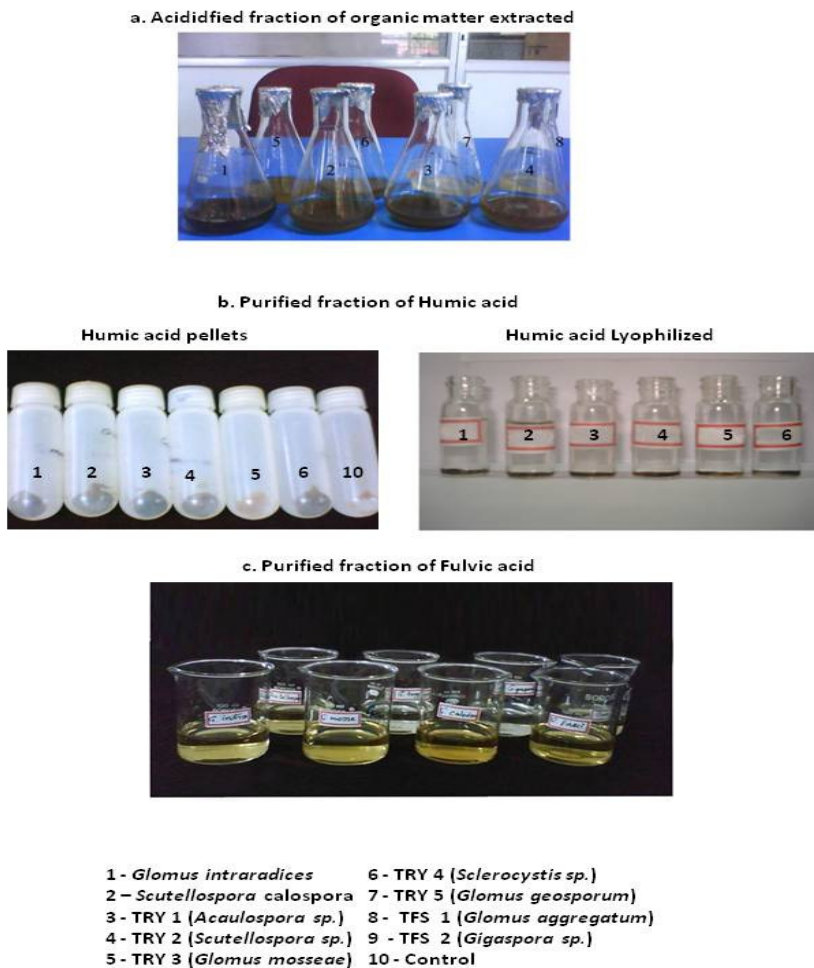


Figure-4

Extraction and purification of organic carbon fractions from sodic soil under 24 weeks continuous culture

Table-5
Effect of AM fungal isolates on Bradford reactive soil protein (BRSP) of Glomalin in post harvest soil

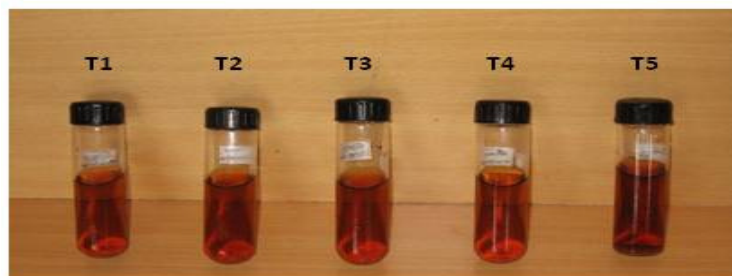
Treatments	Culture	BRSP (μg of protein g^{-1} of soil)				Water stable aggregates (%)	Per cent increase over control
		Total Extractable Glomalin	Per cent increase over control	Easily Extractable Glomalin	Per cent increase over control		
T ₁	<i>G. intraradices</i>	62±0.72 ^a	520.0	29±0.33 ^a	163.6	53.03±3.06	234.8
T ₂	<i>S. calospora</i>	59±0.68 ^b	490.0	28±0.32 ^b	154.5	50.14±2.90	216.5
T ₃	<i>Acaulospora</i> sp	49±0.57 ^c	390.0	22±0.25 ^d	100.0	52.60±3.03	232.1
T ₄	<i>Scutellospora</i> sp.	46±0.53 ^d	360.0	22±0.25 ^d	100.0	35.56±2.05	124.5
T ₅	<i>Glomus mosseae</i>	43±0.50 ^e	330.0	25±0.29 ^c	127.3	57.87±3.34	265.3
T ₆	<i>Sclerocystis</i> sp.	40±0.46 ^f	300.0	20±0.23 ^c	81.8	34.14±1.97	115.5
T ₇	<i>Glomus geosporum</i>	32±0.37 ^h	220.0	20±0.23 ^c	81.8	29.90±1.72	88.7
T ₈	<i>Glomus aggregatum</i>	35±0.40 ^g	250.0	19±0.22 ^f	72.7	24.70±1.42	55.9
T ₉	<i>Gigaspora</i> sp.	31±0.36 ⁱ	210.0	18±0.21 ^g	63.6	20.46±1.20	29.2
T ₁₀	Control	10±0.11 ^j		11±0.13 ^h		15.84±0.91	
	S.Ed	3.873		1.972		3.60	
	CD (0.05)	8.080		4.115		7.50	

Table-6
a. Correlation analysis between the soil organic carbon pools

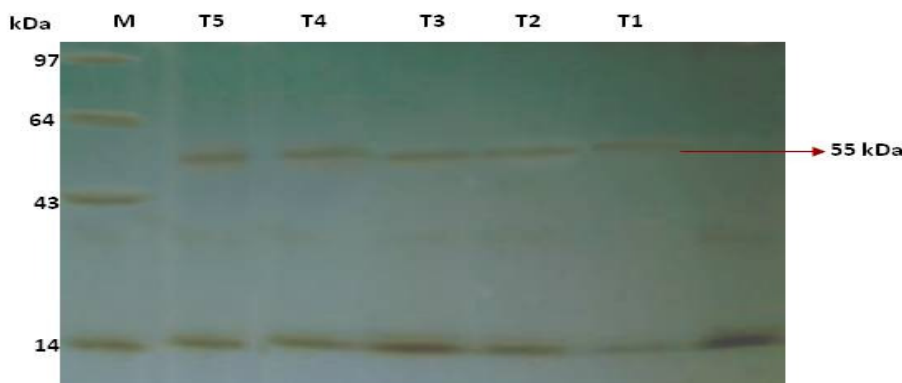
Correlation	SOC	POM	HA	FA	TG	WSC
SOC	1	0.759	0.682	0.778	0.793	0.732
POM		1	0.831	0.907	0.846	0.781
HA			1	0.825	0.795	0.811
FA				1	0.943	0.868
TG					1	0.896
WSC						1

OC- soil organic carbon (%); POM – particulate organic matter (mg); HA – Humic acid (mg); FA – Fulvic acid (mg); TG – total glomalin (µg of protein); WSA – Water Soluble Carbohydrate (mg).

a. Total glomalin extracted (Crude)



b. SDS PAGE profile of purified total glomalin (TEG)



Lane
 T1 - *Glomus intraradices*
 T2 - *Scutellospora* sp.
 T3 - TRY 1 (*Acaulospora* sp.)
 T4 - TRY 2 (*Scutellospora* sp.)
 T5 – TRY 3 (*Glomus mosseae*)

Figure-5
Total glomalin (TEG) extracted from soil under 24 weeks continuous culture

Percent Water Stable Aggregates (WSA): In the present study, soil under T₅ held the maximum aggregate stability of 57.87 % and this result was obtained similar to a study²⁸ that registered 400 % increase in soil aggregation by *Glomus mosseae* in a silt loam soil. The present study also showed increased soil organic matter content due to mycorrhizal

inoculation and this proved that this fungus influenced the carbon allocation between the plant and the soil appeared to gain carbon at the expense of carbon lost by the plant. The aggregate stability of the experimented soil samples varied widely ranging from 20.4 to 57.8 % where all the treatments significantly influenced the water stable aggregates. The

treatments inoculated with T₅ and T₁ showed significant influence on aggregation, 57.87±3.34 and 53.03±3.06 % respectively table-5 and figure-6. This promotion in soil aggregation due to AM fungal inoculations is due to the production of the glomalin related soil proteins in the rhizosphere which glues the soil particles together and lead to build up of aggregate particles and with more stability due to the undisturbed condition that prevailed over 24 weeks time in the pots.

Correlation analysis: Among the parameters analysed in the 24 weeks continuous culture study, a correlation analysis was worked out within the carbon fractions and glomalin (table-6a) as well as with aggregate stability Table-6b. Noticeably positive correlations were observed and the reasons are discussed below.

Table-6

b. Correlation analysis of soil parameters with aggregate stability

S.No	Hyphal biomass	Soil organic carbon	Glomalin content	Iron content	Microbial population
Aggregate stability	0.836	0.635	0.865	0.777	0.788

Level of significance – 5%

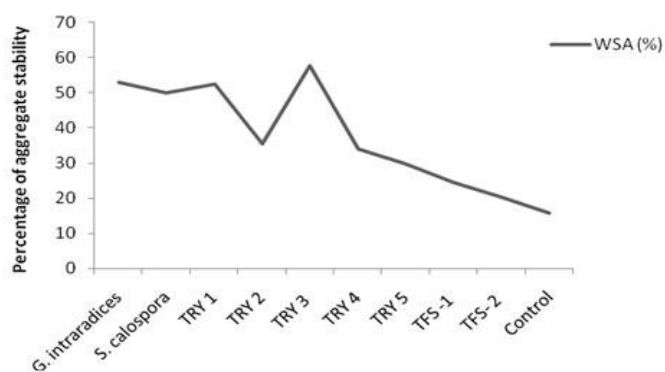


Figure-6

Effect of AM fungal isolates on aggregate stability in post harvest soil

Hyphal biomass vs. aggregate stability: The hyphal biomass yielded through compartmentalized system has showed a strong positive correlation with that of aggregate stability (r=0.836) and the supportive literatures are cited here. Both plant roots and fungal hyphae may initiate aggregate formation by enmeshing or cross-linking organic matter and coarse fragments, acting as nucleation sites and/or by supplying substrates to the microbial community. Significant correlations existed between hyphal biomass of the AM fungi (total hyphal length and hyphal density) with mean diameter of the aggregates, glomalin and aggregate stability²⁹. The different ability of AM fungal isolates to influence glomalin concentration and to form extensive and dense mycelial networks are known to be involved in the binding of micro aggregates with macro aggregates which may directly affect soil

aggregates stability suggests the possibility of selecting the most efficient isolates to be utilized for soil quality improvement and land restoration programs.

Glomalin vs. aggregate stability: In the present study, TEG and aggregate stability showed a strong positive correlation (r=0.832) and this result is supported by various phenomenon and some previous studies. Glomalin in soil being hydrophobic forms a conglomeration with root fragments and organic matter and its recalcitrant nature helps to stabilize aggregates, which in turn help to physically protect organic matter within aggregates from degradation and appears to be highly correlated with stability of the aggregates. This showed a positive correlation between the GRSP concentration and soil aggregate stability.

Iron content vs. aggregate stability: In this study, iron content in soil correlated highly (r=0.889) with glomalin content and with aggregate stability (r = 0.777) and this is in line with a report³⁰ proved that the changes in Fe percentage were significantly correlated with the changes in glomalin weight and carbon %. 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. These organo-mineral complexes are important in the long-term stability of aggregates and may help native and agricultural soils sequester carbon. Within aggregates, the formation of clay-metal-humic linkages (where O-containing hydrophilic groups in humic substances orient the hydrophobic moiety to the aggregate surface) creates a water-repellent coating on aggregates³¹.

Microbial population vs. aggregate stability: Total microbial population in the rhizosphere was positively correlated with soil aggregation (r=0.788). The rhizosphere hosts a large population of micro- and macro-organisms that contribute to soil organic matter content which indirectly provides energy for microorganisms and directly the products produced by microbial degradation act as binding agents in aggregate formation. Chemically, roots enhance aggregation by releasing a variety of compounds, which have a cementing effect on soil particles. Root mucilage such as polygalacturonic acid may stabilize aggregates by increasing bond strength and decreasing wetting rate. Roots can also alter the ionic and osmotic balance in the rhizosphere through nutrient uptake and rhizodeposition, which can affect aggregation.

Conclusion

AM fungi play important roles in crop rhizosphere through their spread of extraradical mycelium and with the help of the glomalin related soil proteins secreted, they aggregate the soil particles marking a positive sign of soil health. In the present study, the AM inoculations have shown good correlations of the GRSP with the organic pool in the rhizosphere that had a positive influence ad relative stability on soil aggregation.

Among the treatments, the isolates of sodic soils especially *Glomus mosseae* (T₃) and *Glomus geosporum* (T₅) have registered the next best and sometimes performed on par with the standard cultures. These underline the fact that the native isolates from the sodic soil which used for the experiment, have remarkable potential and can even compete with the standard isolates in improving soil aggregation, when multiplied and exploited appropriately.

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