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Combined Effect of Arbuscular Mycorrhiza, *Rhizobium* and *Sclerotium rolfsii* on Grass Pea (*Lathyrus sativus*)

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Abstract
The present study was carried out to evaluate the combined effect of indigenous arbuscular mycorrhizal (AM) fungi, *Rhizobium* and *Sclerotium rolfsii* on Grasspea (*Lathyrus sativus*) in the net house of Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during 2014-2015 through 2015-2016. The experiment was designed in RCBD with 8 treatments and 4 replications. Grasspea variety BARI Khesari-1 was used as a test crop. Peat based rhizobial inoculum (BARI RLs-10) was used in this experiment @ 50 g kg^{-1} seed. The AM fungi used in this experiment were *Glomus fusianum, Glomus macrocarpum, Glomus warcuppi, Acaulospora foveata, Acaulospora denticulate, Gigaspora albida, Gigaspora rosea, Glomus* spp. etc. Soil based AM inoculum containing about approximate 252 spores and infected root pieces of the host plant was used pot^{-1}. There were eight treatments viz. T_1: Arbuscular mycorrhiza (AM), T_2: *Rhizobium*, T_3: AM + *Rhizobium*, T_4: *Sclerotium rolfsii*, T_5: *Sclerotium rolfsii* + AM, T_6: *Sclerotium rolfsii* + *Rhizobium*, T_7: *Sclerotium rolfsii* + AM + *Rhizobium* and T_8: Control. Dual inoculation (AM + *Rhizobium*) significantly increased germination (%), nodule number and dry weight, root colonization by AM fungi and spore population in rhizosphere soils of grasspea compared to single inoculation or any other treatments. Dual inoculation increased germination after 23 DAS (20% in 2014-2015 and 23% in 2015-2016) compared to control. It increased nodule number $plant^{-1}$ (172% in 2014-2015 and 72% in 2015-2016) over AM treatment, and (112% in 2014-2015 and 26% in 2015-2016) over *Rhizobium* treatment. It also increased root infection (20% in 2014-2015 and 56% in 2015-2016) over AM treatment, and (200% in 2014-2015 and 100% in 2015-2016) over *Rhizobium* treatment. It reduced foot and root rot disease (48% in 2014-2015 and 44% in 2015-2016) compared to control. On the contrary, *Sclerotium rolfsii* + *Rhizobium*, *Sclerotium rolfsii* + AM, and *Sclerotium rolfsii* + AM + *Rhizobium* reduced 12-17%, 16-20% and 28-31% foot and root rot disease, respectively compared to only *Sclerotium rolfsii* treatment. Therefore, use of these bio-control agents could be promoted as an active component of bio-intensive Integrated Disease Management Program (IDMP) under organic mode.

Keywords: Single and dual inoculation, diseases, germination percent, nodule number, *Lathyrus sativus*.

1. Introduction

Lathyrus sativus L., commonly known as grasspea, is an annual plant widely grown as a pulse crop and its dried seeds are harvested and consumed as a human food since ancient times (Tamburino *et al*., 2012). It belongs to the family Fabaceae and is considered as a highest protein content pulse crop in Bangladesh. In 2013-2014,

about 111,498 ha of land are under grasspea cultivation and the total production is about 110,197 metric tons and in 2014-2015, about 112,397 ha of land is under grasspea cultivation and the total production is about 120,714 metric tons (BBS, 2015).

Arbuscular mycorrhizal (AM) symbiosis, formed by most angiosperms and fungi of the order Glomeromycota, occurs widely in terrestrial ecosystems, where it plays a significant role in plant phosphorus nutrition and the carbon cycle and consequently impacts ecosystem productivity (Smith and Read, 2008). Most terrestrial plants form symbiosis with one or more kinds of mycorrhizal fungi, of which 80% of plant species are being associated with AM fungi (Smith and Smith, 2011). AM fungi can contribute to plant growth by enhancing water and nutrient uptake, especially phosphorus (Watts-Williams *et al*., 2014). It may also contribute to soil fertility by enhancing soil structure and protecting crops from root pathogens (Sharma *et al*., 2013). AM fungi are the major component of the rhizosphere of most of the plants and play a very important role as bio control agent and help in decreasing plant disease incidence (Akthar and Siddiqui, 2008). In addition, biocontrol potential of AM fungi against various phytopathogens is well documented (St-Arnaud and Vujanovic, 2007; Xavier and Boyetchko, 2014).

Rhizobium biofertilizer is a significant technology for improving crop productivity and soil fertility because we can use it as a replacement of nitrogenous fertilizer that is not only economically feasible but also environmentally sustainable. It improved nodulation and as well as nitrogen fixation even under adverse soil conditions. Several studies are available on the interaction between AM fungi and rhizobia for chickpea (Solaiman *et al*., 2005), pea (Geneva *et al*., 2006) and pigeon pea (Bhattacharjee and Sharma, 2012).

Foot and root rot caused by *Sclerotium rolfsii* is considered as an important and destructive disease of pulses in almost all legume-growing countries of the world. It causes seedling death at early stage resulting very poor plant stand which ultimately produces very low yield. Though this disease can be controlled by using chemical pesticide but it causes environmental pollution, health hazards and also is not economical. Hence, biological control agents like AM fungi and *Rhizobium* can be used for green, safe and sustainable agriculture. A synergistic effect of dual inoculation with AM fungi and rhizobia might have on growth and nutrition in pulses. Mycorrhizal infection might help grasspea to obtain the required phosphorus for nodulation.

Keeping in view the above information, present investigation was undertaken to investigate the potential of AM fungi alone and in combination with bio inoculants i.e. *Rhizobium* to find out the best combination on germination, nodulation, and sporulation of grasspea in a given soil condition and as well as their bio control against grasspea foot and root rot disease caused by *Sclerotium rolfsii*.

2. Materials and Methods

2.1 Seed collection and Soil preparation

The pot experiment was carried out during rabi season from December, 2014 to April, 2015 and December, 2015 to April, 2016 in the net house of Soil Science Division, BARI, Joydebpur, Gazipur (23° 59'38" N latitude, 90° 24'89" E longitude and 8.4 m elevation). Seeds of grasspea (BARI Khesari-1) were collected from Pulse Research Centre, BARI, Gazipur. The silted (sandy clay loam) soils were collected from the bank of Turag river at Kodda, Gazipur mixed with cowdung at 5:1 ratio and was used as the potting media. Each pot (25 cm in diameter and 21 cm in height) was filled with approximately 6-kg soil leaving upper 3 inches of pot was vacant to facilitate watering. The pH of cowdung was 6.7 and the nutrient contents were: organic matter 14.1%, N 0.8%, P 1.26%, K 0.88%, Ca 1.55%, Mg 0.82%, S 0.62%, Fe 0.25% and Mn 0.112%. The physical and chemical properties of the soil are presented in

Table 1. The soil contained 12 AM (100^{-1} g soil) spores of indigenous mixed AM fungal species. The soil and cowdung was autoclaved for minimizing the chance of any contamination.

2.2 Soil analysis

Soil pH was measured by a combined glass calomel electrode (Jackson, 1958). Organic carbon was determined by Wet Oxidation Method (Walkley and Black, 1934). Total N was determined by modified Kjeldahl method (Jackson, 1962). Calcium, K and Mg were determined by NH_4OAc extraction method (Black, 1965). Copper, Fe, Mn and Zn were determined by DTPA extraction followed by AAS reading. Boron was determined by $CaCl_2$ extraction method. Phosphorus was determined by Modified Olsen method (Neutral + Calcareous soils). Sulphur was determined by $CaH_4(PO_4)_2$. H_2O extraction followed by turbidimetric turbidity method with $BaCl_2$.

2.3 Fertilizer application

Chemical fertilizers @ 6.3 mg P: 9.5 mg K: 1.002 mg S kg^{-1} soil were applied (BARC, 2012). Phosphatic fertilizer (TSP), Potassic fertilizer (MoP) and Sulphatic fertilizer (Gypsum) were used as a source of P, K and S, respectively. All fertilizers were applied as basal during final land preparation. Peat based rhizobial inoculum (BARI RLs-10) was used in this experiment @ 50 g kg^{-1} seed.

2.4 Collection of pathogen Sclerotium rolfsii and Rhizobium inoculum

Pathogen *Sclerotium rolfsii* were collected from Plant Pathology Division, BARI, Gazipur which was grown on non seed barley. Non seed barley collected from Plant Breeding Division, BARI, Gazipur. Pathogen *Sclerotium rolfsii* along with non seed barley 50 g was used per *Sclerotium* treatment pot. After disease development, pathogen sclerotia mixed with soil. *Rhizobium* strain BARI RLs-10 were collected from Soil Microbiology Laboratory, BARI, Gazipur and mixed properly with the seed before sowing when necessary.

2.5 Preparation of mycorrhizal inoculum

The arbuscular mycorrhizal inoculum was prepared from the roots and rhizosphere soils of sorghum. Mycorrhizal species was originally isolated from different AEZ region, using the wet sieving and decanting method. The spores were left to multiply for 6 months on sorghum plants using unsterilized soil, collected from the same site, in the net house of Soil Science Division, BARI. Plants were irrigated with tap water as needed. A mixture of infected sorghum root and soil which contained spores was used as mycorrhizal inoculum. The AM fungi used in this experiment were *Glomus fusianum, Glomus macrocarpum, Glomus warcuppi, Acaulospora foveata, Acaulospora denticulate, Gigaspora albida, Gigaspora rosea, Glomus* spp. etc. The soil based AM fungal inoculum containing 120 g of rhizosphere soil (approximate 209.67 ± 5.5 spores/100 g soil) and infected sorghum root fragments with a minimum infection level was inoculated to each mycorrhizal pot. The mycorrhizal inoculum were first placed in each pot at 3-5 cm depth and was covered with a thin soil layer of 1 cm immediately prior to the seed sowing of grasspea to facilitate fungal colonization of plant roots.

2.6 Identification of AM fungal spore

For the identification of AM fungal spore, single spore or sporocarps were easily picked up from the filter paper with the help of syringe or fine point camel brush and mounted on a glass slide with a drop of polyvinyl lactophenol (PVL) and a cover slip was placed. Subsequently, recovered spores were identified with the help of manual and different taxonomic keys proposed by different workers. Spore morphology, size, shape and peridium of spore, sporocarps colour, wall ornamentation, subtending hyphae and mode of attachment are considered for identification of spore or sporocarps.

2.7 Design of experiment and treatments

The experiment was designed in RCBD with 8 treatments and 4 replications. Fifteen seeds were sown in each pot at 1 cm soil depth. The 8 treatments were: T_1: Arbuscular mycorrhiza

(AM), T_2: *Rhizobium*, T_3: AM + *Rhizobium*, T_4: *Sclerotium rolfsii*, T_5: *Sclerotium rolfsii* + AM, T_6: *Sclerotium rolfsii* + *Rhizobium*, T_7: *Sclerotium rolfsii* + AM + *Rhizobium* and T_8: Control.

2.8 Determination of germination percentage

The germination test was carried out according to ISTA rules (ISTA, 1976). For each treatment, 100 seeds were put into Petri dishes. The Petri dishes were put on a laboratory table at room temperature (25 ± 2°C). After 8 days, normal, abnormal and diseased seeds were counted. Germination of grasspea seed in the laboratory table was 95%. Fifteen seeds were sown in each pot. After 11, 15, 19 and 23 days germinated seeds were observed and counted. Germination percentage was calculated by the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds in each pot}}{\text{Total number of seeds sown in each pot}} \times 100$$

2.9 Determination of nodule number, nodule weight and root infection (%)

Three plants were harvested after 82 days after sowing ie. at the time of 50% flowering stage for collecting nodule number, nodule weight and root infection parameters according to Khanam *et al.* (2005).

2.10 Determination of pre and post-emergence foot and root rot (%)

Pre-emergence foot and root rot was calculated at 11 days after sowing (DAS) and post-emergence foot and root rot was calculated at 11, 15, 19 and 23 DAS by the following formula:

$$P_1\ (\%) = \frac{N_1}{G_1} \times 100$$

$$P_2\ (\%) = \frac{N_2}{G_2} \times 100$$

Where,

P_1 = Pre -emergence foot and root rot

P_2 = Post -emergence foot and root rot

N_1 = Number of non-germinated seeds in each pot at 11 DAS

G_1 = Total number of seeds sown in each pot

N_2 = Number of abnormal or disease infected or dead seedlings in each pot at 23 DAS

G_2 = Total number of seedlings present in each pot at 11 DAS

2.11 Plant harvest

Grasspea were harvested after 132 days after sowing.

2.12 Assessment of spore population density at rhizosphere soil of grasspea

Assessment of spore population was done following the Wet Sieving and Decanting Method (Gerdemann and Nicolson, 1963). Soil samples from the rhizosphere of the respective plant species were mixed thoroughly by breaking up any large lumps. Any large unwanted particles such as stone, roots, twigs etc. were removed from the soil. Then 100 g of mixed soil was kept in a bucket (8 litres) and filled three quarters with tap water. The soil with water was agitated by stirring vigorously by hand and washed into the bucket and left to settle for one minute. The suspension was sieved by following the wet sieving and decanting method (Gerdemann and Nicolson, 1963). Two sieves (400 µm and 100 µm mesh) were used throughout the experiment. The supernatant was poured through a 100 µm sieve into the second bucked (10 litres) to avoid the loss of useful materials. After allowing the suspension to settle for one minute, the supernatant was decanted into the 400 µm sieve. This time water was discarded and the material was back washed from the sieve into a beaker (250 mL) with a small quantity of water. The solution with spores was distributed in 4 equal size test tubes evenly and balanced up the tubes with water for equal weight. The tubes were plugged properly and then centrifuged for 4 minutes at 3,000 rpm. The supernatant was poured in test tubes and the test tubes were filled with sucrose solution and stirred vigorously with the round-ended spatula to re-suspend the precipitate. The test tubes were

balanced properly to equal weight and they were plugged. Then the plugged test tubes were centrifuged for 15 seconds at 3,000 rpm. After centrifuge, the sucrose supernatant was poured through a 400 µm sieve and rapidly washed with water to remove the sucrose from AM spores by back washing the materials from the sieve into watch glass for observation.

All the AM spores were isolated from the extract with the help of a fine forcep into a watch glass with small quantity of water. The extract, with AM spores, was observed under stereomicroscope and the number of spores was counted. Spore numbers from the three replicates per samples were averaged and the result was expressed as number per 100 g of dry soil basis.

2.13 Assessment of root colonization infection

The percentage of AM infection was estimated by root slide technique (Read *et al*., 1976). One hundred root segments were examined for each sample. The stained root pieces were mounted in acidic glycerol on slides and the cover slip was placed and slightly pressed. The roots were observed under microscope. A root segment was considered as positively infected, if it showed mycelium, vesicles and arbuscules or any other combination of these structural characteristics of AM infection. The presence or absence of infection in the root pieces was recorded and the percent infection was calculated as follows:

$$\% \text{ root colonization} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments scored}} \times 100$$

2.14 Statistical analysis

Data were statistically analyzed using Analysis of Variance (ANOVA) following Statistix 10 package.

3. Results and Discussion

3.1. Germination (%)

Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on germination (%) of grasspea have been presented in Table 2 and Figure 1. Significant differences were found in case of germination (%) at 11, 15, 19 and 23 DAS in both of the years.

The highest germination (78.33, 80.00, 80.00 and 80.00%) at 11, 15, 19 and 23 days after sowing (DAS) respectively in 2014-2015 and (75.00, 76.67, 78.33 and 80.00%) at 11, 15, 19 and 23 days after sowing (DAS) respectively in 2015-2016 were observed in AM + *Rhizobium* treatment (Table 2 and Figure 1). The lowest germination (26.67, 30.00, 30.00 and 21.67%) at 11, 15, 19 and 23 DAS respectively in 2014-2015 and (6.67, 6.67, 3.33 and 5.00%) at 11, 15, 19 and 23 DAS respectively in 2015-2016 were observed in *Sclerotium* treatment (Table 2 and Figure 1). The highest germination at 11, 15 and 19 DAS in 2014-2015 was found in AM + *Rhizobium* treatment which was significantly higher over *Rhizobium*, *Sclerotium*, *Sclerotium* + AM, *Sclerotium* + *Rhizobium*, *Sclerotium* + AM + *Rhizobium* and control treatments but identical to AM treatment while the highest germination at 11, 19 and 23 DAS in 2015-2016 was found in AM + *Rhizobium* treatment which was significantly higher over all other treatments. The highest germination at 23 DAS in 2014-2015 was found in AM + *Rhizobium* treatment which was significantly higher over all other treatments but identical to AM and *Rhizobium* treatments while the highest germination at 15 DAS in 2015-2016 was found in AM + *Rhizobium* treatment which was significantly higher over all other treatments but identical to AM treatment.

3.2. Plant dry weight, nodule number, nodule weight, root colonization by AM fungi and spore population

Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on plant dry weight, nodule number, nodule weight (dry), root colonization by AM fungi and spore population in rhizosphere soils of grasspea have been presented in Table 3. Figure 2 represents effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on nodule number of grasspea. Figure 3

represents effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on root colonization by AM fungi and Figure 4 showing different structure of AM fungi found in the rhizosphere soils and root cortex of grasspea. Significant differences were found in case of plant dry weight, nodule number, nodule weight (dry), root colonization (%) and spore population/100 g soil.

The highest plant dry weight (1.87 g plant^{-1}), nodule number (33.35 plant^{-1}), nodule weight (42.69 mg plant^{-1}), root colonization (30.00%) and spore population/100 g soil (101.17) in 2014-2015 and plant dry weight (1.95 g plant^{-1}), nodule number (27.84 plant^{-1}), nodule weight (35.63 mg plant^{-1}), root colonization (46.67%) and spore population/100 g soil (130.00) in 2015-2016 were observed in AM + *Rhizobium* treatment (Table 3 and Figure 3). The lowest plant dry weight (1.36 g plant^{-1}), nodule number (1.00 plant^{-1}), nodule weight (1.28 mg plant^{-1}), root colonization (05.00%) and spore population/100 g soil (56.17) in 2014-2015 and plant dry weight (1.14 g plant^{-1}), nodule number (2.00 plant^{-1}), nodule weight (2.56 mg plant^{-1}), root colonization (08.34%) and spore population/100 g soil (60.00) in 2015-2016 were observed in *Sclerotium* treatment (Table 3 and Figure 3).

Table 1. Initial fertility status of the soil samples

Soil Properties	Texture	pH	OM (%)	Ca	Mg	K	Total N (%)	P	S	B	Cu	Fe	Mn	Zn
				meq 100 g^{-1}				µg g^{-1}						
Result	Sandy clay loam	7.6	0.32	6.6	2.3	0.09	0.017	12	25	0.10	1.0	14	1.3	0.85
Critical level	-	-	-	2.0	0.5	0.12	-	10	10	0.20	0.2	4.0	1.0	0.60

Table 2. Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on germination (%) of grasspea

Treatments	Germination (%)					
	11 DAS		15 DAS		19 DAS	
	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
AM	73.33a	66.67b	78.33a	68.33ab	75.00ab	68.33b
Rhizobium	66.67b	56.67c	73.33b	65.00b	70.00bc	65.00b
AM + *Rhizobium*	78.33a	75.00a	80.00a	76.67a	80.00a	78.33a
Sclerotium	26.67e	6.67f	30.00f	6.67f	30.00f	3.33f
Sclerotium + AM	41.67d	25.00e	36.67e	30.00d	38.33e	31.67d
Sclerotium + *Rhi.*	31.67e	21.67e	33.33ef	20.00e	33.33ef	20.00e
Scle. + AM + *Rhi.*	53.33c	43.33d	51.67d	43.33c	53.33d	41.67c
Control	58.33c	55.00c	65.00c	63.33b	65.00c	63.33b
SE (±)	1.96	2.51	1.63	2.94	2.74	2.66
F test	**	**	**	**	**	**
CV (%)	7.33	11.46	5.82	12.60	9.84	11.46

AM: Arbuscular mycorrhiza; *Rhi.: Rhizobium; Scle.: Sclerotium rolfsii*. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01

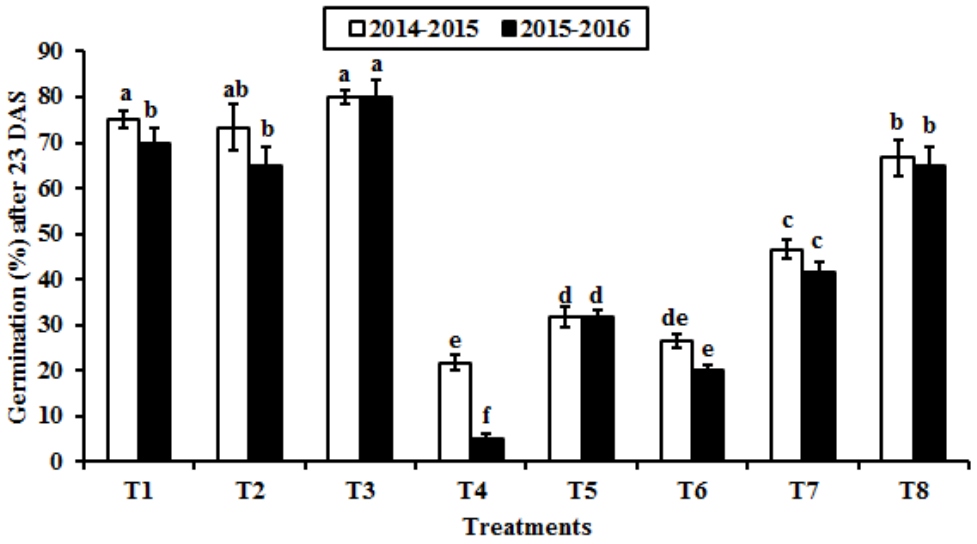

Figure 1. Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on germination (%) after 23 DAS of grasspea. T_1: Arbuscular mycorrhiza (AM), T_2: *Rhizobium*, T_3: AM + *Rhizobium*, T_4: *Sclerotium rolfsii*, T_5: *Sclerotium rolfsii* + AM, T_6: *Sclerotium rolfsii* + *Rhizobium*, T_7: *Sclerotium rolfsii* + AM + *Rhizobium* and T_8: Control

Table 3. Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on plant dry weight, nodule number, nodule weight and spore population in rhizosphere soils of grasspea

Treatments	Plant dry weight (g plant^{-1})		Nodule number plant^{-1}		Nodule weight (mg plant^{-1})		Spore population (100 g^{-1} soil)	
	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
AM	1.66abc	1.55c	12.27cd	15.17e	15.70d	19.41e	91.33b	118.00ab
Rhizobium	1.74abc	1.60bc	15.70c	20.67c	21.35c	26.46c	84.67c	106.00b
AM + *Rhizobium*	1.87a	1.95a	33.35a	26.09a	42.69a	35.63a	101.17a	130.00a
Sclerotium	1.36d	1.14d	1.00e	2.00h	1.28f	2.56h	56.17f	60.00d
Sclerotium + AM	1.64abc	1.54c	9.25d	11.75f	11.09e	15.04f	84.33c	104.00b
Sclerotium + *Rhi.*	1.62bc	1.38cd	13.50c	17.50d	18.23cd	22.40d	77.33d	84.00c
Scle. + AM + *Rhi.*	1.83ab	1.83ab	22.75b	24.00b	29.12b	30.72b	92.33b	108.50b
Control	1.51cd	1.46c	1.50e	4.46g	1.92f	5.71g	69.67e	103.50b
SE (±)	0.18	0.08	1.22	0.77	1.10	0.99	1.82	4.95
F test	**	**	**	**	**	**	**	**
CV (%)	9.66	10.60	17.80	8.29	12.81	9.74	4.44	9.74

AM: Arbuscular mycorrhiza; *Rhi.: Rhizobium; Scle.: Sclerotium rolfsii.* The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01

Figure 2. Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on nodule number of grasspea

The highest plant dry weight in 2014-2015 was found in AM + *Rhizobium* treatment which was significantly higher over *Sclerotium*, *Sclerotium* + *Rhizobium* and control treatments but identical to rest of the treatments while the highest plant dry weight in 2015-2016 was found in AM + *Rhizobium* treatment which was significantly higher over all other treatments but identical to *Sclerotium* + AM + *Rhizobium* treatment. The highest nodule number, nodule weight, root colonization and spore population/100 g soil in 2014-2015 were observed in AM + *Rhizobium* treatment which was significantly higher over all the treatments. In contrast, the highest nodule number, nodule weight and root colonization in 2015-2016 were observed in AM + *Rhizobium* treatment which was significantly higher over all the treatments and the highest spore population were observed in AM + *Rhizobium* treatment which was identical to AM treatment but significantly different from rest of the treatments.

Associative action of mycorrhizal fungi in legumes has a great impact on root and shoots development and plant dry weight which results in the enhancement of nodulation and nitrogen fixation. Dual inoculation produced significantly higher nodule number as compared to single inoculation. The results were in good agreement with Geneva *et al*. (2006) who reported that dual inoculation of pea plants significantly increased

the plant biomass, photosynthetic rate, nodulation, and nitrogen fixation activity in comparison with single inoculation with *Rhizobium leguminosarum.* Hernandez and Hernandez (1996) recorded significantly increased nodule number and nodule weight of soybean at flowering stage with AM and *Rhizobium* inoculation. Khanam *et al*. (2005) reported higher percentage of root colonization and spore population at 50% flowering stage in chickpea by dual inoculation. Higher percentage of root colonization and spore population were found in dual inoculation treatment. This result was supported by Khanam *et al*. (2005) and Geneva *et al*. (2006).

3.3. Foot and root rot disease infection with Sclerotium rolfsii in grasspea seedlings

Effect of dual inoculation of AM and *Rhizobium* on foot and root rot disease infection with *Sclerotium rolfsii* in grasspea seedlings have been presented in Table 4 and Figure 5. Significant differences were found in case of pre-emergence foot and root rot (%) and post-emergence foot and root rot (%).

The highest pre-emergence foot and root rot (73.33%), total post-emergence foot and root rot (13.33%) and highest pre + post emergence foot and root rot (83.33%) in 2014-2015 were observed in *Sclerotium*, *Sclerotium* + AM + *Rhizobium* and *Sclerotium* treatment, respectively. In contrast, the highest pre-emergence foot and root rot (93.33%), total post-emergence foot and root rot (5.01%) and highest pre + post emergence foot and root rot (98.34%) in 2015-2016 were observed in *Sclerotium* treatment (Table 4 and Figure 5). The lowest pre-emergence foot and root rot (21.67%), total post-emergence foot and root rot (1.67%) and lowest pre + post emergence foot and root rot (23.33%) in 2014-2015 and the lowest pre-emergence foot and root rot (25.00%), total post-emergence foot and root rot (0.00%) and lowest pre + post emergence foot and root rot (25.00%) in 2015-2016 was observed in AM + *Rhizobium* treatment (Table 4 and Figure 5).

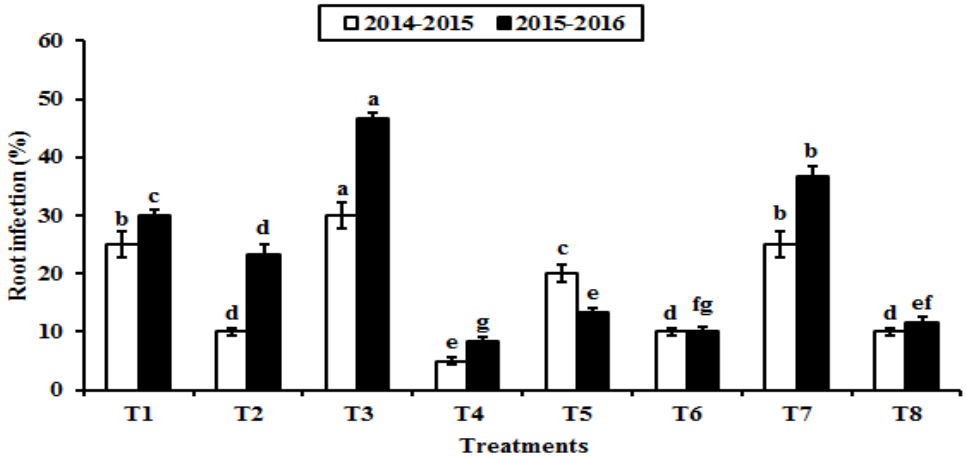

Figure 3. Effect of inoculation of AM, *Rhizobium* and *Sclerotium rolfsii* on root infection of grasspea. T_1: Arbuscular mycorrhiza (AM), T_2: *Rhizobium*, T_3: AM + *Rhizobium*, T_4: *Sclerotium rolfsii*, T_5: *Sclerotium rolfsii* + AM, T_6: *Sclerotium rolfsii* + *Rhizobium*, T_7: *Sclerotium rolfsii* + AM + *Rhizobium* and T_8: Control

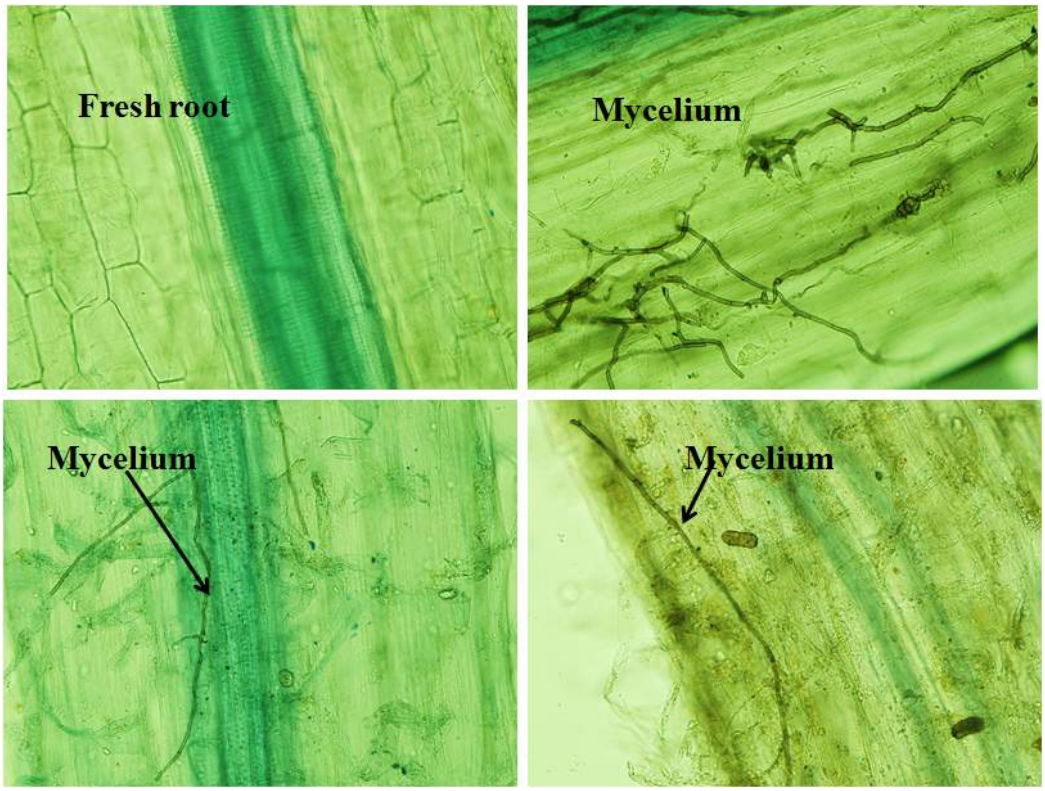

Figure 4. Different mycorrhizal structure found in the rhizosphere soils and root cortex of grasspea

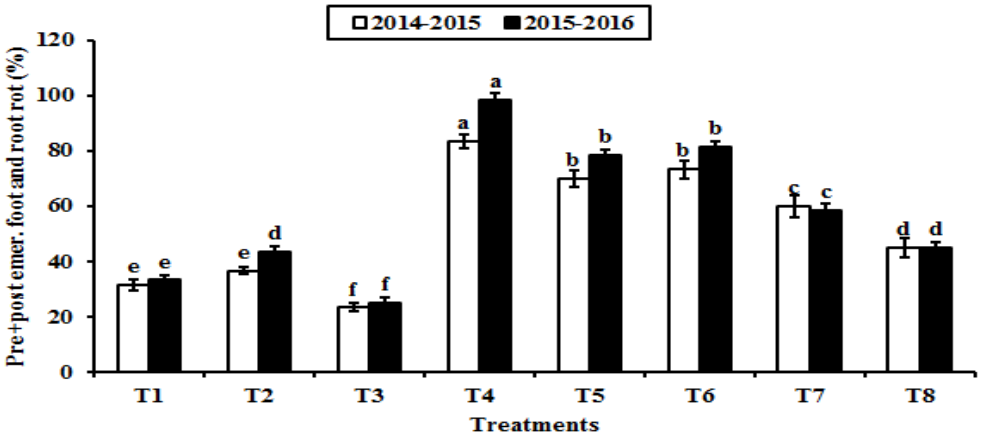

Figure 5. Effect of dual inoculation of AM and *Rhizobium* on pre + post emergence foot and root rot disease% in grasspea. T_1: Arbuscular mycorrhiza (AM), T_2: *Rhizobium*, T_3: AM + *Rhizobium*, T_4: *Sclerotium rolfsii*, T_5: *Sclerotium rolfsii* + AM, T_6: *Sclerotium rolfsii* + *Rhizobium*, T_7: *Sclerotium rolfsii* + AM + *Rhizobium* and T_8: Control

Table 4. Effect of dual inoculation of AM and *Rhizobium* on foot and root rot disease infection with *Sclerotium rolfsii* in grasspea seedlings

Treatments	Pre-emergence foot and root rot (%)	Post-emergence foot and root rot (%)				
		11 DAS	15 DAS	19 DAS	23 DAS	Total
2014-2015						
AM	26.67de	0.00	0.00c	3.33a	1.67d	5.00
Rhizobium	33.33d	0.00	0.00c	3.33a	0.00e	3.33
AM + *Rhizobium*	21.67e	0.00	0.00c	0.00c	1.67d	1.67
Sclerotium	73.33a	0.00	0.00c	1.67b	8.33a	10.00
Sclerotium + AM	58.33b	0.00	5.00a	0.00c	6.67b	11.67
Sclerotium + *Rhi.*	68.33a	0.00	0.00c	0.00c	5.00c	5.00
Scle. + AM + *Rhi.*	46.67c	0.00	3.33b	3.33a	6.67b	13.33
Control	41.67c	0.00	0.00c	1.67b	1.67d	3.33
SE (±)	2.41	-	0.14	0.15	0.32	-
F test	**	-	**	**	**	-
CV (%)	10.42	-	27.04	18.39	16.05	-
2015-2016						
AM	33.33e	0.00	0.00c	0.00c	0.00	0.00
Rhizobium	43.33d	0.00	0.00c	0.00c	0.00	0.00
AM + *Rhizobium*	25.00f	0.00	0.00c	0.00c	0.00	0.00
Sclerotium	93.33a	0.00	1.67b	3.34a	0.00	5.01
Sclerotium + AM	75.00b	0.00	3.34a	0.00c	0.00	3.34
Sclerotium + *Rhi.*	78.33b	0.00	3.34a	0.00c	0.00	3.34
Scle. + AM + *Rhi.*	56.67c	0.00	0.00c	1.67b	0.00	1.67
Control	45.00d	0.00	0.00c	0.00c	0.00	0.00
SE (±)	2.42	-	0.11	0.10	-	-
F test	**	-	**	**	-	-
CV (%)	8.59	-	21.29	32.45	-	-

AM: Arbuscular mycorrhiza; *Rhi.: Rhizobium; Scle.: Sclerotium rolfsii*. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. **Significant P≤0.01.

The highest pre-emergence foot and root rot in 2014-2015 was found in *Sclerotium* treatment which was significantly higher over AM, *Rhizobium*, *Sclerotium*, *Sclerotium* + AM, *Sclerotium* + AM + *Rhizobium* and control treatments but identical to *Sclerotium* + *Rhizobium* treatment while the highest pre-emergence foot and root rot in 2015-2016 was found in *Sclerotium* treatment which was significantly higher over all the treatments. The highest pre + post emergence foot and root rot (%) in 2014-2015 and 2015-2016 was found in *Sclerotium* treatment which was significantly higher over all the treatments.

Different studies revealed that simultaneous inoculation with AM and *Rhizobium leguminosarum* increased plant tolerance to a variety of pathogens causes seedling disease (Lynd and Ansman, 1994). Arbuscular mycorrhizal colonization may also protect host roots, especially under nutrient limitation (Graham, 2001). Larsen and Bodker (2001) found that arbuscular mycorrhizal fungi played

positive role in the areas of disease suppression. In a recent study, the presence of arbuscular mycorrhiza in pea roots is known to reduce the disease and the effect on a pathogen was measured by recording the enzymatic activity of the pathogen under influence of the AM fungus (Kjøller and Rosendahl, 1996).

4. Conclusions

The findings of this study suggest among all treatments, dual combination of AM plus *Rhizobium* significantly increased germination (%), nodule number and dry weight, root colonization by AM fungi and spore population in rhizosphere soils of grasspea compared to single inoculation or any other treatments. Dual inoculation increased germination after 23 DAS (20% in 2014-2015 and 23% in 2015-2016) compared to control. It increased nodule number plant^{-1} (172% in 2014-2015 and 72% in 2015-2016) over AM treatment, and (112% in 2014-2015 and 26% in 2015-2016) over *Rhizobium* treatment. It also increased root infection (20% in 2014-2015 and 56% in 2015-2016) over AM treatment, and (200% in 2014-2015 and 100% in 2015-2016) over *Rhizobium* treatment. Dual inoculation reduced 44-48% foot and root rot disease compared to control. On the other hand, *Sclerotium rolfsii* + *Rhizobium*, *Sclerotium rolfsii* + AM, and *Sclerotium rolfsii* + AM + *Rhizobium* reduced 12-17%, 16-20% and 28-31% foot and root rot disease, respectively compared to only *Sclerotium rolfsii* treatment. Therefore, combinations of AM fungi and *Rhizobium* were able to control foot and root rot disease of grasspea more effectively than either bio control agent applied alone which would be the important basis of sustainable agricultural systems. Interactions between these two microbial agents should be researched deeply to understand the mechanisms involved in belowground and above-ground community via plants. This combination can be further tested under field conditions and can be recommended to the farmers after proper confirmation.

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