

## Original Article

# Biocontrol of Foot and Root Rot Disease of Grasspea (*Lathyrus sativus*) by Dual Inoculation with *Rhizobium* and Arbuscular Mycorrhiza

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The present study was carried out to evaluate the effect of indigenous arbuscular mycorrhizal fungi (AMF) and *Rhizobium* (R) on plant growth and their biocontrol against grasspea foot and root rot disease caused by *Sclerotium rolfsii*. The bio-control potential of these bio-agents against foot and root rot pathogen was carried out under pot culture condition using AMF alone or in combination with rhizobial inoculum in the nethouse 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<sup>-1</sup> 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<sup>-1</sup>. There were eight treatments viz. T<sub>1</sub>: Arbuscular mycorrhiza (AM), T<sub>2</sub>: *Rhizobium*, T<sub>3</sub>: AM + *Rhizobium*, T<sub>4</sub>: *Sclerotium rolfsii*, T<sub>5</sub>: *Sclerotium rolfsii* + AM, T<sub>6</sub>: *Sclerotium rolfsii* + *Rhizobium*, T<sub>7</sub>: *Sclerotium rolfsii* + AM + *Rhizobium* and T<sub>8</sub>: Control. Dual inoculation (AM + *Rhizobium*) increased 20-25% germination, 50-100% seed yield and 36-98% stover yield compared to control. Dual inoculation reduced 44-48% foot and root rot disease 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, arbuscular mycorrhizal fungal species and its combination with rhizobial inoculum were significant both in the formation and effectiveness of AM symbiosis and the reduction of foot and root rot incidence in grasspea plants. Use of these bio-control agents could be promoted as an active component of bio-intensive Integrated Disease Management Program (IDMP) under organic mode.

**Key words:** Foot and root rot, Grasspea (*Lathyrus sativus*), *Rhizobium* and Arbuscular mycorrhiza

## Introduction

*Sclerotium rolfsii* are soil-borne pathogens causing root and foot rot of many crops that commonly occur in the tropical and sub-tropical regions of the world. Foot rot (caused by *Fusarium oxysporum* and *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 arbuscular mycorrhizal fungi and *Rhizobium* can be used for green, safe and sustainable agriculture. Arbuscular mycorrhizal fungi (AMF) that form symbiotic relationships with the roots of most terrestrial plants are known to improve the nutritional status of their host and to protect plants against several soil-borne plant pathogens<sup>1-2</sup>. The major effect of mycorrhizal fungi in undisturbed

ecosystems is to improve the growth of mycorrhizal plants compared to non-mycorrhizal plants<sup>3</sup>. It covers the root of plants so it makes protective physical barrier against diseases also<sup>4-5</sup>. Induce local and systemic resistance against pathogens using a variety of mechanisms including increased mineral nutrition, and the expression of plant genes related to resistance or direct anti-fungal effects<sup>6</sup>. AMF are currently studied as biological control agents against soil-borne diseases<sup>7</sup>. In this way the use of AMF as inoculants to benefit plant growth and health could contribute to a reduction of the inputs of pesticides and other environmentally harmful agrochemical products currently required for optimal plant growth and health<sup>8</sup>.

There are many disease management methods such as crop rotation, use of resistant varieties and chemical pesticides. However, frequent and indiscriminate use of these pesticides affects the physical, chemical and biological property of the soil. It also affects the non-target organisms and has developed

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resistance among the pathogen against these chemicals<sup>9</sup>. Biocontrol potential of AM fungi against various phytopathogens is well documented<sup>10-11</sup>. Arbuscular Mycorrhizal Fungi (AMF) are the major component of the rhizosphere of most of the plants and play a very important role as biocontrol agent and help in decreasing plant disease incidence<sup>12</sup>. *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.

*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<sup>13</sup>. It belongs to the family Fabaceae. In Bangladesh, during 2015-2016 about 113,528 hectare of land is under grasspea cultivation and the total production is about 122,408 metric tons<sup>14</sup>. At the present time, grasspea cover highest land area in terms of production among other pulses and it is also interesting that it contains highest amount of protein than other pulses that partially fulfill the protein requirement of poor livelihood in our Bangladeshi people. Keeping in mind recent world demand for sustainable agricultural development or green agriculture, biocontrol or renewable natural resources must be emphasized. The present study hypothesized that the combined use of AMF and *Rhizobium* can improve plant growth and provides protection against the pathogen *Sclerotium rolfsii* within the context of a sustainable soil-plant system. Therefore, the present investigation was undertaken to investigate the potential of AMF alone and in combination with bio inoculants i.e. *Rhizobium* to find out the best combination on plant growth, and their biocontrol against grasspea foot and root rot caused by *Sclerotium rolfsii*.

## Materials and Methods

### Seed collection and Soil preparation

The 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° 59'378" N latitude, 90° 24'886" 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 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<sup>-1</sup> g soil) spores of indigenous mixed AM fungal species and the experiment was conducted under sterilized soil condition.

### Soil analysis

Soil pH was measured by a combined glass calomel electrode<sup>15</sup>. Organic carbon was determined by Wet Oxidation Method<sup>16</sup>. Total N was determined by modified Kjeldahl method<sup>17</sup>. Calcium, K and Mg were determined by NH<sub>4</sub>OAc extraction method<sup>18</sup>. Copper, Fe, Mn and Zn were determined by DTPA extraction followed by AAS reading. Boron was determined by CaCl<sub>2</sub> extraction method. Phosphorus was determined by Modified Olsen method (Neutral + Calcareous soils) according to Olsen *et al.*<sup>19</sup>. Sulphur was determined by CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>.H<sub>2</sub>O extraction followed by turbidimetric turbidity method with BaCl<sub>2</sub>.

### Fertilizer application

Chemical fertilizers @ 6.3 mg P: 9.5 mg K: 1.002 mg S kg<sup>-1</sup> soils were applied<sup>20</sup>. 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<sup>-1</sup> seed.

### Collection of the 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.

### 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 soil based AM fungal

**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 <sup>-1</sup>								µg g <sup>-1</sup>			
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

inoculum containing approximate 252 spores and infected sorghum root fragments with a minimum infection level was inoculated to each mycorrhizal pot. Figure 1 represents different mycorrhizal spore identified in the Soil Microbiology Laboratory, Soil Science Division, BARI and used for the experiment. 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.

*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

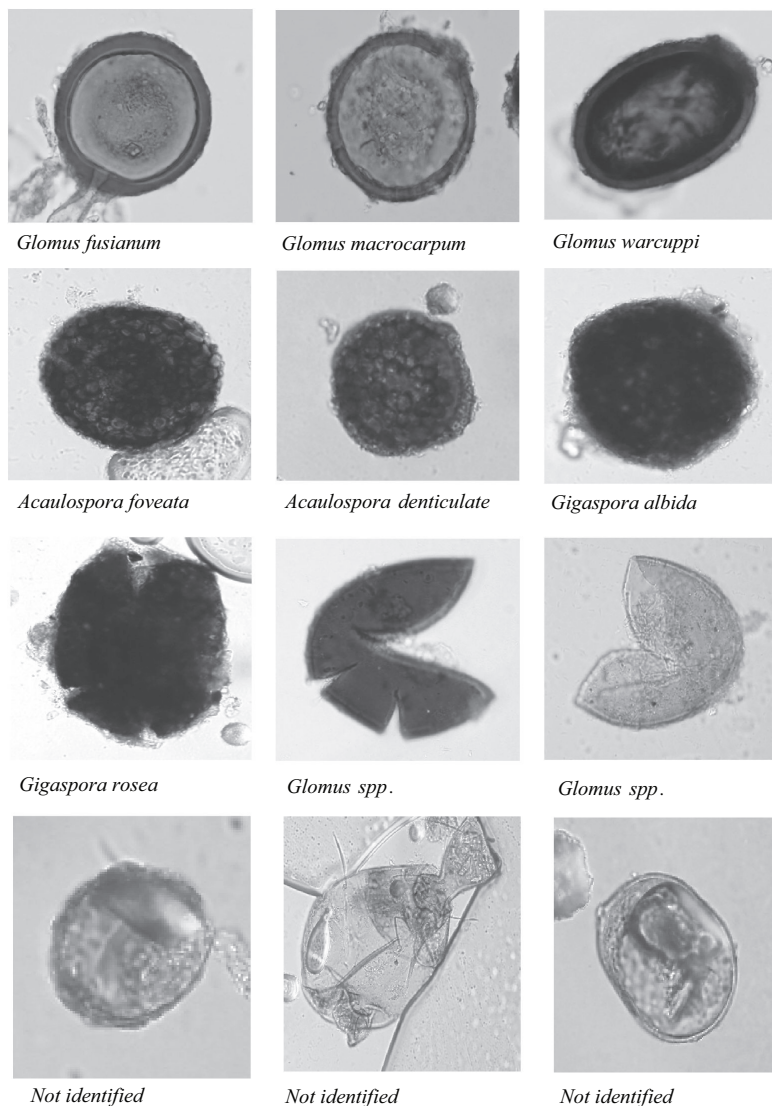
of spore, sporocarps colour, wall ornamentation, subtending hyphae and mode of attachment are considered for identification of spore or sporocarps.

*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<sub>1</sub>: Arbuscular mycorrhizal fungi (AMF), T<sub>2</sub>: *Rhizobium* (R), T<sub>3</sub>: AMF + *Rhizobium*, T<sub>4</sub>: *Sclerotium rolfsii*, T<sub>5</sub>: *Sclerotium rolfsii* + AMF, T<sub>6</sub>: *Sclerotium rolfsii* + *Rhizobium*, T<sub>7</sub>: *Sclerotium rolfsii* + AMF + *Rhizobium* and T<sub>8</sub>: Control.

*Determination of germination percentage*

The germination test was carried out according to ISTA rules<sup>21</sup>. 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



**Figure 1.** Different mycorrhizal spore identified in the Soil Microbiology Laboratory, Soil Science Division, BARI and used for the experiment

were counted. Germination of grasspea seed in the laboratory table was 95%. Fifteen seeds were sown in each pot. After 23 days germinated seeds were observed and counted. Germination percentage was calculated by the following formula:

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

#### 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

#### Plant harvest

Grasspea were harvested after 132 days after sowing. Different growth parameters like root length and shoot length, root length + shoot length, plant dry weight, pods plant<sup>-1</sup>, seed pod<sup>-1</sup>, total seed weight plant<sup>-1</sup>, 1000-seed weight, seed yield pot<sup>-1</sup> and stover yield pot<sup>-1</sup> were measured.

#### Statistical analysis

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

## Results and Discussion

#### Germination % and growth parameters

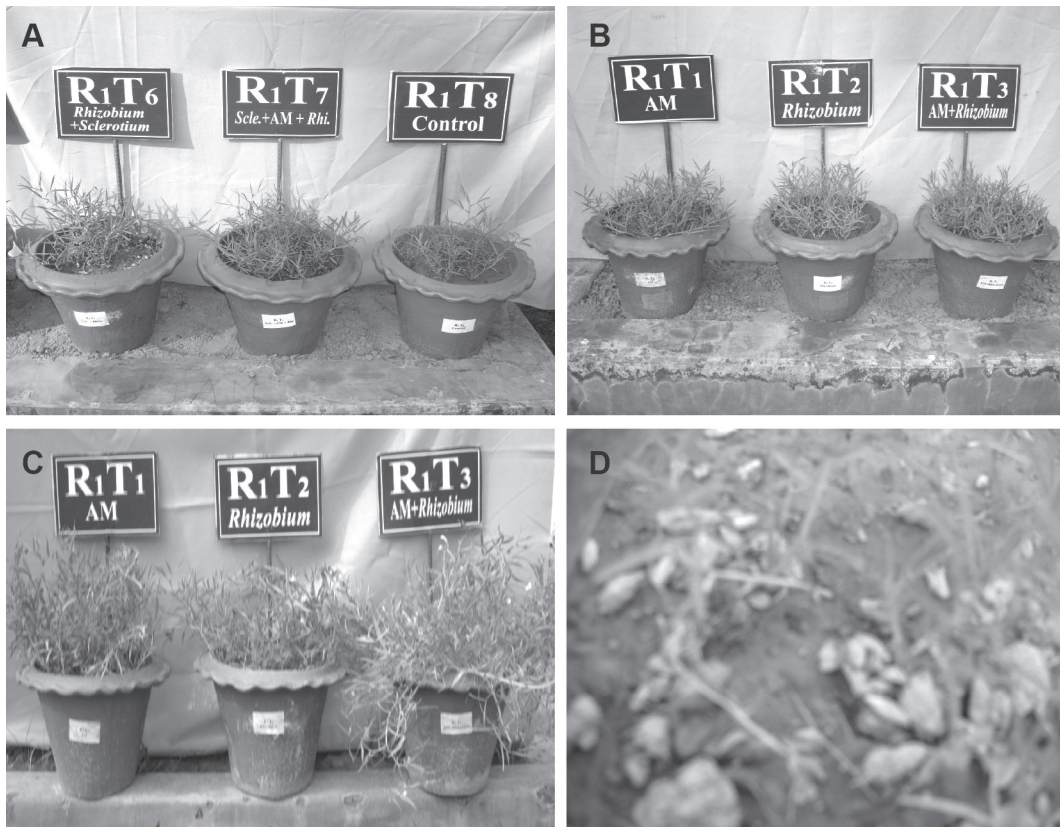
Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on germination % and growth parameters of grasspea have been presented in Table 2 and Figure 2. Significant differences were found in case of germination %, root length (cm), shoot length (cm), root + shoot length (cm) at harvest and plant dry weight (g plant<sup>-1</sup>).

The highest germination (80%) after 23 DAS, root length (7.27 cm), shoot length (32.58 cm), root + shoot length (39.85 cm)

**Table 2.** Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on germination % and growth parameters of grasspea

Treatments	Germination (%)	Root length	Shoot length	Root + shoot length	Plant dry weight
after 23 DAS		(cm at harvest)			(g plant <sup>-1</sup> )
2014-2015					
AM	75.00a	6.18b	29.56ab	35.73abc	1.66abc
<i>Rhizobium</i>	73.33ab	6.44ab	29.67ab	36.11abc	1.74abc
AM + <i>Rhizobium</i>	80.00a	7.27a	32.58a	39.85a	1.87a
<i>Sclerotium</i>	21.67e	2.57d	21.83c	24.40d	1.36d
<i>Sclerotium</i> + AM	31.67d	3.70c	27.96b	31.66c	1.64abc
<i>Sclerotium</i> + <i>Rhi.</i>	26.67de	4.02c	30.25ab	34.27abc	1.62bc
<i>Scle.</i> + AM + <i>Rhi.</i>	46.67c	6.54ab	31.25ab	37.79ab	1.83ab
Control	66.67b	6.10b	27.08b	33.18bc	1.51cd
SE (±)	2.61	0.32	1.57	2.08	0.18
F test	**	**	**	**	**
CV (%)	9.89	11.84	10.89	12.17	12.48
2015-2016					
AM	70.00b	7.08a	31.83ab	38.91a	1.55c
<i>Rhizobium</i>	65.00b	6.53a	31.13ab	37.66ab	1.60bc
AM + <i>Rhizobium</i>	80.00a	7.49a	33.78a	41.28a	1.95a
<i>Sclerotium</i>	5.00f	4.22b	16.71e	20.93d	1.14d
<i>Sclerotium</i> + AM	31.67d	5.26b	29.28bcd	34.54bc	1.54c
<i>Sclerotium</i> + <i>Rhi.</i>	20.00e	7.16a	26.38d	33.54c	1.38cd
<i>Scle.</i> + AM + <i>Rhi.</i>	41.67c	7.24a	26.94cd	34.18bc	1.83ab
Control	65.00b	6.85a	30.79abc	37.64ab	1.46c
SE (±)	2.99	0.37	1.34	1.38	0.08
F test	**	**	**	**	**
CV (%)	11.56	11.56	9.46	7.92	10.60

AM: Arbuscular Mycorrhiza, *Rhi.*: *Rhizobium*; *Scle.*: *Sclerotium*. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. \*\*Significant Pd<sup>0.01</sup>



**Figure 2.** Effect of inoculation of AMF and Rhizobium on grasspea (A and B: Early growth stage, C: Flowering stage, D: *Sclerotium rolfsii* affected young plants)

and plant dry weight ( $1.87 \text{ g plant}^{-1}$ ) in 2014-2015 and germination (80%) after 23 DAS, root length (7.49 cm), shoot length (33.78 cm), root + shoot length (41.28 cm) and plant dry weight ( $1.95 \text{ g plant}^{-1}$ ) in 2015-2016 were observed in AM + *Rhizobium* treatment (Table 2). The lowest germination (21.67%) after 23 DAS, root length (2.57 cm), shoot length (21.83 cm), root + shoot length (24.40 cm) and plant dry weight ( $1.36 \text{ g plant}^{-1}$ ) in 2014-2015 and germination (5.0%) after 23 DAS, root length (4.22 cm), shoot length (16.71 cm), root + shoot length (20.93 cm) and plant dry weight ( $1.14 \text{ g plant}^{-1}$ ) in 2015-2016 were observed in *Sclerotium* treatment (Table 2). The highest germination in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*', '*Sclerotium* + AM + *Rhizobium*' and control treatments but identical to AM and *Rhizobium* treatments while the highest germination in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over rest of the treatments. The highest root length in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over AM, *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*' and control treatments but identical to *Rhizobium* and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest root length in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium* and '*Sclerotium* + AM' treatments but identical to AM, *Rhizobium*, '*Sclerotium* + *Rhizobium*', '*Sclerotium* + AM + *Rhizobium*' and control treatments. The highest shoot length in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over

*Sclerotium*, '*Sclerotium* + AM' and control treatments but identical to AM, *Rhizobium*, '*Sclerotium* + *Rhizobium*' and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest shoot length in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*' and '*Sclerotium* + AM + *Rhizobium*' treatments but identical to AM, *Rhizobium* and control treatments. The highest root + shoot length in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium*, '*Sclerotium* + AM' and control treatments but identical to AM, *Rhizobium*, '*Sclerotium* + *Rhizobium*' and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest root + shoot length in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*' and '*Sclerotium* + AM + *Rhizobium*' treatments but identical to AM, *Rhizobium* and control treatments. The highest plant dry weight in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over '*Sclerotium* + *Rhizobium*' and control treatments but identical to AM, *Rhizobium*, '*Sclerotium* + AM' and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest plant dry weight in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was identical to '*Sclerotium* + AM + *Rhizobium*' treatment but significantly higher over rest of the treatments.

Mycorrhizal treatments significantly increased germination (%) because AMF entangle soil particles within the hyphae, tapping carbon resources, reduce damage caused by pathogen, influence soil microbial activity, increased mobilization and transfer of nutrients

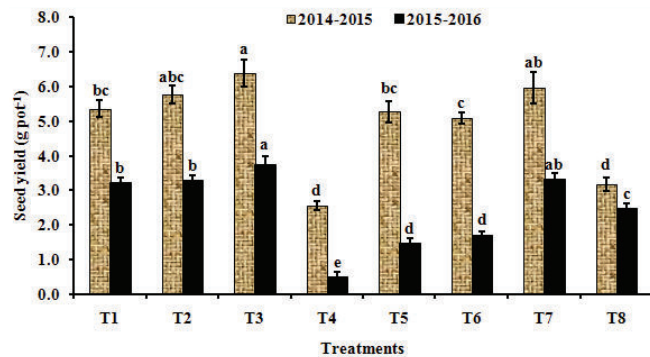
and increased availability of added or fixed phosphorus. Researches in the past few decades on various aspects of root symbionts have shown that dual interaction of AM fungi and *Rhizobium* has improved the growth, nodulation and yield<sup>22-23</sup>. Increased nitrogen fixation in chickpea due to dual inoculation with *G. fasciculatum* and *Rhizobium* was reported by Subba Rao et al.<sup>24</sup>.

### Yield attributes

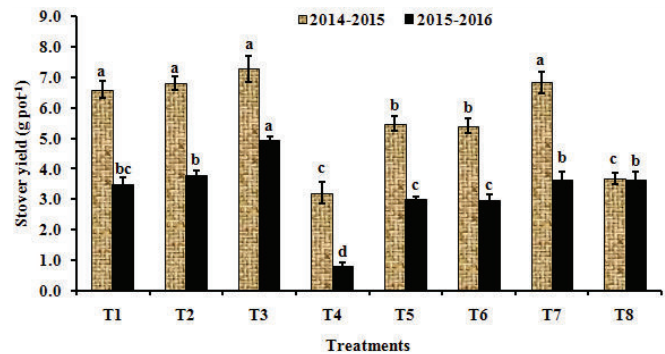
Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on yield and yield contributing characters of grasspea have been presented in Figures 3, 4 and Table 3. Significant differences

were found in case of pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, total seed weight (g plant<sup>-1</sup>), 1000-seed weight (g), seed yield (g pot<sup>-1</sup>) and stover yield (g pot<sup>-1</sup>).

The highest number of pods (7.33 plant<sup>-1</sup>), number of seeds (3.35 pod<sup>-1</sup>), total seed weight (1.02 g plant<sup>-1</sup>), 1000-seed weight (72.25 g), seed yield (6.39 g pot<sup>-1</sup>) and stover yield (7.28 g pot<sup>-1</sup>) in 2014-2015 and number of pods (6.50 plant<sup>-1</sup>), number of seeds (3.20 pod<sup>-1</sup>), total seed weight (0.74 g plant<sup>-1</sup>), 1000-seed weight (51.25 g), seed yield (3.73 g pot<sup>-1</sup>) and stover yield (4.93 g pot<sup>-1</sup>) in 2015-2016 were observed in AM + *Rhizobium* treatment (Figures 3, 4 and Table 3). The lowest number of pods (4.13



**Figure 3.** Effect of inoculation of AMF and *Rhizobium* on seed yield of grasspea. T<sub>1</sub>: Arbuscular mycorrhizal fungi (AMF), T<sub>2</sub>: *Rhizobium* (R), T<sub>3</sub>: AMF + *Rhizobium*, T<sub>4</sub>: *Sclerotium rolfsii*, T<sub>5</sub>: *Sclerotium rolfsii* + AMF, T<sub>6</sub>: *Sclerotium rolfsii* + *Rhizobium*, T<sub>7</sub>: *Sclerotium rolfsii* + AMF + *Rhizobium* and T<sub>8</sub>: Control.



**Figure 4.** Effect of inoculation of AMF and *Rhizobium* on stover yield of grasspea. T<sub>1</sub>: Arbuscular mycorrhizal fungi (AMF), T<sub>2</sub>: *Rhizobium* (R), T<sub>3</sub>: AMF + *Rhizobium*, T<sub>4</sub>: *Sclerotium rolfsii*, T<sub>5</sub>: *Sclerotium rolfsii* + AMF, T<sub>6</sub>: *Sclerotium rolfsii* + *Rhizobium*, T<sub>7</sub>: *Sclerotium rolfsii* + AMF + *Rhizobium* and T<sub>8</sub>: Control.

**Table 3.** Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on yield contributing characters of grasspea

Treatments	Number of pods(plant <sup>-1</sup> )	Number of seeds(pod <sup>-1</sup> )	Total seed weight(g plant <sup>-1</sup> )	1000-seed weight(g)
2014-2015				
AM	6.42ab	3.15	0.78b	68.00ab
<i>Rhizobium</i>	5.17cde	3.10	0.79b	63.25ab
AM + <i>Rhizobium</i>	7.33a	3.35	1.02a	72.25a
<i>Sclerotium</i>	4.13e	2.90	0.62c	58.50bc
<i>Sclerotium</i> + AM	5.33cd	3.10	0.75b	59.75bc
<i>Sclerotium</i> + <i>Rhi.</i>	5.01de	3.08	0.71bc	63.00ab
<i>Scle.</i> + AM + <i>Rhi.</i>	6.17bc	3.30	0.99a	66.75ab
Control	4.90de	3.10	0.67bc	51.50c
SE (±)	0.36	0.17	0.04	3.50
F test	**	NS	**	*
CV (%)	12.81	10.56	10.67	11.12
2015-2016				
AM	5.25bc	2.85ab	0.65a	45.25abc
<i>Rhizobium</i>	5.09c	2.90ab	0.68a	41.88bcd
AM + <i>Rhizobium</i>	6.50a	3.20a	0.74a	51.25a
<i>Sclerotium</i>	4.25d	2.25c	0.49b	38.50d
<i>Sclerotium</i> + AM	5.11c	2.60bc	0.53b	41.25bcd
<i>Sclerotium</i> + <i>Rhi.</i>	4.58cd	2.90ab	0.49b	41.88bcd
<i>Scle.</i> + AM + <i>Rhi.</i>	5.83ab	3.15a	0.70a	47.38ab
Control	5.17bc	2.85ab	0.52b	40.88cd
SE (±)	0.25	0.14	0.04	2.17
F test	**	**	**	*
CV (%)	9.43	10.19	12.12	9.99

AM: Arbuscular Mycorrhiza, *Rhi.*: *Rhizobium*; *Scle.*: *Sclerotium*. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. \*\*Significant Pd<sup>0.01</sup>, \*significant Pd<sup>0.05</sup>, NS non significant.

plant<sup>-1</sup>), number of seeds (2.90 pod<sup>-1</sup>), total seed weight (0.62 g plant<sup>-1</sup>), 1000-seed weight (58.50 g), seed yield (2.54 g pot<sup>-1</sup>) and stover yield (3.20 g pot<sup>-1</sup>) in 2014-2015 and number of pods (4.25 plant<sup>-1</sup>), number of seeds (2.25 pod<sup>-1</sup>), total seed weight (0.49 g plant<sup>-1</sup>), 1000-seed weight (38.50 g), seed yield (0.50 g pot<sup>-1</sup>) and stover yield (0.79 g pot<sup>-1</sup>) in 2015-2016 were observed in *Sclerotium* treatment (Figures 3, 4 and Table 3). The highest pods plant<sup>-1</sup> in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was identical to AM treatment but significantly higher over rest of the treatments while the highest pods plant<sup>-1</sup> in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was identical to '*Sclerotium* + AM + *Rhizobium*' treatment but significantly higher over rest of the treatments. The highest seeds pod<sup>-1</sup> in 2014-2015 was found in 'AM + *Rhizobium*' treatment which were non-significant among the treatments while the highest seeds pod<sup>-1</sup> in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium* and '*Sclerotium* + AM' treatments but identical to AM, *Rhizobium*, '*Sclerotium* + *Rhizobium*', '*Sclerotium* + AM + *Rhizobium*' and control treatments. The highest total seed weight (g plant<sup>-1</sup>) in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was identical to '*Sclerotium* + AM + *Rhizobium*' treatment but significantly higher over rest of the treatments while the highest total seed weight (g plant<sup>-1</sup>) in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was identical to AM, *Rhizobium*, '*Sclerotium* + AM + *Rhizobium*' treatment but significantly higher over rest of the treatments. The highest 1000-seed weight (g) in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium*, '*Sclerotium* + AM' and control treatments but identical to AM, *Rhizobium*, '*Sclerotium* + *Rhizobium*' and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest 1000-seed weight (g) in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Rhizobium*, *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*' and control treatments but identical to AM and '*Sclerotium* + AM + *Rhizobium*' treatments. The highest seed yield in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over AM, *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*' and control treatments but identical to *Rhizobium* and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest seed yield in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was identical to '*Sclerotium* + AM + *Rhizobium*' but significantly higher over rest of the treatments. The highest stover yield in 2014-2015 was found in 'AM + *Rhizobium*' treatment which was significantly higher over *Sclerotium*, '*Sclerotium* + AM', '*Sclerotium* + *Rhizobium*' and control treatments but identical to AM, *Rhizobium* and '*Sclerotium* + AM + *Rhizobium*' treatments while the highest stover yield in 2015-2016 was found in 'AM + *Rhizobium*' treatment which was significantly higher over all the treatments.

These are more or less similar to the findings of the following researchers. Dual inoculation of AM fungi and *Rhizobium* had improved the growth, nodulation and yield<sup>22-23</sup> and also nutrient status<sup>23, 25</sup> in legumes. Increased nitrogen fixation in chickpea due to dual inoculation with *G. fasciculatum* and *Rhizobium* was reported by Subba Rao *et al.*<sup>24</sup>.

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

Effect of dual inoculation of AMF 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 (%).

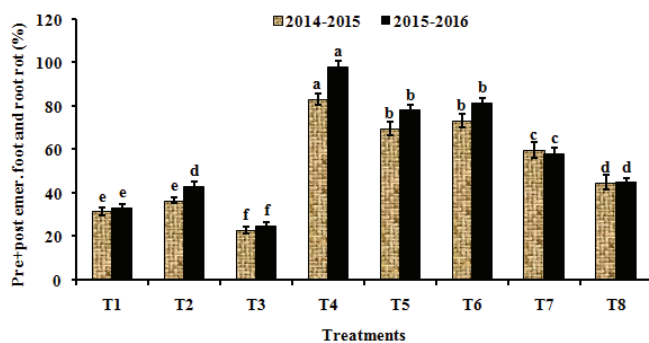
The highest pre-emergence foot and root rot (73.33%), total post-emergence foot and root rot (13.33%) and highest 'pre + post' emergence foot and root rot (83.33%) in 2014-2015 were observed in *Sclerotium*, '*Sclerotium* + AM + *Rhizobium*' and *Sclerotium* treatment, respectively. In contrast, the 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). 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 post-emergence foot and root rot (%) at 15 DAS in 2014-2015 was found in '*Sclerotium* + AM' treatment which was significantly higher over all the treatments while the highest post-emergence foot and root rot (%) at 15 DAS in 2015-2016 was found in '*Sclerotium* + AM' treatment which was significantly higher over all the treatments but identical to '*Sclerotium* + *Rhizobium*' treatment. The highest post-emergence foot and root rot (%) at 19 DAS in 2014-2015 was found in AM, *Rhizobium* and '*Sclerotium* + AM + *Rhizobium*' treatments which was significantly higher over remaining treatments while the highest post-emergence foot and root rot (%) at 19 DAS in 2015-2016 was found in *Sclerotium* treatment which was significantly higher over remaining treatments. The highest post-emergence foot and root rot (%) at 23 DAS in 2014-2015 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 causing seedling diseases<sup>26</sup>. Arbuscular mycorrhizal colonization may also protect host roots, especially

**Table 4.** Effect of dual inoculation of AMF 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 (%)				Total
		11 DAS	15 DAS	19 DAS	23 DAS	
2014-2015						
AM	26.67de	0.00	0.00c	3.33a	1.67d	5.00
<i>Rhizobium</i>	33.33d	0.00	0.00c	3.33a	0.00e	3.33
AM + <i>Rhizobium</i>	21.67e	0.00	0.00c	0.00c	1.67d	1.67
<i>Sclerotium</i>	73.33a	0.00	0.00c	1.67b	8.33a	10.00
<i>Sclerotium</i> + AM	58.33b	0.00	5.00a	0.00c	6.67b	11.67
<i>Sclerotium</i> + <i>Rhi.</i>	68.33a	0.00	0.00c	0.00c	5.00c	5.00
<i>Scl.</i> + AM + <i>Rhi.</i>	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 ( $\pm$ )	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
<i>Rhizobium</i>	43.33d	0.00	0.00c	0.00c	0.00	0.00
AM + <i>Rhizobium</i>	25.00f	0.00	0.00c	0.00c	0.00	0.00
<i>Sclerotium</i>	93.33a	0.00	1.67b	3.34a	0.00	5.01
<i>Sclerotium</i> + AM	75.00b	0.00	3.34a	0.00c	0.00	3.34
<i>Sclerotium</i> + <i>Rhi.</i>	78.33b	0.00	3.34a	0.00c	0.00	3.34
<i>Scl.</i> + AM + <i>Rhi.</i>	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 ( $\pm$ )	2.42	-	0.11	0.10	-	-
F test	**	-	**	**	-	-
CV (%)	8.59	-	21.29	32.45	-	-

AM: Arbuscular Mycorrhiza, *Rhi.*: *Rhizobium*; *Scl.*: *Sclerotium*. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. Test Statistix 10. \*\*Significant Pd<sup>0</sup>0.01.



**Figure 5.** Effect of dual inoculation of AMF and *Rhizobium* on 'pre+post' emergence foot and root rot disease % in grasspea. T<sub>1</sub>: Arbuscular mycorrhizal fungi (AMF), T<sub>2</sub>: *Rhizobium* (R), T<sub>3</sub>: AMF + *Rhizobium*, T<sub>4</sub>: *Sclerotium rolfsii*, T<sub>5</sub>: *Sclerotium rolfsii* + AMF, T<sub>6</sub>: *Sclerotium rolfsii* + *Rhizobium*, T<sub>7</sub>: *Sclerotium rolfsii* + AMF + *Rhizobium* and T<sub>8</sub>: Control.

under nutrient limitation<sup>27</sup>. Larsen and Bodker<sup>28</sup> 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 was shown 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<sup>29</sup>.

### Conclusions

Dual inoculation increased 20-25% germination, 50-100% seed yield and 36-98% stover yield compared to control. 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, arbuscular mycorrhizal fungal species and its combination with rhizobial inoculum were significant both in the formation and effectiveness of AM symbiosis and the reduction of foot and root rot incidence in grasspea plants. The findings of this study suggest among all treatments, dual combination of AMF plus *Rhizobium* was most effective in increasing germination (%), growth parameters, and yield contributing characters. Furthermore, combinations of AMF 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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