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Biocontrol of Foot and Root Rot Disease of Groundnut (*Arachis hypogaea*) by Dual Inoculation with *Rhizobium* and Arbuscular Mycorrhiza

Mominur Rahman[1*], Faridul Alam[2], Md. Nazrul Islam[3], Md. Farid Ahammed Anik[1], Mohammad Eyakub Ali[1]

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ABSTRACT

The present study was carried out to investigate the potential of AM (Arbuscular mycorrhiza) fungi alone and in combination with bioinoculants i.e., ***Rhizobium*** to find out the best combination on dry biomass, nodulation, colonization, and yield, along with their biocontrol against groundnut foot and root rot caused by *Sclerotium rolfsii*. The study was carried out under pot culture conditions in the net house of the Soil Science Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur in 2020 and 2021. The experiment was designed in RCBD with eight treatments and four replications. Peat-based rhizobial inoculum (BARI RAh-801) was used @ 1.5 kg ha^{-1} in this experiment. Soil-based AM inoculum containing approximately 252 spores and infected root pieces of the host plant was used in pot^{-1}. The treatments were Arbuscular mycorrhiza (AM), ***Rhizobium***, AM+***Rhizobium***, *Sclerotium rolfsii*, *Sclerotium rolfsii*+AM, *Sclerotium rolfsii*+***Rhizobium***, *Sclerotium rolfsii*+AM+***Rhizobium*** and Control. Dual inoculation (AM+***Rhizobium***) significantly increased dry biomass, nodulation, colonization, yield, and yield attributes of groundnut compared to single inoculation or other treatments. The result showed that dual inoculation (AMF+***Rhizobium***) increased nut yield (59.61% in 2020 and 26.32% in 2021) and stover yield (23.21% in 2020 and 33.74% in 2021) compared to control. On the contrary, *Sclerotium rolfsii*+AMF+***Rhizobium*** increased nut yield (65.50% in 2020 and 52.94% in 2021) and stover yield (36.45% in 2020 and 99.35% in 2021) compared to only *Sclerotium rolfsii* treatment. The plant dry biomass, nodulation, colonization, nutrient concentration and uptake were increased by dual inoculation under pathogenic and non-pathogenic conditions leading to an improved yield of groundnut. Therefore, AM fungi species and its combination with rhizobial inoculum were significant in the formation and effectiveness of AM symbiosis. They also increased yield and reduced the incidence of foot and root rot disease in groundnut plants.

INTRODUCTION

Foot rot (caused by *Fusarium oxysporum* and *Sclerotium rolfsii*) is considered an essential and destructive disease of pulses and oilseeds in almost all countries of the world (Anonymous, 1986). *Sclerotium rolfsii* are soil-borne pathogens that commonly occur in the tropics and subtropic regions of the world, causing root and foot rot in many crops (Aycock, 1966). It causes early seedling death, resulting in an inferior plant stand, producing a meager yield. Though chemical pesticides can control this disease, it causes environmental pollution and health hazards and is not economical. Hence, biological control agents like arbuscular mycorrhizal fungi and ***Rhizobium*** can be used for green, safe, 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 protect plants against several soil-borne plant pathogens. (Smith & Read, 1997; Bi *et al*., 2007). The significant effect of mycorrhizal fungi in undisturbed ecosystems is to improve the growth of mycorrhizal plants compared to non-mycorrhizal plants (Planchette *et al*., 1983). It covers the root of plants, making it a protective physical barrier against diseases (McAllister *et al*., 1997; Karagiannidis *et al*., 2002). 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 (Aghighi *et al*., 2004). AMF are currently studied as biological control agents against soil-borne diseases (Hooker *et al*., 1994). Their impact on plant-pathogen interactions ranges from disease reduction (Smith & Read, 1997; Rahman *et al*., 2017a) to a neutral action (Baath & Hayman, 1984). In this way, using AMF as inoculants to benefit plant growth and health could contribute to reducing the inputs of pesticides and other environmentally harmful agrochemical products currently required for optimal plant growth and health (Barea *et al*., 1997).

Many disease management methods exist, such as crop rotation use of resistant varieties, and chemical pesticides. However, frequent and indiscriminate use of these pesticides affects the Soil's physical, chemical, and biological properties. It also affects non-target organisms and has developed resistance among pathogens against these chemicals (Arwry & Quandt, 2003). The Biocontrol potential of AM fungi against various phytopathogens is well documented (Xavier & Boyetchko, 2014; Rahman *et al*., 2017b). Arbuscular Mycorrhizal Fungi (AMF) are the major component of the rhizosphere of most

[1] Soil Microbiology Laboratory, Soil Science Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2] Soil Unit, Bangladesh Agricultural Research Council, Farmgate, Dhaka-1215, Bangladesh
[3] Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[*] Corresponding author's email: mominurbau@gmail.com

plants and play a significant role as a biocontrol agent and help decrease plant disease incidence (Akthar & Siddiqui, 2008). On the other hand, *Rhizobium* is an important biofertilizer that can fix atmospheric nitrogen and is widely utilized in agricultural practices to reduce inorganic nitrogenous fertilizer inputs on legume crops. It is also employed as an alternative method to agricultural pesticides for use in plant disease management (Volpiano *et al*., 2019).There is a lot of information out there regarding the dual inoculation of AM fungi and *Rhizobium* that enhances plant growth and yield in different plants due to nutrient uptake, increases biological activity in the rhizospheric area, increases nitrogen fixation efficiency, etc., is a more familiar figure in the scientific world (Meng *et al*., 2015 and Rahman *et al*., 2017b). There have some ancient findings that showed that in some cases enhanced mineral nutrition of mycorrhizal plants has no affect against pathogens (Graham & Egel, 1988; Caron *et al*., 1986). Improved nutrient status of the host plant, changes in root growth and morphology, competition for colonization sites and host photosynthates, microbial changes in the mycorrhizosphere, and activation of plant defense mechanisms are the reason for reduced damage for the fungal pathogens caused by AM fungi. On the flip side, physiological and biochemical changes, changes in root growth and morphology, and activation of plant defense mechanisms are the reason for reduced damage for the fungal pathogens caused by root-nodule bacteria *Rhizobium* (Akhtar *et al*., 2011). Akkopru & Demir (2005) conducted biocontrol of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. lycopersici by AMF *Glomus intraradices* and some rhizobacteria. But, the potential of AM fungi and *Rhizobium* in controlling foot and root rot disease in groundnut remains broadly unexplored. Moreover, the present demanding idea supports a green, safe, and sustainable agricultural system, which enhances economically sustainable production systems in similar areas worldwide. Hence, keeping in view the above information, the present investigation was undertaken to investigate the potential of AM fungi alone and in combination with bioinoculants i.e., *Rhizobium* to find out the best combination on dry biomass, nodulation, colonization, and yield, along with their biocontrol against groundnut foot and root rot caused by *Sclerotium rolfsii*.

MATERIALS AND METHODS

Seed collection and soil preparation

The experiment was carried out during the rabi season from December 2019 to April 2020 and December 2020 to May 2021 in the net house of Soil Science Division, BARI, Joydebpur, Gazipur (230 59'378" N latitude, 900 24'886" E longitude and 8.4 m elevation). Seeds of groundnut (BARI Chinabadam-8) were collected from Regional Agricultural Research Station, Jamalpur. The silted (sandy clay loam) soils were collected from the bank of Turag river at Kodda, Gazipur, mixed with cow dung at a 5:1 ratio, and were used as the potting media. Each pot (25 cm in diameter and 21 cm in height) was filled with approximately 6 kg soil leaving the upper 3 inches of the pot vacant to facilitate watering.

The pH of cow dung 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, and the experiment was conducted under sterilized soil conditions.

Table 1: Initial fertility status of the soil and cowdung used in the investigational pot

Samples	Texture	pH	OM	Ca	Mg	K	Total N(%)	P	S	B	Cu	Fe	Mn	Zn
				meq 100 g^{-1}				µg g^{-1}						
Soil	Sandy clay loam			7.2	2.5	0.11	0.026	9.9	21.1	0.22	1.8	15	1.1	0.38
Cowdung	-	6.7	14.1	1.55	0.82	0.88	0.84	1.26	0.62	0.02	0.01	0.25	0.11	0.02
Critical level	-	-	-	2.0	0.5	0.12	-	10	10	0.20	0.2	4.0	1.0	0.60

Methods of chemical analysis

Soil pH was measured by a combined glass calomel electrode (Jackson, 1958). Organic carbon was determined by the Wet Oxidation Method (Walkley & Black, 1934). Total N was determined by the modified Kjeldahl method (Jackson, 1962). Calcium, K, and Mg were determined by the NH_4OAc extraction method (Black, 1965). Copper, Fe, Mn, and Zn were determined by DTPA extraction followed by AAS reading. Boron was determined by the $CaCl_2$ extraction method. Phosphorus was determined by the Modified Olsen method (Neutral + Calcareous soils) according to Olsen *et al.* (1954). Sulfur was determined by $CaH_4(PO_4)_2.H_2O$ extraction followed by turbidimetric turbidity method with $BaCl_2$.

Chemical fertilizers were applied as soil test basis according to the method described in the fertilizer recommendation guide (BARC, 2018). Half of N and all of P, K, S, Mg, Zn, B, and Mo were applied as basal during final land preparation, and the remaining N was used as top dressing at the flowering stage.

Collection of pathogens *Sclerotium rolfsii* and *Rhizobium* inoculum

Pathogen *Sclerotium rolfsii* was 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* and non-seed barley 50 g was used per *Sclerotium*

treatment pot. After disease development, pathogen sclerotia are mixed with Soil. *Rhizobium* strain BARI RAh-801 was collected from Soil Microbiology Division, BARI, Gazipur, and mixed correctly with the seed before sowing. The used inoculum rate was 1.5 kg $ha^{-1.}$

Preparation of mycorrhizal inoculum

The arbuscular mycorrhizal inoculum was prepared from Sorghum's roots and rhizosphere soils. Mycorrhizal species were initially isolated from different AEZ regions using the wet sieving and decanting method. A mixture of infected sorghum root and Soil, which contained spores, was used as mycorrhizal inoculum. The spores were left to multiply for six months on sorghum plants using unsterilized Soil collected from the same site in the net house of the Soil Science Division, BARI. Plants were rinsed with tap water as needed. The soil-based AM fungal inoculum having approximately 252 spores and colonized sorghum root fragments with a minimum colonization level was inoculated to each mycorrhizal pot. The species was *Glomus fusianum, Acaulospora foveata, Gigaspora albida, and Acaulospora denticulate*) were used for the preparation of AM fungal inoculum. The mycorrhizal inoculum was placed in each pot at a 3-5 cm depth. It was covered with a thin soil layer of 1 cm immediately before the seed sowing of groundnut to facilitate fungal colonization of plant roots.

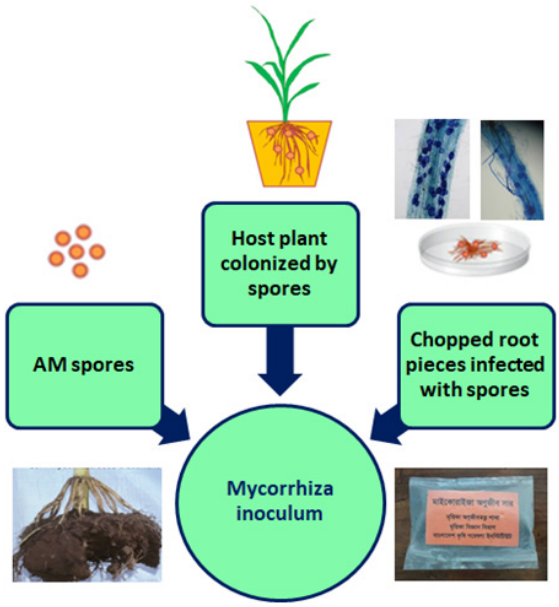

Figure 1: Mycorrhizal inoculum production

Experimental design

The experiment was designed in RCBD with eight treatments and four replications. Fifteen seeds were sown in each pot at 1 cm soil depth. The eight (08) treatments were: T_1: Arbuscular mycorrhizal fungi (AMF), T_2: *Rhizobium* (R), T_3: AMF+*Rhizobium*, T_4: *Sclerotium rolfsii*, T_5: *Sclerotium rolfsii*+AMF, T_6: *Sclerotium rolfsii*+*Rhizobium*, T_7: *Sclerotium rolfsii*+AMF+*Rhizobium* and T_8: Control. The treatment was sustained with 06 vigorous seedlings in pot, and the other seedlings were removed. Three plants each were collected for nodulation (Khanam *et al.*, 2005) and colonization data, and rest three plants were kept finally in each pot for yield and yield contributing measurements.

Plant harvest

Groundnuts were harvested after maturity, and yield parameters were measured.

Assessment of spore population density and root colonization

The spore population was assessed following the Wet Sieving and Decanting Method (Gerdemann & Nicolson, 1963). All the AM spores were isolated from the extract with the help of fine forceps into a watch glass with little water. The extract, with AM spores, was observed under a stereomicroscope, and the number of spores was counted. Spore numbers from the three replicates per sample were averaged, and the result was expressed as a number per 100 g of dry soil basis. The root slide technique estimated the percentage of AM colonization (Read *et al.*, 1976). A root segment was considered positively infected if it showed mycelium, vesicles, arbuscules, or any other combination of these structural characteristics of AM colonization. The presence or absence of colonization in the root pieces was recorded. The percent colonization was calculated by dividing the number of AM-positive segments by the total number of segments scored and multiplying this value by 100.

Statistical analysis

Data were analyzed via RCBD model. All data were statistically assessed with the open source software R version 4.2.2 and RStudio. In the manuscript the tests of normality of the parameters were also done with R software and all were normally distributed. Means

were separated by Duncan's multiple range tests by the least significant contrast strategy and correlation by the Pearson method utilizing RStudio. Data were statistically analyzed using Analysis of Variance (ANOVA) following the software R 4.2.2 and RStudio.

RESULTS AND DISCUSSION

Nodulation, fresh and dry biomass, and growth parameters

Significant differences were found in the case of nodulation, fresh and dry biomass, and growth parameters of groundnut in both of the years (Table 2). In the year 2019-2020, the highest nodule number (30.58 plant^{-1}), nodule weight (75.83 mg plant^{-1}), fresh root weight (0.64 g plant^{-1}), fresh shoot weight (16.92 g plant^{-1}), dry root weight (0.19 g plant^{-1}), dry shoot weight (3.71 g plant^{-1}), plant height (18.11 cm), and no. of branch plant^{-1} (6.17) were observed in AM+*Rhizobium* treatment. The lowest nodule number (16.17 plant^{-1}), nodule weight (36.67 mg plant^{-1}), fresh root weight (0.44 g plant^{-1}), fresh shoot weight (11.63 g plant^{-1}), dry root weight (0.14 g plant^{-1}), dry shoot weight (2.23 g plant^{-1}), plant height (15.48 cm), and no. of branch plant^{-1} (4.25) were observed in *Sclerotium* treatment. In all cases, *Sclerotium*+AM+*Rhizobium* treatment produced higher parameters compared to *Sclerotium* treatment.

In the year 2020-2021, the highest nodule number (13.13 plant^{-1}), nodule weight (80.00 mg plant^{-1}), fresh root weight (1.42 g plant^{-1}), fresh shoot weight (46.42 g plant^{-1}), dry root weight (0.49 g plant^{-1}), dry shoot weight (12.14 g plant^{-1}), plant height (41.00 cm), and no. of branch plant^{-1} (5.50) were observed in AM+*Rhizobium* treatment. The lowest nodule number (6.38 plant^{-1}), nodule weight (31.25 mg plant^{-1}), fresh root weight (0.97 g plant^{-1}), fresh shoot weight (35.34 g plant^{-1}), dry root weight (0.32 g plant^{-1}), dry shoot weight (8.71 g plant^{-1}), plant height (36.75 cm), and no. of branch plant^{-1} (4.13) were observed in *Sclerotium* treatment. In all cases,

Table 2: Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on nodulation, fresh and dry biomass, and growth parameters of groundnut during 2020 and 2021

Treatments	Nodule number plant^{-1}	Nodule weight (mg plant^{-1})	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})		Plant height (cm)	No. of branch plant^{-1}
			Root weight	Shoot weight	Root weight	Shoot weight		
2019-2020								
AM	24.58bc	59.17bc	0.57a	15.27a	0.16bcd	3.55ab	17.43ab	5.92abc
Rhizobium	25.17bc	63.33ab	0.61a	15.88a	0.17abc	3.61ab	17.83ab	6.00ab
AM+*Rhizobium*	30.58a	75.83a	0.64a	16.92a	0.19a	3.71a	18.11a	6.17a
Sclerotium	16.17d	36.67e	0.44b	11.63c	0.14d	2.23d	14.70c	4.25d
Sclerotium+AM	21.42c	47.50cde	0.47b	12.79bc	0.15cd	2.58d	16.92abc	5.17bc
Sclerotium+*Rhi.*	21.67c	54.17bcd	0.57ab	14.93ab	0.15bcd	2.69cd	17.41ab	5.58abc
Scle.+AM+*Rhi.*	27.25ab	66.67ab	0.59a	15.94a	0.17ab	3.16bc	18.03a	6.00ab
Control	16.50d	40.83de	0.50bc	12.93bc	0.15bcd	2.45d	16.00bc	5.08cd
SE (±)	1.40	4.60	0.03	0.78	0.01	0.17	0.63	0.30
F test	***	***	***	***	*	***	.	**
CV (%)	12.19	16.56	9.49	10.68	12.52	11.09	7.40	10.93
2020-2021								
AM	10.38b	61.50b	1.10bcd	37.69d	0.43ab	10.08bcd	40.15a	5.25a
Rhizobium	11.00b	65.00b	1.22b	40.94c	0.45a	11.36ab	40.25a	5.25a
AM+*Rhizobium*	13.13a	80.00a	1.42a	46.42a	0.49a	12.14a	41.00a	5.50a
Sclerotium	6.38d	31.25e	0.97d	35.34d	0.32c	8.71e	36.75b	4.13b
Sclerotium+AM	10.13b	42.50cd	1.08bcd	36.88d	0.36bc	9.00de	39.50a	5.13a
Sclerotium+*Rhi.*	10.25b	45.00c	1.14bc	42.95bc	0.43ab	10.91abc	39.50a	5.25a
Scle.+AM+*Rhi.*	13.00a	62.50b	1.21b	45.43ab	0.47a	11.57a	40.63a	5.38a
Control	8.13c	33.75de	1.06cd	36.51d	0.34c	9.87cde	39.75a	5.25a
SE (±)	0.49	3.00	0.05	0.91	0.03	0.44	0.81	0.16
F test	***	***	***	***	**	***	*	***
CV (%)	9.53	11.37	8.99	4.52	13.08	8.49	4.07	6.24

*AM: Arbuscular Mycorrhiza, Rhi.: Rhizobium; Scle.: Sclerotium. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. R 4.2.2 and RStudio. '***'Significant P≤0.001. '**'Significant P≤0.01. '*'significant P≤0.05. '.'Significant P≤0.1.*

Sclerotium+AM+*Rhizobium* treatment produced higher parameters compared to *Sclerotium* treatment.

Nodule number, nodule weight, fresh weight of root and shoot, dry weight of root and shoot, plant height, and branch number were positively correlated with yield (r = 0.72***, r = 0.62***, r = 0.57***, r = 0.66***, r = 0.47**, r = 0.76***, r = 0.59***, and r = 0.70***) and stover yield (r = 0.62***, r = 0.53**, r = 0.67***, r = 0.47**, r = 0.50**, r = 0.59***, r = 0.43*, and r = 0.54**) in 2020 (Figure 6 and 7). This finding is supported by the findings of Akkopru & Demir (2005), who conducted biocontrol of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. lycopersici by AMF *Glomus intraradices* and some rhizobacteria. They suggested that suitable combinations of these biocontrol agents may ameliorate plant growth and health by enhancing root dry weight.

Root colonization, spore population, yield, and yield attributes

Results on the effect of inoculation of AMF, *Rhizobium*, and *Sclerotium rolfsii* on root colonization, spore population, yield, and yield attributes of groundnut are presented in Table 3, Figure 3 and Figure 4. Significant differences were found in all the parameters mentioned above. In the year 2019-2020, the highest root colonization (45.00%), spore population (109.00, 100 g^{-1} soil), nut (18.42 $plant^{-1}$), kernel (26.08 nut^{-1}), kernel weight (11.50 g plant nut^{-1}), nut yield (16.52 g $plant^{-1}$) and stover yield (16.88 g $plant^{-1}$) were observed in AM+*Rhizobium* treatment. The lowest root colonization (00.00%), spore population (57.00, 100 g^{-1} soil), nut (09.17 $plant^{-1}$), kernel (12.83 nut^{-1}), kernel weight (5.98 g plant nut^{-1}), nut yield (8.84 g $plant^{-1}$) and stover yield (12.21 g $plant^{-1}$) were observed in *Sclerotium* treatment. In all cases, *Sclerotium*+AM+*Rhizobium* treatment produced higher parameters compared to *Sclerotium* treatment. Dual inoculation (AMF+*Rhizobium*) increased 59.61% nut yield and 23.21% stover yield compared to control. On the contrary, *Sclerotium rolfsii*+AMF+*Rhizobium* increased 65.50% nut yield and 36.45% stover yield compared to only *Sclerotium rolfsii* treatment.

In the year 2020-2021, the highest root colonization (45.00%), spore population (61.50, 100 g^{-1} soil), nut (14.58 $plant^{-1}$), kernel (20.67 nut^{-1}), kernel weight (8.09 g plant nut^{-1}), nut yield (12.24 g $plant^{-1}$) and stover yield (27.71 g $plant^{-1}$) were observed in AM+*Rhizobium* treatment. The lowest root colonization (00.00%), spore population (41.50, 100 g^{-1} soil), nut (6.75 $plant^{-1}$), kernel (9.25 nut^{-1}), kernel weight (3.64 g plant nut^{-1}), nut yield (7.65 g $plant^{-1}$) and stover yield (13.75 g $plant^{-1}$) were observed in *Sclerotium* treatment. In all cases *Sclerotium*+AM+*Rhizobium* treatment produced higher parameters compared to *Sclerotium* treatment. Dual inoculation (AMF+*Rhizobium*) increased 26.32% nut yield and 33.74% stover yield compared to control. On the contrary, *Sclerotium rolfsii*+AMF+*Rhizobium* increased 52.94% nut yield and 99.35% stover yield compared to only *Sclerotium rolfsii* treatment.

Figure 2 shows root colonization of arbuscular mycorrhizal fungi where we see fresh root cortex and mycorrhiza colonized root cortex of groundnut. High colonization rate indicates high vigorous mycorrhizal spore that lead to better productivity. However, Nut $plant^{-1}$, kernel plant nut^{-1}, kernel weight, spore population and root colonization were positively correlated with nut yield (r = 0.90***, r = 0.87***, r = 0.84***, r = 0.65***, and r = 0.46**) and stover yield (r = 0.66***, r = 0.72***, r = 0.73***, r = 0.70***, and r = 0.42*) in 2020 (Figure 6 and 7). This finding is supported by the findings of Akkopru & Demir (2005), and Rahman, *et al.*, (2017ab). They found that suitable combinations of these biocontrol agents increase plant growth, yield, and yield traits by enhancing root colonization and sporulation of mycorrhizal spores.

Table 3: Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on root colonization, spore population, yield and yield attributes of groundnut during 2020 and 2021

Treatments	Root Colonization (%)	Spore population (100 g^{-1} soil)	Nut $plant^{-1}$	Kernel plant nut^{-1}	Kernel weight (g plant nut^{-1})
2019-2020					
AM	40.00ab	93.50b	16.83ab	24.67ab	10.29ab
Rhizobium	0.00d	77.00cd	17.17ab	25.25ab	10.56ab
AM+*Rhizobium*	45.00a	109.00a	18.42a	26.08a	11.50a
Sclerotium	0.00d	57.00e	9.17c	12.83c	5.98c
Sclerotium+AM	35.00b	82.50bc	14.75b	21.25b	9.23b
Sclerotium+*Rhi.*	10.00c	75.00cd	16.33ab	22.58ab	9.42b
Scle.+AM+*Rhi.*	35.00b	94.50ab	17.33ab	25.75a	10.68ab
Control	5.00cd	67.50de	10.75c	13.67c	6.41c
SE (±)	2.67	5.00	1.12	1.39	0.64
F test	***	***	***	***	***
CV (%)	25.15	12.20	14.83	12.89	13.78
2020-2021					

AM	40.00ab	55.00ab	11.75bc	15.42b	7.21ab
Rhizobium	15.00c	53.00b	11.83b	15.92b	7.47ab
AM+*Rhizobium*	45.00a	61.50a	14.58a	20.67a	8.09a
Sclerotium	0.00d	41.50c	6.75d	9.25c	3.64c
Sclerotium+AM	35.00b	52.50b	9.83c	10.08c	4.63c
Sclerotium+*Rhi.*	15.00c	51.50b	10.92bc	14.17b	6.25b
Scle.+AM+*Rhi.*	35.00b	61.00a	14.25a	19.50a	7.97a
Control	0.00d	43.50c	11.50bc	15.17b	6.85ab
SE (±)	2.56	2.45	0.66	0.76	0.42
F test	***	***	***	***	***
CV (%)	22.13	9.36	11.50	10.09	13.04

*AM: Arbuscular Mycorrhiza, Rhi.: Rhizobium; Scle.: Sclerotium. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. R 4.2.2 and RStudio. '***'Significant P≤0.001. '**'Significant P≤0.01.*

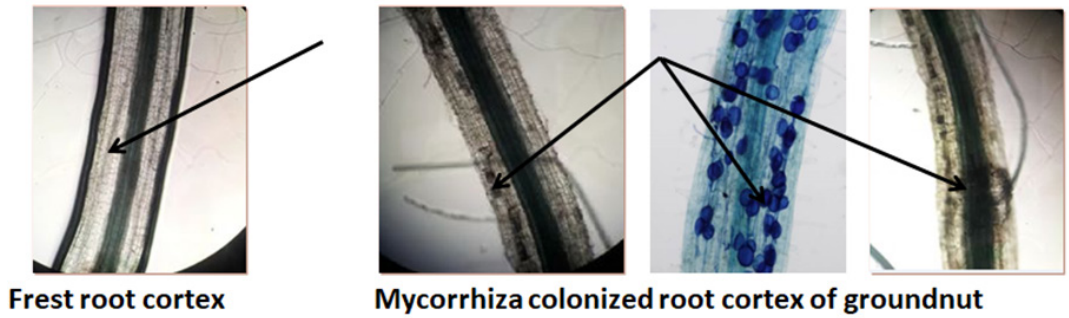

Figure 2: Root colonization of arbuscular mycorrhizal fungi

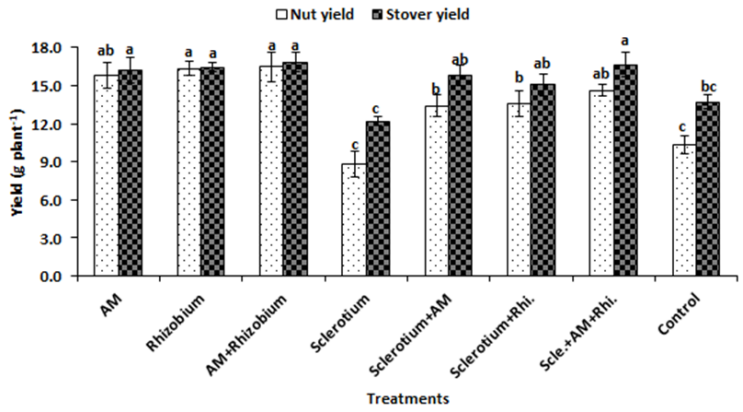

Figure 3: Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on nut yield and stover yield of groundnut during 2020

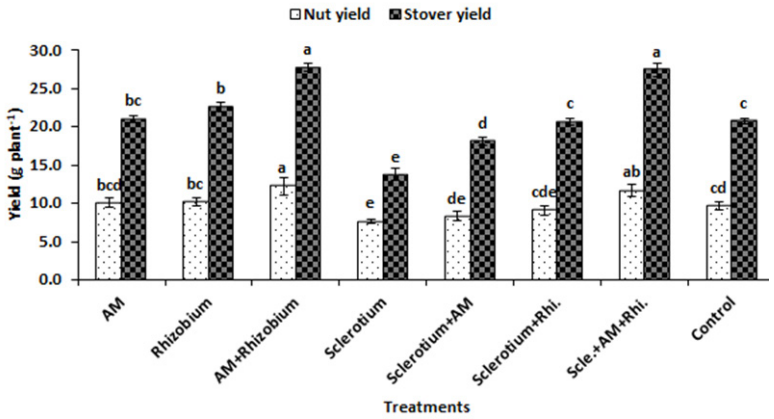

Figure 4: Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on nut yield and stover yield of groundnut during 2021

Plant dry biomass and nutrient content

Results on the effect of inoculation of AMF, *Rhizobium*, and *Sclerotium rolfsii* on plant dry biomass, and nutrient content of groundnut during 2020 are presented in Table 4. Significant differences were found in all the parameters except S and Zn content. The highest plant dry biomass (3.90 g plant^{-1}), P content (0.41%), K content (1.85%), S content (0.27%), B content (71.50%), and Zn content (55.90%) were observed in AM+*Rhizobium* treatment. The lowest plant dry biomass (2.37 g plant^{-1}), P content (0.23%), K content (1.52%), S content (0.25%), B content (66.50%), and Zn content (54.00%) were observed in *Sclerotium* treatment. In all cases, *Sclerotium*+AM+*Rhizobium* treatment produced higher parameters compared to *Sclerotium* treatment. Dual inoculation (AMF+*Rhizobium*) increased 50% plant dry biomass, 70.83% P content, 18.59% K content, 8% S content, 4.38% B content and 1.64% Zn content compared to control. On the contrary, *Sclerotium rolfsii*+AMF+*Rhizobium* increased 40.51% plant dry biomass, 47.83% P content, 21.05% K content, 8% S content, 6.02% B content and 2.78% Zn content compared to only *Sclerotium rolfsii* treatment. Sulphur, B, and Zn content had no correlation with both nut yield and stover yield in 2020. But, plant dry biomass, P content, and K content had positively correlated with nut yield (r = 0.76***, r = 0.66*** and r = 0.76***) and stover yield (r = 0.59***, r = 0.57***and r = 0.65***) of groundnut in 2020 (Figure 6 and 7). This finding is supported by the findings of Akkopru & Demir (2005), who suggested that suitable combinations of these biocontrol agents may ameliorate plant growth and health by enhancing root dry weight and P content. This result also supported by Akhter *et al.*, (2011), who said that Arbuscular mycorrhizal bacteria improve nutrient acquisition in plants. Thus, inhibit pathogens, acquire mineral nutrients and modify plant root growth. They also said that combined use of these microorganisms is more beneficial than their use alone.

Table 4: Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on plant dry biomass and nutrient content of groundnut during 2020

Treatments	Plantdry biomass (g plant^{-1})	Macronutrient concentration (%)			Micronutrient concentration (ppm)	
		P	K	S	B	Zn
AM	3.72 ± 0.18ab	0.33 ± 0.02b	1.84 ± 0.02a	0.27 ± 0.01	71.0 ± 3.46ab	55.00 ± 0.58
Rhizobium	3.78 ± 0.26ab	0.32 ± 0.02b	1.76 ± 0.02b	0.27 ± 0.02	70.5 ± 3.18bc	55.00 ± 0.58
AM+*Rhizobium*	3.90 ± 0.18a	0.41 ± 0.02a	1.85 ± 0.03a	0.27 ± 0.01	71.50 ± 3.75a	55.90 ± 0.52
Sclerotium	2.37 ± 0.22d	0.23 ± 0.01c	1.52 ± 0.02c	0.25 ± 0.01	66.50 ± 3.18e	54.00 ± 0.58
Sclerotium+AM	2.72 ± 0.13d	0.34 ± 0.02b	1.84 ± 0.02a	0.26 ± 0.01	70.00 ± 2.89c	55.00 ± 0.58
Sclerotium+*Rhi.*	2.85 ± 0.14cd	0.32 ± 0.02b	1.80±0.02ab	0.26 ± 0.01	69.00 ± 3.46d	55.00 ± 0.58
Scle.+AM+*Rhi.*	3.33 ± 0.03bc	0.34 ± 0.02b	1.84 ± 0.01a	0.27 ± 0.01	70.5 ± 3.18bc	55.50 ± 0.29
Control	2.60 ± 0.20d	0.24 ± 0.02c	1.56 ± 0.01c	0.25 ± 0.02	68.50 ± 3.75d	55.00 ± 0.58
SE (±)	0.17	0.02	0.02	0.02	0.31	0.52
F test	***	***	***	ns	***	ns
CV (%)	10.72	12.63	2.38	11.59	0.88	1.88

*AM: Arbuscular Mycorrhiza, Rhi.: Rhizobium; Scle.: Sclerotium. The values represent means of 04 replicates. Different letters within each column indicate significant differences between treatments. R 4.2.2 and RStudio. '***'Significant P≤0.001. ns: Non significant.*

Nutrient uptake

Results on the effect of inoculation of AMF, *Rhizobium*, and *Sclerotium rolfsii* on nutrient uptake of groundnut during 2020 are presented in Figure 5. Significant differences were found in all the parameters. The highest P uptake (16.00 mg plant^{-1}), K uptake (72.21 mg plant^{-1}), S uptake (10.34 mg plant^{-1}), B uptake (279 mg plant^{-1}), and Zn uptake (218 mg plant^{-1}) were observed in AM+*Rhizobium* treatment. The lowest P uptake (5.33 mg plant^{-1}), K uptake (35.97 mg plant^{-1}), S uptake (5.80 mg plant^{-1}), B uptake (157 mg plant^{-1}), and Zn uptake (128 mg plant^{-1}) were observed in *Sclerotium* treatment. In all cases, *Sclerotium*+AM+*Rhizobium* treatment produced higher parameters compared to *Sclerotium* treatment. Dual inoculation (AMF+*Rhizobium*) increased 159% P uptake, 78.16% K uptake, 59.08% S uptake, 56.80% B uptake and 52.68% Zn uptake compared to control. On the contrary, *Sclerotium rolfsii*+AMF+*Rhizobium* increased 113% P uptake, 70.0% K uptake, 52.2% S uptake, 49.2% B uptake and 44.7% Zn uptake compared to only *Sclerotium rolfsii* treatment.

Phosphorus (P), K, S, B, and Zn uptake had positively correlated with nut yield (r = 0.78***, r = 0.85***, r = 0.71***, r = 0.69***, and r = 0.80***) and stover yield (r = 0.63***, r = 0.69***, r = 0.53**, r = 0.57***, and r = 0.64**) of groundnut in 2020 (Figure 6 and 7). This result is supported by Akhter *et al.*, (2011), who said that Arbuscular mycorrhizal bacteria improve plant nutrient acquisition. Thus, inhibit pathogens, acquire mineral nutrients, and modifies plant root growth. They also said that the combined use of these microorganisms is more beneficial than their use alone.

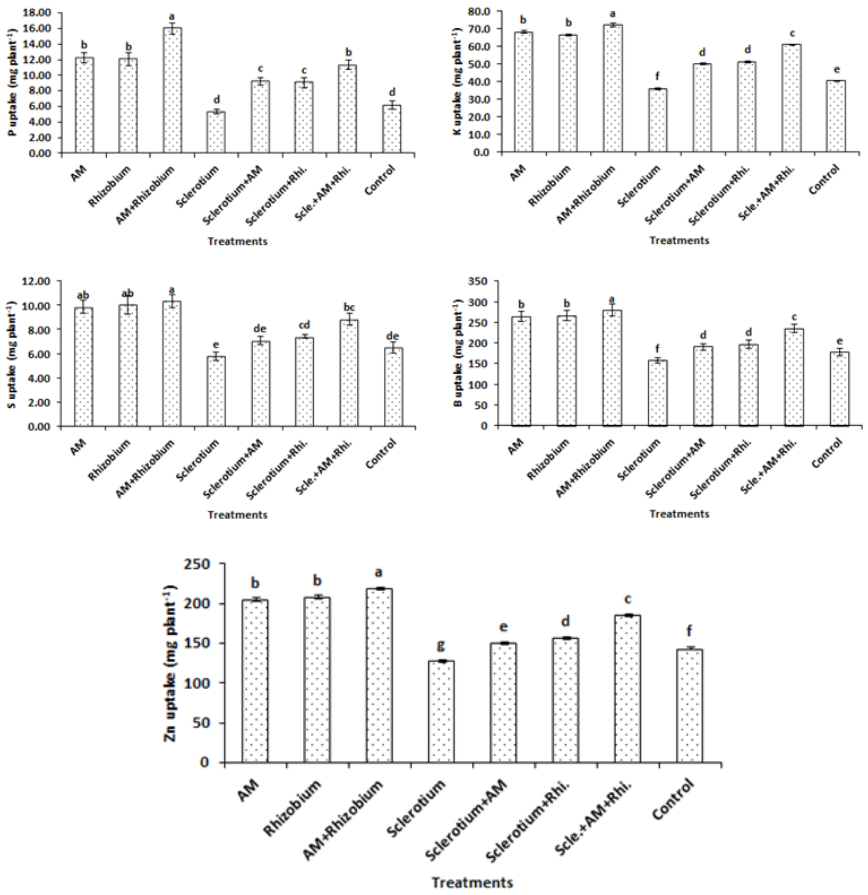

Figure 5: Effect of inoculation of AMF, *Rhizobium* and *Sclerotium rolfsii* on P, K, S, B and Zn uptake of groundnut during 2020

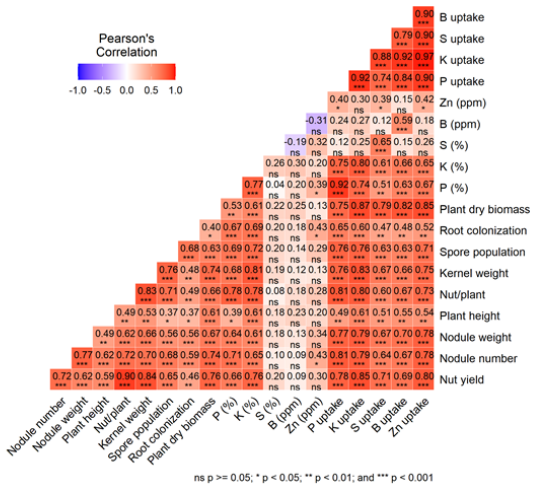

Figure 6: Correlation between nut yield and other parameters of groundnut plants (n = 32)

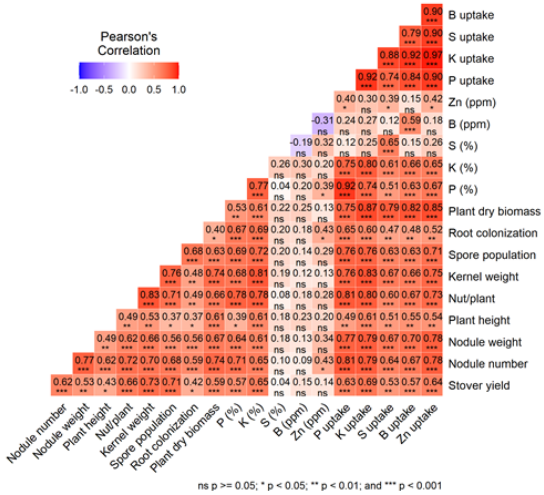

Figure 7: Correlation between stover yield and other parameters of groundnut plants (n = 32)

CONCLUSION

The findings of this study suggest among all treatments, the dual combination of AMF plus *Rhizobium* was most effective in increasing biomass, nodulation, colonization, yield, and yield attributes in rhizosphere soils of groundnut. Dual inoculation (AMF+*Rhizobium*) increased nut yield (59.61% in 2020 and 26.32% in 2021) and stover yield (23.21% in 2020 and 33.74% in 2021) compared to control. On the contrary, *Sclerotium rolfsii*+AMF+*Rhizobium* increased nut yield (65.50% in 2020 and 52.94% in 2021) and stover yield (36.45% in 2020 and 99.35% in 2021) compared to only *Sclerotium rolfsii* treatment. The plant dry biomass, nodulation, colonization, nutrient concentration and uptake were increased by dual inoculation under pathogenic and non-pathogenic conditions leading to an improved yield of groundnut. Thus, AMF and *Rhizobium* combinations increase yield and control groundnut foot and root rot

disease more effectively than either biocontrol agent applied alone, which would be the vital basis of green, safe and hazardous-free sustainable agricultural systems as enhancing economically sustainable production systems worldwide. Even though we have constraints in the pure culture strain of AM fungal inoculum production, requiring optimization and control of both fungal development and host growth, we justified our research objectives and got superb results. Benefits include controlling groundnut seedlings' mortality, saving capital costs, and helping to achieve sustainable agriculture through these symbionts. It is strongly suggested to study host, fungal pathogens, symbionts, and environmental factors addressed altogether for further clarification.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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