Journal of Agricultural and Crop Research
Vol. 9(9), pp. 212-221, September 2021
doi: 10.33495/jacr_v9i9.21.148
ISSN: 2384-731X
Research Paper

Mycorrhizae as a biological method for improving soil fertility and controlling *Rhizobium-inoculated* soybean collar rot disease in Benin

Howell B Houngnandan[1], Adélaïde H. Fagnibo[2], Appolinaire Adandonon[1,2*], Leslie DR Bossou[1], Mahougnon Charlotte C Zoundji[2], Félix Kouèlo[3], Tobi Moriaque Akplo[3] and Pascal Houngnandan[1,2,3]

[1]Laboratory of Plant, Horticultural and Forest Sciences (LaSVHF), National University of Agriculture (UNA), Republic of Benin.
[2]School of Crop and Seed Production and Management, National University of Agriculture of Porto Novo; BP 43 Kétou, Republic of Benin.
[3]Laboratory of Soil Microbiology and Microbial Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi (UAC); 01 BP 711 Abomey-Calavi, R. Benin.

*Corresponding author. E-mail: adanappo@yahoo.fr; adanappo2013@gmail.com.

Accepted 18th July, 2021.

Abstract. The current study aims to improve soybean productivity without mineral fertilizer inputs by testing the effect of indigenous species of arbuscular mycorrhizal fungi (AMF) in double inoculation with *Bradyrhizobium* on soybean collar rot (SCR). The trial was conducted in North Benin and included 11 treatments, namely eight AMF species in double inoculation with *Bradyrhizobium* and three controls, namely *Bradyrhizobium* + 100 $kg{\cdot}ha^{-1}$ of P_2O_5 (RP100), *Bradyrhizobium* without mycorrhiza (RM0) and the untreated control without either *Bradyrhizobium* or mycorrhiza (R0M0). The results showed R0M0 to have the highest incidence of collar rot disease. The causal agent of the disease was identified in the current study as *Sclerotium rolfsii*. When not treated, the disease incidence in the current study was more than 70%, while, when treated with AMF as *Racocetra crispa, Gigaspora margarita, Scutellospora savannicola, Paraglomus occultum, Rhizophagus partial (*Unc) and *Diversispora* sp., this disease incidence was significantly ($p < 0.05$) as low as 6% and was not more than 27%. The treatment RP100 as positive control also resulted in a disease incidence lower than 27%. The AMF species *Racocetra crispa* gave the highest plant height while *R. crispa*, *P. occultum*, *S. savannicola* and *R. partial* (*Unc*) gave the highest grain yields, best symbiotic parameters and high soil phosphorus and nitrogen levels at harvest. This is the first report of *S. rolfsii*-caused collar rot disease in soybean in Benin and also of the effect of indigenous arbuscular mycorrhizal fungi on the disease incidence.

Keywords: *Bradyrhizobium*, collar rot disease, Mycorrhization, *Sclerotium rolfsii*, soybean.

INTRODUCTION

Soybean, (*Glycine max* L.), is a very important seed legume in terms of nutritional and agronomic traits (Zoundji *et al.*, 2015). Nutritionally, soybean is the richest in protein, about 40%, compared to other legumes. Agronomically, soybean, like other seed legumes, can fix atmospheric nitrogen through its nodules, thus contributing to an improvement of soil fertility (Houngnandan *et al.*, 2008). However, soybean yields are low, around 1100 kg per hectare (IITA, 2020), compared to a potential yield of 4000 $kg.ha^{-1}$ per hectare (Kpenavoun *et al.*, 2018). These low yields obtained by soybean producers are often due to low soil nutrients,

non-adapted agricultural practices (Zoundji *et al.*, 2015), unfavorable weather conditions and absence of phytosanitary protection measures against insect pests and diseases. Moreover, the often used mineral fertilizers, while improving soil fertility and crop yields, further, degrade the soil (Didagbé *et al.*, 2014), posing a problem of sustainability of soybean farming systems.

Various studies have reported that *Rhizobium* inoculation to soybean results in higher crop yield improvement when combined with the mineral phosphorous application (Javaid and Mahmood, 2010; Houngnandan *et al.*, 2020). However, mineral phosphorus applied to plants remains inaccessible and only about 15% is often available and assimilated by plants (Ismail *et al.*, 2013), the remaining 85% will certainly pollute the environment. An alternative to the application of mineral phosphorus could be the use of arbuscular mycorrhizal fungi (AMF) reported making available to the plant nutrients such as phosphorus and water (Javaid, 2009; Diédhiou *et al.*, 2016). They (AMF) are also known to be effective in controlling insect pests (Koricheva *et al.*, 2009) and diseases of various crops, and this efficacy is said to be enhanced when the soybean crop is firstly seed-inoculated with *Rhizobium* (Rahman *et al.*, 2017). Overall, mycorrhized plants experience improved growth and health and gain increased protection against plant diseases and environmental conditions unfavorable to their survival (Rahman *et al.*, 2017). Thus, AMF plays an important role in protecting plants against pathogens, for example, those causing seedling collar rot (Sharma *et al.*, 2011), damping-off and wilting caused by pathogens such as *Sclerotium rolfsii* Sacc., *Verticilium* sp. and *Fusarium* sp (Rahman *et al.*, 2017). Inoculation of plants with mycorrhizal fungi may therefore offer the dual benefit of soil fertilization and protection against pests and diseases, and be a good alternative to the use of mineral phosphorus. Thus, identifying AMF species that are effective for a specific crop or in double inoculation with *Rhizobium* to boost crop yields becomes a challenge at any cost (Javaid *et al.*, 1993, 1994; Javaid and Khan, 2019).

A survey in the major soybean production areas in northern Benin revealed symptoms of wilting with collar rot of soybean plants (Adandonon, pers comm.). No research work was done before to report about the incidence of the disease with the associated causal agents. Moreover, so far, no measure was identified to control the soybean collar rot disease in Benin. During the recent survey, in 2020, in the soybean-producing areas, diverse AMF species in the rhizosphere of the crop (Adoho, 2020) were identified and revealed eight AMF species found in mostly all agro-ecological zones surveyed in the country. Although studies were carried out on mycorrhizal fungi and rhizobial inoculation in Benin, to the best of our knowledge, there was little or no information in Benin on the use of local mycorrhizae combined with *Bradyrhizobium* to control pests or diseases like collar rot diseases in soybean. The objective of the current study was to evaluate the effect of these eight indigenous mycorrhizae on collar rot disease and the yield of *Bradyrhizobium* - inoculated soybean.

MATERIALS AND METHODS

Experimental site

The trial was set up in August 2020 in Ina, Bembèrèkè, Department of Borgou, Benin. The soil types were tropical ferruginous soils and relatively well-drained, medium concretion ferralitic soils (Kombienou *et al.*, 2015). The soil characteristics of the study area were the following (Fagnibo, 2021): Water 6.4, Nitrogen 0.03%, Carbon 0.72%, C/N 26, Assimilable Phosphorous 8.57, Limon 11.08%, Clay 23.33%, Sand 63.47%.

The climate is Sudanese, gradually changing to North Sudanese in the far north. This part of the country has one dry season from November to March and one rainy season from April to October with an annual rainfall of around 1,200 mm. Monthly rainfall during the experimental period from August to November 2020 varied between 100 to 350 mm with an average of around 225 mm per month.

Plant material

The soybean variety used was TGX 1910-14F (Ferme semencière Alafiarou, Parakou, Bénin) with a cycle of 90 to 100 days.

Microbiological material

The *Rhizobium* inocula and mycorrhizae species used were obtained from the Laboratoire de Microbiologie des Sols et d'Ecologie Microbienne (LMSES, UAC, Calavi, Benin). The rhizobial strain was *Bradyrhizobium diazoefficiens* USDA 110. Eight species of mycorrhizae were tested: *Acaulospora* sp. (white without hyphae), *Racocetra crispa* (black with hyphae), *Acaulospora denticulate* (yellow without hyphae), *Gigaspora magarita* (white with hyphae), *Scutellospora savannicola* (brown without hyphae), *Paraglomus occultum* (yellow with hyphae), *Rhizophagus partial* (Unc) (brown with hyphae) and *Diversispora* sp. (black without hyphae). These indigenous mycorrhizal fungi were collected from the fields in earlier research in Benin (Adoho, 2020) and were multiplied using a bait plant in pot soil in the laboratory (LMSES, UAC, Calavi, Benin). Inoculum of these AMF was then provided by the laboratory and tested for their dual performance in the field for collar rot disease control

to improve soil fertility improvement.

Soil tests for nitrogen and phosphorus status before and after mycorrhizae application

Soil samples (500 g) were taken at the beginning and end of the trial at different locations on the experimental site to a depth of 20 cm. Composites were made after a homogeneous mixing of the samples. Total nitrogen was determined by the Kjeldahl 1883 method (Houngnandan *et al.*, 2020). Assimilable phosphorus was determined using the Bray method (Menage and Pridmore, 1973).

Experimental design and setting up of the trial

The experimental set-up was the Complete Randomized Block (CRB) comprising 11 treatments repeated four times. The 11 treatments were: eight species of mycorrhizal fungi in double inoculation each with *Bradyrhizobium* and three controls, firstly no inoculum application, secondly *Bradyrhizobium* inoculation alone and thirdly *Bradyrhizobium* inoculation plus the application of 100 kg of phosphorus Pentoxide **(**P2O5 46%). A flat ploughing was carried out and the experimental units of size 6 m × 4 m were demarcated from each other by a 2-m distance between two consecutive blocks. The sowing density was 0.50 m × 0.20 m with three seeds per hole. During the trial, two weeding were carried out, the first 14 days after sowing and the second 60 days after sowing.

Inoculation of the mycorrhizal fungus and sowing

Mycorrhizal inoculum (10 g) of each species was placed in the seeding hole while the soybean seeds were previously inoculated by coating with *Bradyrhizobium* inoculum (25%) (LMSEM, FSA, UAC). In a liquid medium, *Bradyrhizobium* species take 3 to 5 days to create moderate turbidity and 6 to 8 hours to double in population size. *Bradyrhizobium* was inoculated by coating the seeds at the rate of 100 g *Bradyrhizobium* for 15 kg of soybean seeds.

Effect of mycorrhizal fungi on collar rot of *Bradyrhizobium*-inoculated soybean plants

Each experimental unit was observed and the number of plants showing a characteristic of collar rot was recorded to determine the incidence of the disease. Observations were made on the two diagonals at two different dates (four and five weeks after sowing). All soybean plants within each diagonal were examined and the number of plants showing symptoms of stem collar rot was recorded and tape marked. Disease incidence was expressed as the percentage of diseased plants calculated as a percentage of the number of diseased plants out of the total number of observed plants in the experimental unit.

Samples of diseased plants (stems and roots) were collected, three diseased plants per experimental unit, and brought back to the laboratory for isolation, identification and pathogenicity of the disease causal agents. Portions (2 × 2 mm^2) of the diseased plant samples were placed on PDA culture medium and incubated in the laboratory to isolate the various associated microorganisms. The isolated microorganisms were pure cultured and stored at 4°C (Adandonon *et al*., 2005a,b). The identification of the microorganisms was done using the mycological identification key of Stevens (1974) taking into account the shapes of the colonies on the culture medium.

The pathogenicity test was conducted under greenhouse conditions at the Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC), using the various isolated microorganisms. Soybean seeds were surface disinfected with 1% sodium hypochlorite (NaOCl) for 2 min, rinsed twice with sterile distilled water and then sown in the pots (14 cm diameter × 18 cm height) at four seeds per pot filled to ¾ of pasteurized soil. The identified microorganisms were inoculated to healthy soybean plants under a greenhouse 10 days after sowing (Adandonon *et al.*, 2005a). For the inoculation, 1 × 1 cm block of PDA with mycelium was cut from a pure culture of the fungus on PDA and deposited at the plant collar by mixing the medium block with soil around the collar of the plant. This plant was then inoculated with the fungus being tested for pathogenicity. Another block of PDA without the fungus culture was also cut out and mixed with soil around the collar of another plant and served as an uninoculated control. The plants were watered every two days and monitored for the development of disease symptoms (Adandonon *et al.*, 2005a). Stems and roots from plants with collar rot symptoms were sampled from the inoculated plants and grown for confirmation of the causal agent.

Effect of mycorrhizae on height growth, plant chlorophyll content and grain yield of *Bradyrhizobium*-inoculated soybeans

Six weeks after sowing, height growth and chlorophyll content of the plants were evaluated (Sharma *et al*., 2011) on eight plants per experimental unit with 2 plants randomly selected per line. Plant height was measured from the collar to the last leaflet at the top of the plant and leaf chlorophyll content was recorded with a SPAD at six weeks after sowing. The SPAD Chlorophyll Meter instantly measures chlorophyll content or “greenness” of

plants to reduce the risk of yield-limiting deficiencies. The SPAD chlorophyll meter was simply clamped over leafy tissue and it was received an indexed chlorophyll content reading (-9.9 to 199.9) in less than 2 seconds. Nitrogen needs were assessed by comparing in-field SPAD readings to university guidelines (Uddling *et al.*, 2007).

Grain yield was assessed at harvest. From an area of 6 m^2 per treatment, the soybean pods were collected and brought back to the laboratory. The pods were oven-dried at 65°C for 72 h. Soybeans pods and seeds were weighed and the formula of Sharma *et al.* (2011) was used to determine the grain yield as:

$$R_G \text{ (kg DW/ha)} = \frac{(go * gt)}{(g1 * Au)} * 10.000$$

g0 = Dry weight of the full pod sample;
*g*1 = Fresh weight of the full pod sample;
gt = Total fresh weight of full pods on the working area;
Au = considered area (m^2) for pod harvest.

Effect of mycorrhizal fungi on symbiotic parameters of *Bradyrhizobium*-inoculated soybean

Roots were sampled 45 days after sowing (flowering stage) in a 1-m^2 area delineated in each experimental unit. The collected roots were brought back to the laboratory to evaluate the symbiotic parameters.

For nodulation parameters, the fresh weight of nodules per plant was assessed using the modified method of Sharma *et al*. (2011). The fresh weight of nodules was taken and the nodules were kept on silica gel and cotton.

The mycorrhization parameters were assessed after staining the roots taken from the sampled plants, following the method of Phillips and Hayman (1970). After washing with tap water, fine roots were selected and cut into about 1-cm long fragments. They were then soaked in a KOH solution (10%), and heated in a 90°C water bath for 45-60 min. They were then rinsed with water, immersed in an acidic trypan blue solution (0.5%) before being rinsed again for microscopic observation. Fungal structures such as arbuscules, vesicles and mycelium turn blue. The annotation of the mycorrhizal infection was done following the method of Trouvelot *et al.* (1986). For each treatment, 60 fine root fragments of about 1 cm in length were mounted between slides in glycerol.

The mycorrhization frequency or infection frequency (F%), as well as the mycorrhization intensity (I%) of the root system, were assessed under the light microscope. The degree of endomycorrhizal colonization of each fragment by vesicles or arbuscules was estimated using a six-class scale rated from 0 to 5.

The mycorrhization frequency, F%, is determined by the following formula Trouvelot et al. (1986):

F% = (number of mycorrhizal fragments / total number of fragments observed) × 100

The mycorrhization intensity, I %, is determined by the formula Trouvelot *et al.* (1986):

I% = (95n5 + 70n4 + 30n3 + 5n2 + n1) / total number of fragments observed

where n5 = number of fragments scored 5, n4 = number of fragments scored 4, n3 = number of fragments scored 3, n2 = number of fragments scored 2 and n1 = number of fragments scored 1.

Statistical analysis

The statistical analyses were carried out with SAS software version 9.4. after checking the conditions of normality and equality of variances. An analysis of variance (ANOVA) of the data was then carried out using the general linear model (GLM) and the separation of the means was carried out on the basis of the Student-Newman-Keuls test at the 5% threshold.

RESULTS

Effect of mycorrhizal fungi on the incidence of collar rot disease in *Bradyrhizobium*-inoculated soybean plants

Table 1 shows the incidence of collar rot in soybean plants as a function of the mycorrhizae species applied. It shows that the untreated control (no *Bradyrhizobium* and no mycorrhiza inoculation) has the most significantly ($p < 0.05$) high disease incidence ranging from 69 to 78%. The lowest incidence ($p < 0.05$) ranging from 6 to 27% was recorded in the treatments with *Bradyrhizobium* inoculation doubled with the application of mycorrhizae species *R. crispa*, *G. margarita, S. savannicola*, *P. occultum*, *R. partial* (Unc) and *Diversispora* sp. The fungus *S. rolfsii* was isolated from all diseased plants and the pathogenicity test indicated that it was the causal agent responsible for the soybean collar rot disease observed during the current study. The observed symptoms of the disease were wilting and collar rot of the plants with white mycelia and sclerotia in the lesion and on the soil around the diseased plants. This is the first time that *S. rolfsii* has been identified as causing soybean collar rot in Benin.

Effect of mycorrhizae on height growth, leaf chlorophyll content and grain yield of soybean previously inoculated with *Rhizobium*

Table 2 shows the effect of double inoculation on plant

Table 1. Incidence of collar rot in soybean plants according to mycorrhizal species tested in double inoculation with *Rhyzobium*[a].

Treatments	Disease incidence (%)	
	21/09/20	29/09/20
R0M0	69.34 ± 0.80c	78.35 ± 0.80d
RM0	16.13 ± 0.90ab	41.08 ± 0.50bc
RM1	27.09 ± 2.10ab	48.29 ± 1.80c
RM2	11.15 ± 1.00ab	25.17 ± 0.80a
RM3	18.02 ± 0.50b	38.30 ± 0.50b
RM4	6.07 ± 1.10a	19.01 ± 0.50a
RM5	14.12 ± 0.50ab	22.26 ± 0.70a
RM6	9.31 ± 0.90ab	23.51 ± 0.70a
RM7	11.12 ± 0.50ab	20.21 ± 0.50a
RM8	11.02 ± 0.80ab	27.30 ± 0.50a
RP100	8.26 ± 0.70ab	22.11 ± 0.60a
F value	58.54	76.39
p value	<.05	<.05

[a]In each column, means followed by the same letter (a, b, c or d) are not significantly different (5%) using SAS software and the Student-Newman-Keuls test. Means ± Standard Error. RM1 = *Acaulospora sp,* RM2 = *Racocetra crispa,* RM3 = *Acaulospora denticulate,* RM4 = *Gigaspora margarita,* RM5 = *Scutellospora savannicola,* RM6 = *Paraglomus occultum,* RM7 = *Rhizophagus partial (Unc),* RM8 = *Diversispora sp, where R = application of Rhizobium,* RP100 = application of *Rhyzobium* and 100 kg phosphorus, RM0 = application of *Rhizobium* without mycorrhiza, R0M0 = absolute control without any inoculation

Table 2. Effect of Rhizobium-inoculated soybeans and mycorrhizal fungi application on Plant height, chlorophyll content at six weeks after sowing and grain yield at harvest[a].

Treatments	Height (cm)	Chlorophyll content (%)	Grain yield (kg.ha^{-1})
R0M0	51.90 ± 1.43cde	38. 40 ± 084	582.30 ± 47.52f
RM0	56.40 ± 1.29bcd	41.79 ± 0.57	1443.60 ± 129.90e
RM1	51.53 ± 2.50de	40.29 ± 0.77	2649.50 ± 359.62c
RM2	63.78 ± 0.95a	41.89 ± 0.47	3857.30 ± 182.91a
RM3	48.17 ± 1.49e	39.11 ± 0.90	1659.30 ± 234.31de
RM4	60.10 ±0.85ab	41.92 ± 0.43	2171.00 ± 43.88cde
RM5	57.12 ±0.89bc	40.29 ± 0.65	3102.20 ± 198.03abc
RM6	56.51 ± 1.51bcd	39.69 ± 1.31	3537.40 ± 148.27ab
RM7	59.82 ± 0.82ab	40.75 ± 0.52	2948.90 ± 434.30bc
RM8	56.35 ± 1.07bcd	41.26 ± 0.69	2316.90 ± 281.74cde
RP100	61.04 ± 1.39ab	38.66 ± 0.96	2477.80 ± 78.06cd
F value	11.38	2.73	17.43
p value	<0.001	0.003	<0.001

[a]In each column, means followed by the same letter (a, b, c or d) are not significantly different at the 5% threshold using SAS software and the Student-Newman-Keuls test. Means ± Standard Error. RM1 = *Acaulospora sp,* RM2 = *Racocetra crispa,* RM3 = *Acaulospora denticulate,* RM4 = *Gigaspora margarita,* RM5 = *Scutellospora savannicola,* RM6 = *Paraglomus occultum,* RM7 = *Rhizophagus partial (Unc),* RM8 = *Diversispora sp, where R is the application of Rhizobium,* RP100 = application of *Rhyzobium* and 100 kg phosphorus, RM0 = application of *Rhizobium* without mycorrhiza, R0M0 = absolute control without any inoculation.

height and chlorophyll content of soybean leaves at six weeks after sowing as well as grain yield at harvest. For plant height, the highest mean (63.78 cm) was obtained in the treatment inoculated with the mycorrhizal fungus species, *R. crispa*, compared to the untreated control (51.90 ± 1.43) and the other mycorrhizal species. There was no significant difference ($p > 0.05$) between the treatment with the mycorrhizal fungal species, *R. crispa*,

Table 3. Effect of double inoculation on soybean symbiotic parameters*.

Treatments	Nodule fresh weight nodules (g)	Frequency of mycorrhization (%)	Intensity of mycorhization (%)
R0M0	11.35 ± 0.83d	8.75 ± 2.55d	0.20 ± 0.05e
RM0	11.19 ± 0.54d	27.50 ± 1.57b	0.44 ± 0.05de
RM1	17.29 ± 0.61bc	20.41 ± 1.79bc	0.34 ± 0.03de
RM2	15.58 ± 1.61bcd	39.16 ± 2.53a	3.63 ± 0.19a
RM3	11.72 ± 0.69d	18.33 ± 1.42c	0.50 ± 0.08de
RM4	18.46 ± 1.19bc	36.66 ± 1.78a	1.02 ± 0.14d
RM5	19.39 ± 1.34ab	38.33 ± 3.04a	3.04 ± 0.21b
RM6	14.24 ± 0.97cd	40.00 ± 2.61a	4.00 ± 0.31a
RM7	22.46 ± 2.05a	27.50 ± 1.99b	0.47 ± 0.4de
RM8	16.00 ± 0.38bcd	20.83 ± 1.04bc	0.44 ± 0.05de
RP100	15.70 ± 0.85bcd	24.58 ± 2.34bc	1.58 ± 0.42c
F value	10.23	22.59	58.01
p value	<0.001	<0.001	<0.001

[a]In each column, means followed by the same letter (a, b, c or d) are not significantly different (5%) using SAS software and the Student-Newman-Keuls test. Means ± Standard Error. RM1 = *Acaulospora sp.,* RM2 = *Racocetra crispa,* RM3 = *Acaulospora denticulate,* RM4 = *Gigaspora margarita,* RM5 = *Scutellospora savannicola,* RM6 = *Paraglomus occultum,* RM7 = *Rhizophagus partial (Unc),* RM8 = *Diversispora sp, with R the application of Rhizobium,* RP100 = application of *Rhyzobium* and 100 kg phosphorus, RM0 = application of *Rhizobium* without mycorrhiza, R0M0 = absolute control without any inoculation.

and the plot fertilized with 100 kg phosphorus (61.04 ± 1.39).

For chlorophyll content, no significant difference ($p>0.05$) was observed between treatments. At harvest, the grain yields evaluated (Table 2) showed that double inoculation had a significant effect ($p < 0.001$) on this parameter regardless of the mycorrhizal fungal species applied. The mycorrhizal species *R. crispa*, *P. occultum* and *S. savannicola* gave the highest grain yields, respectively 3857.3 kg.ha^{-1}, 3537.4 kg.ha^{-1} and 3102.2 kg.ha^{-1}, followed by *Rhizophagus partial* (Unc) (2948.90 kg.ha^{-1}), compared to the yield obtained from the untreated control (582.30 kg.ha^{-1}) and the *Rhizobium* treatment with 100 kg phosphorus application (2477.8 kg.ha^{-1}).

Effect of mycorrhizal fungi on symbiotic parameters of soybean previously inoculated with *Rhizobium*

Table 3 shows the effect of double inoculation on nodulation and mycorrhization parameters. There was a significant effect ($p < 0.001$) observed on nodule fresh weight, mycorrhization frequency and mycorrhization intensity at 6 weeks after sowing. The highest mean fresh weight of nodules per square meter was obtained with *Rhizophagus* partial (Unc) (22.46 g) application compared to the untreated control (11.35 g). There was a significant difference ($p < 0.05$) between this treatment and those with other fungal species and the 100kg phosphorus supply. Regarding the mycorrhization parameters, the best mycorrhization frequencies and intensities were obtained with the application of *P. occultum* and *R. crispa* species with an average of 40% and 39% for the frequency and 4% and 3.6% for the intensity of mycorrhization, respectively, compared to the untreated control. Application of 100 kg phosphorus also led to a mycorrhization frequency of 24.58% and a mycorrhization intensity of 1.58%, which may correspond to the activities of the existing mycorrhizae in the soil before the trial (Table 3).

Physico-chemical characteristics of the soil in the study area at sowing and harvesting of soybeans

The soil test results (Table 4) showed that there was, in general, an increase in soil phosphorus at harvest compared to at sowing in all plots that received both *Rhizobium* inoculation and mycorrhizal fungi. The same is true for the plot that received *Rhizobium* inoculation with an application of 100kg of phosphorus. Plots without *Rhizobium* inoculation and/or mycorrhizal fungi also showed an increase, but more than two times less than plots with both *Rhizobium* and mycorrhizal fungi (Table 4). The trend is similar to that observed for soil nitrogen levels, but in some cases to a lesser extent.

DISCUSSION

Organic farming is nowadays an important issue and useful microorganisms such as arbuscular mycorrhizal fungi are often used to take advantage of the many benefits they offer in terms of ecological crop protection (Rahman *et al.*, 2017) and biological soil fertilization

Table 4. Physico-chemical characteristics of the experimental site soil[a].

Treatments	Ass P at sowing**	Ass P at soybean harvest	Soil N at sowing**	Soil N at soybean harvest
R0M0	8.57	18.590 ± 1.07b	0.03	0.026533 ± 0.01e
RM0	8.57	17.303 ± 1.33b	0.03	0.034400 ± 0.01de
RM1	8.57	45.213 ± 1.88a	0.03	0.059467 ± 0.01ab
RM2	8.57	45.023 ± 0.25a	0.03	0.071733 ± 0.01a
RM3	8.57	45.703 ± 0.77a	0.03	0.042000 ± 0.01cd
RM4	8.57	49.410 ± 2.63a	0.03	0.068133 ± 0.002a
RM5	8.57	43.727 ± 1.59a	0.03	0.048533 ± 0.0009bc
RM6	8.57	45.623 ± 1.23a	0.03	0.060667 ± 0.003ab
RM7	8.57	46.833 ± 0;96a	0.03	0.076000 ± 0.005a
RM8	8.57	45.027 ± 1.67a	0.03	0.063733 ± 0.001a
RP100	8.57	47.797 ± 2.04a	0.03	0.070000 ± 0.002a
F value	-	55.81	-	17.86
p value	-	<.0001	-	<.0001

[a]In each column, means followed by the same letter (a, b, c or d) are not significantly different (5%) using SAS software and the Student-Newman-Keuls test. Means ± Standard Error. RM1 = *Acaulospora sp,* RM2 = *Racocetra crispa,* RM3 = *Acaulospora denticulate,* RM4 = *Gigaspora margarita,* RM5 = *Scutellospora savannicola,* RM6 = *Paraglomus occultum,* RM7 = *Rhizophagus partial (Unc),* RM8 = *Diversispora sp, where R is the application of Rhizobium,* RP100 = application of *Rhyzobium* and 100 kg phosphorus, RM0 = application of *Rhizobium* without mycorrhiza, R0M0 = absolute control without any inoculation. Ass P: Assimilable phosphorus; Soil N = Soil nitrogen

(Houngnandan *et al.*, 2020). Dealing with various constraints of pests and diseases and low soil fertility, mycorrhizae are a good alternative to synthetic chemicals. The present work investigated the influence of different indigenous species of mycorrhizal fungi on the collar rot disease of soybean plants as well as on yield and soil fertility. This disease is known to cause significant damage to crops such as tomato (*Lycopersicon esculentum* Miller), cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.), cowpea (*Vigna unguiculata* (L.) Walp., etc. (Adandonon *et al.*, 2005a,b) and is responsible for soybean yield losses in Benin recorded earlier and during the current study. The disease symptoms in the field included wilting with rotting at the collar of the diseased plants. This is often followed by a general wilting and death of the diseased plants as reported on cowpea by Adandonon *et al.* (2005a,b). The results of the present study showed, through the pathogenicity test, that the causal agent of the disease on soybean is the fungus *S. rolfsii* as reported earlier on cowpea in Benin (Adandonon *et al.*, 2005a). *Sclerotium rolfsii* is a soil-borne fungus whose sclerotia are hosted by plant debris (Punja, 1985). High nitrogen fertilization, lack of potash and phosphorus favor the development of the disease (Institut Ecoumène Golf and Environnement, 2016).

Control of the disease caused by this fungus is often difficult because the pathogen causes the disease to a wide range of crops and its sclerotia can live in the soil for several years (Punja, 1985; Adandonon *et al*., 2005a). Various control methods, such as cultural practices (rotation, crop association, deep ploughing by turning over the soil, destruction of crop debris, etc.) and chemical control, are considered but mostly not very effective. The sclerotia of the pathogen last a long time in the soil, making crop rotation and combination unsuccessful (Punja, 1985; Adandonon *et al.*, 2005a). Chemical methods such as the use of pesticides (fungicides) (Punja, 1985) or the application of phosphorus-based fertilizers (Punja, 1985) control the pathogen, but they are expensive, inaccessible and are likely to cause environmental pollution and health problems for users. Mycorrhizal fungi applied to crops are reported to be able to make nutrients, especially phosphorus, and water available to the plant (Houngnandan *et al.*, 2009; Diédhiou *et al.*, 2016) leading to good plant growth and protection against pests and diseases and consequently improved yields (Koricheva *et al.*, 2009; Rahman *et al.*, 2017). In the current study, indigenous AMF collected from the fields in mostly all agroecological zones surveyed in Benin (Adoho, 2020) were tested for their performance in the field to control collar rot disease and to improve soil fertility. The various eight mycorrhizae species tested, namely *Acaulospora* sp, *R. crispa*, *A. denticulate*, *G. magarita*, *S. savannicola*, *P. occultum*, *R. partial* (Unc) and *Diversispora* sp., and the application of 100 kg of phosphorus led to a reduction of the incidence of collar rot disease in soybean plants previously inoculated with *Rhizobium*. Rahman *et al.* (2017) observed a similar trend during their study on the effect of double inoculation on the incidence of this disease caused to Square Pea (*Lathyrus sativus* L.). These authors reported that the combined action of mycorrhizal fungi applied to the legumes resulted in a

high photosynthetic rate with a significant impact on root and stem development and dry biomass through increased nodulation, nitrogen fixation and mycorrhization. Other authors, such as Geneva *et al.* (2006) on pea (*Pisum sativum* L.)**,** Hernandez and Hernandez (1996) on soybean and Khanam *et al.* (2005) on chickpea (*Cicer arietinum* L.), have reached the same conclusion.

The results in the current study showed that application of mycorrhizae species coupled with rhizobial inoculation led to better collar rot disease control, height growth, high dry weight of nodules, improved grain yield, good nodulation and mycorrhization (frequency and intensity) with high phosphorus and nitrogen at harvest. This confirms that AMF, in symbiotic association with their host roots, represents an asset for agriculture (Rahman *et al.*, 2017) through improved plant growth and a consequent increase in yield. Earlier work has reported several mechanisms said to be involved in the arbuscular mycorrhizal fungi and soil pathogens interactions, but it is still unclear mechanisms for the disease suppression (Linderrnan, 1994). However, Dehne and Schonbeck (1979) indicated that in this association, mycorrhizal roots are more lignified and vigorous, contain more polysaccharides, and show lots of chitinolytic activities. Plants in association with mycorrhizas of pathogenic fungi have root exudations that affect microbial populations (Vance *et al.*, 1987) and these beneficial bacteria or fungi present in this AM environment boost plant growth (Azcon-Aguilar and Barea, 1992). Consequently, the high nutritional capacity of vigorous AM improves plant growth and is said to play an important role in tolerance against the soil-borne pathogen (Dehne, 1982; Alam *et al*., 2008) leading to reduction of disease incidence (Linderman, 1994). This is even more significant when soybean seeds are firstly inoculated with *Rhizobium* (Chakraborty and Purkayastha, 1984) before AMF application. Other reports confirmed this mechanism suggestion (Bhosale and Navale, 2006; Fritz *et al.*, 2006; Alam *et al.*, 2008). The current study results are supported by these mechanisms and could explain the low incidence of collar rot diseases, improved soil nutrient (P and N) status and mycorrhization and soybean yield recorded following application of AMF to *Bradyrhizobium*-inoculated soybean. These are consistent with research works of Khanam *et al.* (2005), Hernandez and Hernandez (1996), Geneva *et al.* (2006) and Rahman *et al.* (2017). The high grain yield and high phosphorus and nitrogen levels obtained at harvest from plots that received double inoculation with mycorrhizae were evidence of the enhanced fertility of the soils that received these mycorrhizae. Graham (2001) indicated that colonization by mycorrhizal fungi could protect plant roots, particularly under conditions of soil nutrient deficiency. In our study, the soil was initially low in phosphorus and nitrogen but experienced an increase in these elements during the trial following the application of mycorrhizal fungi. Thus, the colonization of the mycorrhizal fungi resulted in the mobilization of phosphorus and nitrogen, thus improving the fertility of the soil and thus the ability of the plant to carry out sufficient photosynthesis which led to plant growth and development. According to Gill *et al.* (1985), mycorrhizal fungi solubilise phosphorus inaccessible to the soybean root system, making this nutrient available to the roots, a requirement of legumes including soybeans for their growth. Moreover, various studies have reported that double inoculation with *Rhizobium* plus mycorrhizae led to tolerance of plants against a variety of pathogens causing damping-off and collar rot (Lynd and Ansman, 1994; Graham, 2001; Larsen and Bodker, 2001). Mycorrhizal fungi effect on the pathogen was said to be detectable through a record of enzymatic activity of the pathogen due to the presence of the mycorrhizal fungi (Kjøller and Rosendahl, 1996). The results showed in the current study that although these attributes were recorded in all the mycorrhizal fungi species studied and also reported by Rahman *et al.* (2017), the level of performance is uneven. The best performing local mycorrhizal fungi species during this study was *R. crispa*, followed by *P. occultum*, *S. savannicola* and R. partial (Unc) which gave low incidences of *Bradyrhizobium*-inoculated soybean plant collar rot disease, good nodulation, plant growth, good mycorrhization frequency and/or intensity and best grain yields. The performance of these mycorrhizal fungi species was higher than or similar to that of *Bradyrhizobium* inoculation of soybean with 100 kg phosphorus application in a previous study conducted by Houngnandan *et al.* (2020). Thus, these results confirm the effect of *Bradyrhizobium* inoculation of the crop with 100 kg of phosphorus on soybean yield reported by Houngnandan *et al.* (2020). The results also show the effectiveness of the *Bradyrhizobium*-Mycorrhizae double inoculation on soil fertility, development of the crop, and crop protection against soybean collar rot disease. Thus, these local mycorrhizal fungal species are the most emerging among those studied here with regard to earlier published results (Hernandez and Hernandez, 1996; Khanam *et al*., 2005; Geneva *et al*., 2006; Rahman *et al.*, 2017; Houngnandan *et al.*, 2020). The efficiency of *R. crispa* reported by previous work could be because this species is well adapted to the soybean habitat. Similar work was carried out in Niger on cowpea by Aboubacar *et al.* (2013) and these authors reported a similar performance of *R. crispa*. According to them, double inoculation improves the agronomic performance of cowpea, especially grain yield. The effectiveness of this local *R. crispa* species in Benin was also shown in the current study. As important findings, the inoculum of this species could be produced and applied in the field to improve soybean yield. *Acaulospora* species were the least successful in this

study although these have been shown to confer bio-protective effects on cassava plants against the nematode Meloidogyne spp. ranging from resistance to tolerance (Séry *et al.*, 2016). The low mycorrhization rate recorded in plants inoculated with *A. denticulate* could be intrinsic to this mycorrhizae species and its interaction with the crop as reported by Krisnarini *et al.* (2018). Further studies could be carried out in different agro-ecological zones of soybean production to test the effect of different doses of these species on crop development in the field.

ACKNOWLEDGEMENT

We thank the Laboratoire de Microbiologie du Sol et d'Ecologie Microbienne (LMSEM) for the inocula of the mycorrhizal fungi and rhizobial bacteria used.

REFERENCES

Aboubacar K, Ousmane ZM, Amadou HI, Issaka S, Zoubeirou AM (2013). Effect of co inoculation of rhizobium and mycorrhizae on agronomic performance of cowpea, Niger. J. Appli. Biosci. 72:5846-5854.

Adandonon A, Aveling TAS, Labuschagne N, Ahohuendo BC (2005a). Etiology of and effect of environmental factors on damping-off and stem rot of cowpea in Benin. *Phytoparasitica.* 33 (1):65-72.

Adandonon A, Aveling TAS, Tamo M (2005b). Occurrence and distribution of cowpea damping-off and stem rot and associated fungi in Benin. J. Agric. Sci. 142:561-566.

Adoho T (2020). Study of arbuscular mycorrhizal fungi species associated with soybean (*Glycine max* L. *Merrill*) in major soybean production areas in Benin. *Master thesis*, Faculty of Agronomic Sciences (FSA), University of Abomey Calavi (UAC), Calavi, Benin. p. 81.

Alam F, Khanam D, Bhuiyan MAH, Rahman MME, Salahin N (2008). Effect of Arbuscular Mycorrhiza on Damping off Disease of Tomato. Bangladesh J. Agriculturist. 1(2):187-192.

Azcón-Aguilar C, **Barea JM (1992).** Interactions between mycorrhizal fungi and other rhizosphere microorganisms**.** In: Mycorrhizal functioning. An integrative plant-fungal process, Allen MJ, (Eds.), Chapman and Hall, New York, USA, pp. 163-198.

Bhosale DM, Navale AM (2006). Response to Rangpurlime seedlings to VA-mycorrhizal inoculation. Lnter. J. Plant Sci. 1(2):202-204.

Chakraborty U, Purkayastha RP (1984). Role of rhizobitoxine in protecting soybean roots from Macraphomina phaseolrna infection. Canadian J. Microbiol. 30:285-289.

Dehne HW (1982). Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopath. Z. 72:1115-1119.

Dehne HW, Schonbeck F (1979). The influence of endophytic mycorrhiza on plant diseases ll. Phenol metaboiism and lignification. Phytopath. Z. 95:210-216.

Didagbé OY, Houngnandan P, Sina H, Zoundji CC, Kouelo AF, Lakou J, Toukourou F, Baba- Moussa L (2014). Response of groundnut (*Arachis hypogaea* L.) to exogenous *Bradyrhizobium sp* strains inoculation and phosphorus supply in two agro-ecological zones of Benin, West Africa. J. Exp. Biol. Agric. Sci. 2(6):623-634.

Diédhiou AG, Mbaye FK, Mbodj D, Faye MN, Pignoly S, Ndoye I, Djaman K, Gaye S, Kane A, Laplaze L, Manneh B, Champion A (2016). Field Trials Reveal Ecotype-Specific Responses to Mycorrhizal Inoculation in Rice. *PLoS ONE* 11(12):471-579.

Fagnibo AH (2021). Effect of double rhizobial and mycorrhizal inoculation on the agronomic performance of soybean (*Glycine max L*. *Merill*) sown in Northern Benin. MSc thesis, Faculty of Agronomic Sciences (FSA), UAC, Abomey-Calavi. R. Benin. pp. 53.

Fritz M, Jakobsen I, Lyngkjaer MF, Christensen HT, Kuhnemann JP (2006). Arbuscular mycorrhiza reduces susceptibility to tomato to Alternaria solani. Mycorrhiza 16:413-419.

Geneva M, Zehirov G, Djonova E, Kaloyanova N, Georgiev G, Stancheva I (2006). The effect of inoculation of pea plants with mycorrhizal fungi and *Rhizobium* on nitrogen and phosphorus assimilation. Plant Soil Environ. 52(10):435-440.

Gill MA, Naimat A, Nayyer MM (1985). Relative effect of phosphorus combined with potash and *Rhizobium phaseoli* on the yield of *Vigna radiata* (Mung). J. Agric. Res. 23:279-282.

Graham JH (2001). What do root pathogens see in mycorrhizas? New Phytologist. 148:357-359.

Hernandez A, Hernandez AN (1996). Effect of the AM-Rhizobium interaction in cultivation of soybeans (*Glycine max*). Cult. Tropi. 17:5-7.

Houngnandan HB, Adandonon A, Akplo TM, Zoundji CC, Kouelo AF, Zeze A, Houngnandan P, Bodjrenou R, Dehoue H, Akinocho J (2020). Effect of rhizobial inoculation combined with phosphorus fertilizer on nitrogen accumulation, growth and yield of soybean in Benin. J. Soil Sci. Environ. Manag. 11(4):153-163.

Houngnandan P, Yemadje RGH, Oikeh SO, Djidohokpin CF, Boeckx P, Van Cleemput O (2008). Improved estimation of biological nitrogen fixation of soybean cultivars (*Glycine max* L. Merril) using N-15 natural abundance technique. *Biol Fertil Soils* 45:175-183.

Institut Ecoumène Golf & Environnement, (2016). Bulletin d'alerte N°7 au 27 juin 2016. www.ecoumenegolf.org/xBulletin%20d'alerte%20n%207%20du%2027%20juin%202016.pdf.

International Institute of Tropical Agriculture (IITA) (2004). Soybean crop Improvement. https://www.iita.org/research/our-research-themes/improving-crops/soybean-crop-improvement/.

Ismail Y, McCormick S, Hijri M (2013). The arbuscular mycorrhizal fungus, *Glomus irregulare*, controls the mycotoxin production of *Fusarium sambucinum* in the pathogenesis of potato. FEMS. Microbiol. Let. 348(1):46-51.

Javaid A, Hafeez FY, Iqbal SH (1993). Interaction between vesicular arbuscular (VA) mycorrhiza and *Rhizobium* and their effect on biomass, nodulation and nitrogen fixation in *Vigna radiata* (L.) Wilczek. *Science International (Lahore)* 5(4):395-396.

Javaid A, Iqbal SH, Hafeez FY (1994). Effect of different strains of *Bradyrhizobium* and two types of vesicular arbuscular (VA) mycorrhizae on nodulation in *Vigna radiata* (L.) Wilczek var. NM 20-21. *Science International (Lahore)* 6(1):87-89.

Javaid A (2009). Arbuscular mycorrhizal mediated nutrition in plants. J. Plant Nutr. 32(10):1595-1618.

Javaid A, Mahmood N (2010). Growth, nodulation and yield response of soybean to biofertilizers and organic manures. Pak. J. Bot. 42(2):863-871.

Javaid A, Khan IH (2019). Mycorrhizal fungi associated with mungbean. *Mycopath* 17(1):45-48.

Khanam D, Mridha MAU, Solaiman ARM (2005). Effect of dual inoculation of arbuscular mycorrhiza and *Rhizobium* on root colonization and spore population in chickpea. Banglad. J. Microbiol. 22(1):15-18.

Kombienou PD, Arouna O, Azontondé AH, Mensah GA, Sinsin BA (2015). Characterization of the soil fertility level of the Atakora range in northwest Benin. J. Anim. Plant Sci. 25:3836-3856.

Koricheva J, Gange AC, Jones T (2009). Effects of mycorrhizal fungi on insect herbivores: a meta-analysis. Ecology 90(8):2088-2097.

Kpenavoun S, Chogou FO, Santos F, Hounhouigan DJ (2018). Technical efficiency of soybean farmers in Benin. Ann. Sci. Agro. 22(1):12-35.

Krisnarini K, Rini MV, Timotiwu PB (2018). The Growth of Oil Palm (*Elaeis guineensis* Jacq.) Seedlings with the Application of Different Arbuscular Mycorrhiza Fungi and Various Phosphorous Dosages. J.

Trop. Soils 23(3):117-124.
Larsen J, Bodker L (2001). Interactions between pea root-inhabiting fungi examined using signature fatty acids. New. Phytologist. 149:487-493.
Linderman RG (1994). Role of VAM fungi in biocontrol. In: Mycorrhizae and Plant Health, Pfleger F L and Linderman RG (Eds.), APS, St. Paul, Minne, USA, pp. 1-25.
Lynd JQ, Ansman TR (1994). Lichen mycobionts mycorrhizal symbiosis with nitrogen-fixation of showy partridge pea. J. Plant Nutri. 17(2-3):445-459.
Kjøller R, Rosendahl S (1996). The presence of the arbuscular mycorrhizal fungus *Glomus intraradices* influences enzymatic activities of the root pathogen *Aphanomyces euteiches* in pea roots. Mycorrhiza 6:487-491.
Menage, PMA, Pridmore, B (1973). Automated determination of phosphate using Bray No 1 Extractant. CSIRO Division of Soils. Notes on Soil Techniques, pp 80-82.
Phillips JM, Hayman DS (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.
Punja ZK (1985). The biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathol. 23:97-127.
Rahman M, Ali ME, Alam F, Islam MN, Bhuiyan MAH (2017). Combined Effect of Arbuscular Mycorrhiza, *Rhizobium* and *Sclerotium rolfsii* on Grass *Pea* (*Lathyrus sativus*). The Agriculturists 15(1):143-155.
Sharma R, Singh GP, Sharma VK (2011). Comparison of different media formulations on growth, morphology and chlorophyll content of green alga, *Chlorella vulgaris*. Int. J. Pharm. Bio. Sci. 2:509-516.
Séry DJ-M, Kouadjo ZGC, Voko BRR, Zézé A (2016). Selecting Native Arbuscular Mycorrhizal Fungi to Promote Cassava Growth and Increase Yield under Field Conditions. Front. Microbiol. 7:2063.
Stevens RB (1974). Mycology guidebook. Mycological Society of America, University of Washington Press, USA. pp. 703.
Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986). Mesure du taux de mycorhization VA d'un système radiculaire. Recherches et méthodes d'estimation ayant une signification fonctionnelle. In Physiological and Genetical Aspects of Mycorrhizae,Gianinazzi-Pearson V, Gianinazzi S (Eds.), INRA, Paris, pp. 217-221.
Uddling J, Gelang-Alfredsson J, Piikki K, Pleijel H (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth Res. 91(1):37-46.
Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. Soil. Biol. Biochem. 19:703-704.
Zoundji CC, Houngnandan P, Dedehouanou H, Toukourou F (2015). Determinants of soybean [*Glycine max (L.) Merrill*] production system in Benin. J. Exp. Biol. Agric. Sci. 3(5):430-439.