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Arbuscular Mycorrhizal Fungi as a Biocontrol Agent for Angular Leaf Spot Disease of Common Beans (*Phaseolus vulgaris* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Angular leaf spot disease caused by *Phaeoisariopsis griseola* is the most important disease which caused yield losses up to 80% of common beans. This study aims to induce natural defense of common bean against angular leaf spot disease by mycorrhization. The samples of *Phaeoisariopsis*

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griseola were collected in the field of 02 different agro-ecological zones considered as major bean production area in Cameroon. The *Phaeoisariopsis griseola* were isolated on PDA solid medium, and identified based on morphological. A pot experiment was carried out using a bifactorial device. The first factor was two varieties of common bean (GLP 195-C and PNG). The second factor consisted of eight treatments, namely T0 : absolute control, T1 : mycorrhizal treatment with *Acaulospora tuberculata*, T2 : mycorrhizal treatment with *Gigaspora gigantea*, T3 : mycorrhizal treatment with *Entrophospora infrequens*, T4: Terazeb synthetic fungicide treatment (positive control), T5 : mycorrhizal treatment with *Acaulospora tuberculata* and *Gigaspora gigantea* combination, T6 : mycorrhizal treatment with *Acaulospora tuberculata* and *Entrophospora infrequens* combination, T7 : mycorrhizal treatment with combination of *Gigaspora gigantea* and *Entrophospora infrequens.* Incidence, severity of angular leaf spot disease and biochemical parameters were assessed. The results showed that pots treated with the mycorrhizae *Entrophospora infrequens*, *Gigaspora gigantea*, *Acaulospora tuberculata* and combinations of mycorrhizae *Acaulospora tuberculata* and *Gigaspora gigantea*, *Acaulospora tuberculata* and *Entrophospora infrequens, Gigaspora gigantea* and *Entrophospora infrequens* contributed significantly to improved amino acid content from 44% to 70%, proline content from 20% to 33%, total phenol content from 36% to 60%, protein content from 16% to 41%, flavonoid content from 27% to 82%, tannin content from 60% to 298%, polyphenoloxidase content from 15% to 74% and peroxidase content from 31% to 109% compared with the control in the two common bean varieties. Similarly, the mycorrhizae treatments and the mycorrhizae combination significantly reduced the development of angular leaf spot disease by 20 to 80% compared with the control in both common bean varieties. This work shows that Arbuscular Mycorrhizal Fungi made a significant contribution to reducing the development of angular leaf spot disease in the pots while improving common bean grain yield.

Keywords: Phaseolus vulgaris; angular leaf spot; arbuscular mycorrhizal fungi; bioprotector.

1. INTRODUCTION

Angular leaf spot disease is the largest and most widespread disease affecting common bean production in sub-Saharan Africa [1]. The damage caused by this disease varies from 70% to 80% in Cameroon [2,3]. It is caused by *Phaeoisariopsis griseola (Sacc.) Ferraris,* a cryptogam widely distributed in the tropical and subtropical regions of Central and South America, and East and Central Africa. This fungal agent grows under varying humidity and temperatures between 18 and 25°C [4]. To combat the development of the angular spot disease of common beans, farmers use chemical fungicides (mancozeb, terazeb etc.,). The intensive and systematic use of chemical fungicides causes environmental damage (groundwater and air pollution, presence of residues in soil and plants) and is harmful to both pesticide producers and consumers. The development of sustainable practices as an alternative for crop management to reduce the use of pesticides and chemical fertilizers is an ongoing global challenge in agriculture [5]. Biological control, based on the use of the potential of beneficial symbiotic microorganisms that are antagonistic to plant pathogens, could be a promising solution to improve plant nutrition and resistance and tolerance to biotic stress [6]. A preventive strategy is to activate the natural defenses of plants by using beneficial microbes such as arbuscular mycorrhizal fungi as inoculants, which deserves increasing interest [7]. Arbuscular mycorrhizal fungi belonging to the phylum Glomeromycota are widely distributed in natural ecosystems and colonize the roots of more than 80% of plant species, including major crops [8]. The plants form a mutualistic association with the arbuscular mycorrhizal fungus that benefits both partners. Arbuscular mycorrhizal fungi improve the uptake of water and nutrients, especially phosphorus in plants and also increase the phenotypic and metabolic resistance of plants to biotic and abiotic stress. In return, arbuscular mycorrhizal fungi benefit from the carbohydrates synthesized by the plant Martínez-Medina et al. [9] ; Cameron et al. [10]; Abdelrahman et al. [11]; Sanchez-Bel et al., [12] ; Ferrol et al., [13]; Campo et al., [14]; Mitra et al., [15]; Rivero et al., [16] Indeed, mycorrhization protection against disease has been associated with the accumulation of phenols, phytoalexins and the induction of the activity of specific isoforms of hydrolytic enzymes such as chitinases and 1,3 glucanases in mycorrhizal roots [17]. Numerous studies have proven that arbuscular mycorrhizal fungi (AMF) enhance

plant resistance against various pathogens [18-21] demonstrated that Glomus and Gigaspora sp initiate cell wall defense related to protein production, enzymatic activity, and increased phenolic compound production of common bean against *Ralstonia solani*. This study aims to induce the natural defence of common bean against angular leaf spot disease through mycorrhization.

2. MATERIALS AND METHODS

2.1 Characterization of Study Site

The pot experiment was carried out in the Regional Laboratory of Biological Control and Applied Microbiology of IRAD during the period from March 2022 to June 2022.

2.2 Soil Sampling and Analyses

Composite soil samples were sampled from 0 to 20 cm in three replicates and transferred to the laboratory for chemical analyses. The soil was air dried, sieved to pass through 1-mm mesh and the chemical properties analyzed in 4 analyticals replicates. The soil-pH was measured in aqueous soil suspension (1 : 2.5, v : v) using the electronic pH meter type CG822 after agitating the sample for 16 h. The soil available-P was determined by the Bray-I chemical extraction method. Briefly, 30 ml of Bray-I extractant was added to 3 g of air-dried soil sample and the content (soil solution ratio 1:10) was shaken for 5 min and filtered, and the P concentration was measured after the colorimetric change [22]. Total N concentration was measured in a subsample of 0.5 g after digestion with concentrated H_2SO_4 at 500 °C using a stainless-steel pressure digestion system (BERGHOF Products + Instruments GmbH Labor-Technik Eningen, Germany). Organic C was determined by chromic acid digestion and a spectrophotometric procedure [23]. The N concentrations was determined using a spectrophotometer (Jenway 6310 Scanning Visible Range Spectrophotometer 230 V, Clarkson Laboratory, USA), according to the method described by Novozamsky et al. [24].

2.3 Biological Materials

Two common bean varieties; GLP 195-C and PNG were obtained from the Institute Agricultural and Development Research (IADR) of Dschang were used. The bean variety GLP 195-C has a cycle from 80 to 90 days and the seeds are spotted red color. The variety also originated from CIAT with a yield range between 2 and 2.5 ton/ha. The variety PNG is a glossy black colored seeds, growing cycles of about 90 days, and a yield range between 1.5 and 2.5 ton/ha. The mycorrhizal inoculum *Acaulospora tuberculata, Gigaspora gigantea*, *Entrophospora infrequens* isolates from the rhizosphere of the two sites (Olamze and Soa). The inoculum contained spores and colonized Sorghum root fragments at a concentration of 50 propagules per cubic centimeter. Chemical material used was the Terazeb 80 WP which is a contact and systemic fungicide and content 80 % of maneb and was applied at the recommended dose : 10-15 g/l. spores of phaeoisoriopsis griseola agent causal of angular leaf spot disease.

Fig. 1. Mycorrhizal inoculum

2.4 Methods

2.4.1 Experimental device in pots

The experimental device used in pots was a bifactorial device. The first factor was two varieties of common bean (GLP 195-C and PNG). The second factor consisted of eight treatments, namely T0: absolute control, T1: mycorrhizal treatment with *Acaulospora tuberculata*, T2: mycorrhizal treatment with *Gigaspora gigantea*, T3: mycorrhizal treatment with *Entrophospora infrequens*, T4: Terazeb synthetic fungicide treatment (positive control), T5: mycorrhizal treatment with *Acaulospora tuberculata* and *Gigaspora gigantea* combination, T6: mycorrhizal treatment with *Acaulospora tuberculata* and *Entrophospora infrequens* combination, T7: mycorrhizal treatment with combination of *Gigaspora gigantea* and *Entrophospora infrequens.* Dried soil from the olamze and soa sites was sieved using a 4 mm sieve and mixed with river sand (soil-to-sand ratio 3 : 1 v/v). The substrate thus prepared was autoclaved (121°C, 1 h) twice. The 5-litre pots were filled with disinfected substrate at a rate of 3.5 kg/pot. Half of the pots were given twice the AMF inoculum and seedbed at the beginning and watering the pots 14 days after planting at a dilution of 5 mL/L of water. Four sterilized healthy seeds of each common bean variety were sown in the pots. All the pots were kept outside in natural conditions (daytime temperature 25°C, night temperature 20°C, photoperiod 16h) and watered if necessary. After 4 weeks of AMF inoculation, leaf infestation was done by spraying the inoculum of the pathogen (*Phaeoisoriopsis griseola*) on the leaves. The chemical fungicide used in this study as a positive control was terazeb (80 WP). Three applications were applied as soon as the first symptoms of the disease appeared. The applications were made in the morning between (8am-10am). 100 to 150g of Terazeb 80 WP was poured into a 15 l sprayer fill half with water, shake and add water up to 15 liters and spray on the leaves of the plants fungicide pots.

2.4.2 Identification of foliar disease-causing agents during growth development

The identification of *Phaeoisariopsis griseola (Sacc.) Ferraris,* causal agent of angular leaf spot achieved according to methodology used by Riviera et al*.* [25]. The leaves showing the symptoms of the disease were collect in two agro-ecological zones where the common bean is the most cultivated in Cameroon, namely, the Western Highlands (Dschang) and the bimodal rainfall forest zone (Yaoundé),washed in tap water then cut into 5mm fragments at the front of the growth of the necrose. The fragment were successively disinfected using a 0.5 % sodium hydrochlorite solution for 3 minutes and 70 % ethanol for 3 minutes. Infected fragment were incubated on PDA during three days. Microscopic identification was carried out using the optic microscope.

2.4.3 Quantification of angular leaf spot disease in the pots

The quantification of angular leaf spot disease of common bean is based on incidence and severity. Data were collected every 7 days after 35 days after sowing. The disease was identified in the pot by a visual diagnosis based on precise observations of the symptoms and their evolution in time and space.

2.4.3.1 Evaluation of the incidence of angular leaf spot disease

Diseased plants were counted every week after 35 days after sowing on a sample of 10 plants per treatment. Incidence was assessed using the Tchumakov and Zaharova [26] formula;

$$I\,(\%) = \frac{Ni}{Nt} \times 100$$

Where: I = Incidence in percentage (%); Ni = Number of infected plant; Nt = total number of plant

2.4.3.2 Assessment of the severity of angular leaf spot disease

This was determined by measuring each week after 35 days after sowing on a sample of 10 plants per treatment. The formula of Tchumakov and Zaharova [26] was used to express the severity values:

$$S = \sum (axb)/N$$

Where: S = severity (%); ∑ (a.b) = sum of the number of diseased plants (a) × corresponding degree of infection (b) in %; N = total number of diseased plants. The scale used for the degree of infection (b) is that proposed by Wangungu et al*,* (2011) or 0 = 0% infection of the plant; 1 = infection covering between 1-15% of plant; 2 = infection between 16 - 40% of plant; 3 = infection covering between 41 - 75%; 4 = infection covering 76 - 100% of plant.

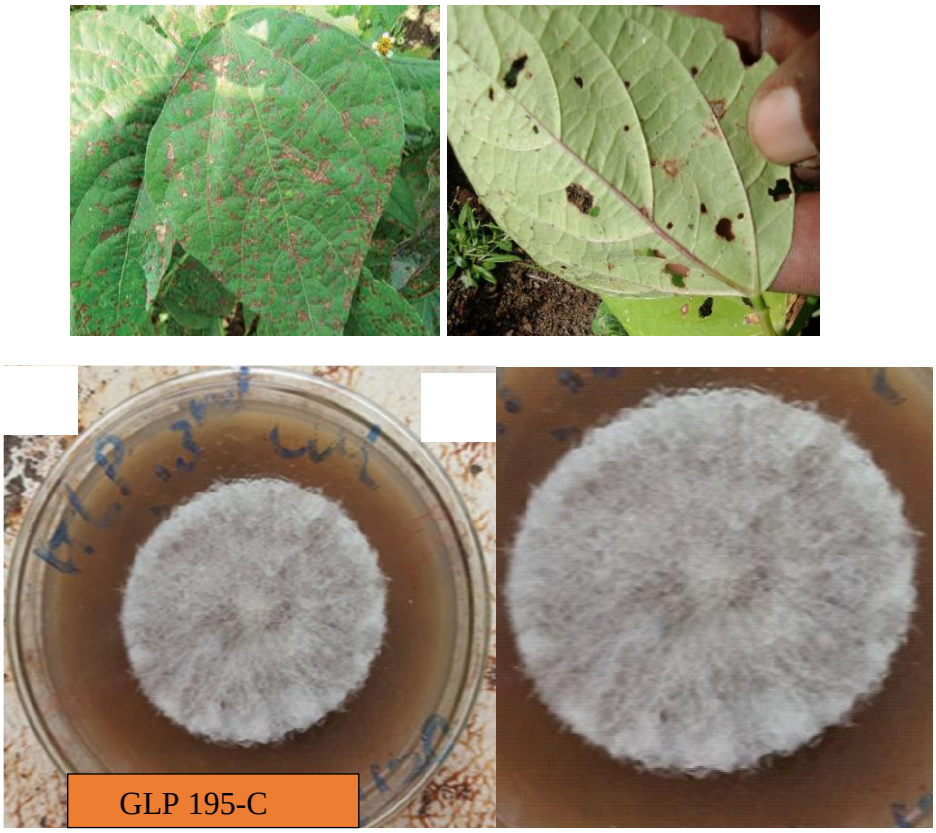


Fig. 2. Identification of the *Phaeoisariopsis griseola* (Sacc.) *Ferraris*

2.4.4 Assessment of mycorrhizal colonisation rate

The modified method of Kormanik and McGraw [27] was used. Roots were immersed in 5% potash (KOH) in labelled test tubes and heated in a water bath for 30 minutes at 90°C, then washed three times with tap water to remove the potash. 1% hydrochloric acid (HCl) was added to the roots and left to soak for 1 hour. The hydrochloric acid was then removed and the dye (lactic acid-glycerol-water) in proportions of 4-1-1 plus 0.05% methylene blue was prepared and added to the tubes. The tubes were heated to 90°C for 30 minutes in a water bath. After heating, the dye was removed and the roots were rinsed three times with tap water. The bleach (lactic acid-glycerol-water) was introduced into the tubes and left to stand for at least 24 hours. Ten root fragments of approximately 1 cm were mounted between slide and coverslip. The mounting was repeated three times for each sample. Observation was carried out using an OLYMPUS JAPAN BH-2 electron microscope with an objective of 10. The number of fragments colonised by fungi out of the 30 per sample was noted.

The rate of mycorrhizal root colonisation (RMC) of the plants was assessed by calculating the percentage of root colonisation using the formula of Mpemboura Nsangou Salamatou, [28]:

$$RMC\ (\%) = \frac{Nc}{No} \times 100$$

Where; RMC = Rate of Mycorrhizal root Colonisation in percentage (%); Nc = Total number of root fragments colonized; No = Total number of root fragments observed.

2.4.5 Assessment of biochemical parameters

2.4.5.1 Extraction and determination of amino acids, proline and phenol

Total amino acids and proline were determined from an ethanoic extract. One-gram fresh bean leaves plants was ground in 5 mL of 80 % ethanol and centrifuged at 5000 ppm at 4°C for 15 min. The recovered supernatant was used for the assay. Total amino acids and proline were determined by the ninhydrin reaction according to the method described by Yemm and Coocking, [29]. Total amino acids and proline underwent oxidative denaturation in the presence

of ninhydrin, with the release of CO_2, NH_3 and an aldehyde molecule. The reaction medium consisted of 50 µL of extract, 0.5 mL of citrate buffer (0.2 M, pH: 5), 1 mL of 80% ethanol and 1 mL of ninhydrin reagent (1% ninhydrin and 0.06% KCN in acetone). The resulting mixture was placed in a boiling water bath for 15 min and then cooled in melting ice. The absorbance of the complex formed was read at 570 nm (for amino acids) and 440 nm (for proline) using a spectrophotometer (Jenway 6310 Scanning Visible Range Spectrophotometer 230V, Clarkson Laboratory, USA). Amino acid and proline content were assessed respectively by reference to a calibration curve performed with pure glycine and proline. Levels were expressed in mg/g fresh matter (FM).

Total phenol concentration was determined by Folin–Ciocalteau method [30]. Half mL of extract was added to 3mL water, 0.5 mL Na_2CO_3 (20 %) and shake for 3 minutes. Subsequently, 0.5 mL of Folin - Ciocalteu reagent was added and the mixture transferred to a water bath set at 40 ^{0}C and incubated for 30 min and the absorbance read at 760 nm using a spectrophotometer (Jenway 6310 Scanning Visible Range Spectrophotometer 230V, Clarkson Laboratory, USA). The concentration of phenol was calculated using gallic acid for a standard curve and expressed in milligrams per gram of fresh leaf matter.

2.4.5.2 Extraction and determination of phenolic compounds

The flavonoid content of the extracts was determined according to the method described by Michel, (2011). The operation consisted of adding 1 ml of extract to 1 ml of 2% aluminium chloride (AlCl3) (prepared in methanol). The resulting mixture was placed in the dark for 10 minutes before the absorbance reading at 450 nm. The results obtained were expressed in mg equivalent of quercetin per gram of fresh material. These concentrations were determined by reference to the calibration curve performed with quercetin prepared in methanol. The total tannin content was determined according to the protocol described by Ndhlala et al*.,* 2007. 0.5 mL of each extract was carefully transferred to a 10 mL test tube, 3 mL of the buthanol-HCL reagent (95 : 5) and 0.2 mL of ferric reagent were added. The mixture was stirred and incubated in a water bath at 100°C for 1 hour. The absorbance was read against a white made making a similar mixture without incubation. The following formula developed by Porter et al*.,* 1986 gives the tannin content, expressed in mg of gallic acid equivalent/100g of fresh matter according to the following formula:

% Tannins = DO x 78.26 x dilution factor / Fresh sample mass * 100

2.4.5.3 Determination of the enzymatic activity of polyphenoloxidases (PPO) and peroxidases (POX)

The determination of the POP was done according to the method described by Mayer et al*.* (1965) modified. In each tube and in the following order, we put 2 ml of the 0.1M phosphate buffer, pH 6.5 and then 10 µl of the enzymatic extract. The reaction was triggered by adding 10µl of the catechol solution (substrate) 0.2 M, the density reading was made after 3 min at 410 nm against a blank in which the extract was replaced by phosphate buffer. For each extract, three readings were performed and the PPO activity is expressed in ΔDO.mn-1. g-1 of PF. The determination of peroxidases was done according to the method described by Thorpe et al*.* (1978). The reaction medium consisted of 1 ml of 0.2% H_2O_2 (V/V) followed by 1 ml of 1% guaiacol (V/V) and 2 ml of phosphate buffer, 0.06 M, pH 6.8. In each test tube, 4 ml of the reaction medium, 10 µl of extract, was introduced. After homogenization, the enzymatic activity of POX was determined by following the formation of tetragaiacol from guaiacol. The absorbance was read at 420 nm on the spectrophotometer against a blank in which the extract was replaced by the extraction buffer. Three readings were taken per sample. The POX content is expressed in ΔDO.mn-1. g-1 of PF (molar extinction coefficient of peroxidases ε = 26.6 M.cm-1).

2.4.5.4 Determination of protein content

The determination of the protein content was evaluated after determining the total nitrogen content and multiplying the total nitrogen content by a factor of 6.25. Total nitrogen was determined using the classical Kjeldhal method (AFNOR, 1984) followed by the colorimetric assay of [31].

Nitrogen dosing : 0.2 ml of mineraliserate, 1.2 ml of sodium acetate solution and 1.6 ml of reactive solution (15 ml of formaldehyde + 8 ml of acetone in 77 ml of distilled water) were successively introduced into a test tube. The mixture was incubated in a water bath (97.5°C)

for 15 minutes. Once cooled in a stream of cold water, the volume of the tube was supplemented to 10 ml by adding 7 ml of distilled water. The absorbance was read at 412 nm against a blank consisting of sodium acetate solutions, reactive solution, and distilled water. The calibration range of the nitrogen solution was established from a solution of ammonium sulphate 0.4 g nitrogen/ml. The amount of nitrogen was determined from the ammonium sulphate calibration curve. The calibration line equation y = 8.9472x was used to calculate the amount of nitrogen from the formula :

$$X = Y \times \frac{Vt \times 100}{Vp \times m \times a}$$

X : Amount of nitrogen (mg); Y: Optical density; Vt (ml): Total volume of mineralisate; Vp (ml): total volume of mineralized dosed; m (g): Mass of the mineralized sample; a: Calibration coefficient (0.006).

Protein content = N content x 6.25

2.5. Data Analysis

The data collected were subjected to one-way and two-way an analysis of variance (ANOVA) using R software version 3.5.1. The comparisons of the means were performed using the Tukey HSD test with a threshold of 5%.

3. RESULTS

3.1 Physico-Chemical Composition of the Soils of the Sites

The soils of the sites are acidic because the pH is below 7. The soil of the Soa site (5.5) is more acidic compared to the soil of the Olamze site (6). The available phosphorus is low 8.12 mg/kg in the locality of Olamze and medium in the locality of Soa 45.33 mg/kg. The low nitrogen content of the soils, 1.08% in Olamze and 0.87% in Soa, is an indicator associated with the history of continuous cultivation with little or no addition of organic or inorganic fertilizers. The soils in the study area are sandy, clayey.

3.2 Influence of Treatments and Varieties on Mycorrhization Parameters

3.2.1 Colonization rate and mycorrhization intensity

The results obtained on the colonization rate and the intensity of mycorrhization showed significant differences ($P < 0.0001$) between the treatments in the two soils. There are no significant differences between varieties and between varieties x treatments interaction (Table 1).

In pots containing Olamze sterilized soil, the highest average mycorrhizal colonization rates were obtained with the T2 (76%) and T7 (71%) mycorrhizal treatments compared to the T4 (00%) synthetic fungicide treatments and the T0 controls (00%). Similarly, the highest average mycorrhizal intensity was obtained with the T2 (14%) and T7 (15.42%) mycorrhizal treatments compared to the T4 (00%) synthetic fungicide treatments and the T0 controls (00%) in the two varieties, respectively. For pots containing sterilized Soa soil, the highest average mycorrhizal colonization rates were obtained with T2 (65.5%) and T7 (73.5%) mycorrhizal treatments compared to T4 (00%) synthetic fungicide treatments (00%) and T0 controls (00%). Similarly, the highest average mycorrhizal intensity was obtained with the T2 (11.42%), T7 (13%), T5 (12.34%) and T6 (12.48%) mycorrhizal treatments compared to the T4 (00%) synthetic fungicide treatments (00%) and the T0 controls (00%) respectively in the two varieties. The highest colonization rate and mycorrhization intensity were obtained with the T7 treatment (Table 1).

3.3 Influence of Treatments and Varieties on the Epidemiological Parameters of Common Bean

3.3.1 Influence of treatments and varieties on the incidence of angular spot disease of common bean in pots

The incidence of angular spot disease varied with treatments, varieties, soil, and weather. In pots containing Olamze sterilized soils, at 5, 6 and 7 SAS, significant differences were observed between treatments ($P < 0.0001$) and between varieties ($P < 0.01$) in the two varieties of common bean. At 7 SAS, the composite mycorrhizal treatments T7 (13.33%), T5 (17.78%) and simple mycorrhizal treatments T2 (15.55%) recorded the lowest incidences compared to plants treated with synthetic fungicides T4 (66.67%) and control T0 (71.11%) in the GLP 195-C variety. For the PNG variety, the T7 (12.22%), T5 (12.22%) and T2 (12.67%) single mycorrhizal treatments recorded the lowest incidences compared to plants treated with synthetic fungicides T4 (46.66%) and the T0 control (66.67%) (Table 2).

Table 1. Influence of AMF on the colonization rate and mycorrhization intensity of common bean roots (%)

Varieties		Olamze		Soa	
	Treatments	Colonization rate	Intensity	Colonization rate	Intensity
GLP 195-C (V1)	T0	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 e
	T1	75.66 ± 1.15 b	15.00 ± 1.50 bc	63.33 ± 2.89 c	9.17 ± 1.53 bc
	T2	83.33 ± 2.89 a	15.50 ± 1.08 ab	70.00 ± 5.00 bc	11.50 ± 1.00 bc
	T3	76.50 ± 1.32 b	15.00 ± 1.30 bc	65.00 ± 2.50 c	10.67 ± 0.58 c
	T4	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 e
	T5	76.67 ± 2.89 b	15.33 ± 1.04 ab	70.00 ± 10.00 bc	13.00 ± 1.00 ab
	T6	76.50 ± 1.32 b	14.50 ± 1.33 bc	66.67 ± 2.89 c	12.50 ± 1.00 ab
	T7	81.67 ± 2.87 a	13.50 ± 0.50 bc	78.33 ± 5.77 a	13.50 ± 0.50 a
PNG (V2)	T0	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 e
	T1	71.67 ± 2.89 c	11.17 ± 0.76 d	65.00 ± 5.00 c	11.33 ± 0.58 bc
	T2	81.67 ± 2.87 a	13.50 ± 1.00 bc	70.00 ± 5.00 bc	11.50 ± 1.00 bc
	T3	71.67 ± 2.89 c	13.00 ± 0.50 cd	65.00 ± 8.66 c	11.96 ± 1.33 cd
	T4	0.00 ± 0.00 d	0.00 ± 0.00 e	0.00 ± 0.00 d	0.00 ± 0.00 e
	T5	76.67 ± 2.89 b	15.67 ± 0.76 ab	66.67 ± 2.89 c	11.96 ± 1.00 abc
	T6	71.67 ± 2.89 c	13.50 ± 1.00 bc	66.67 ± 2.89 c	12.17 ± 1.04 abc
	T7	81.67 ± 1.32 a	17.33 ± 1.12 a	76.67 ± 2.89 ab	12.50 ± 1.00 ab
Pr (>F) V		**0.949ns**	**0.759ns**	**0.963ns**	**0.963ns**
Pr (>F) T		**< 0.0002*** **	**< 0.0002*** **	**< 0.0002*** **	**< 0.0002*** **
Pr (>F) VxT		**0.18ns**	**0.370ns**	**0.991ns**	**0.222ns**

**: p<0.05 (significant effect), **: p< 0.01 (highly significant effect), ***: p< 0.001 (very highly significant effect); ns: not significant; VxT: interaction; V: Varieties; V1: GLP 195-C; V2: PNG; T: Treatments; T0: control, T1 : mycorrhizal treatment with Acaulospora tuberculata, T2 : mycorrhizal treatment with Gigaspora gigantea, T3 : mycorrhizal treatment with Entrophospora infrequens, T4: chemical fungicide Terazeb, T5: mycorrhizal treatment with a combination of Acaulospora tuberculata and Gigaspora gigantea, T6: mycorrhizal treatment with a combination of Acaulospora tuberculata and Entrophospora infrequens, T7: mycorrhizal treatment with a combination of Gigaspora gigantea and Entrophospora infrequens. WAS: Weeks After Sowing. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold*

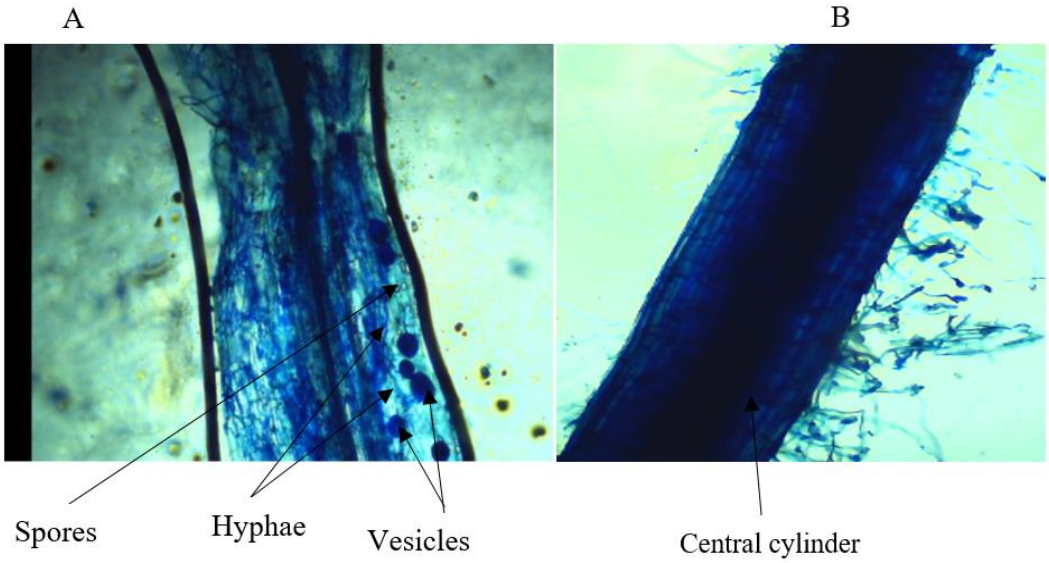


Fig. 3. Common bean roots, A : plants inoculated with AMF, B : plants without AMF inoculation

Table 2. Influence of treatments and variety on the incidence of angular spot disease of common bean in pots (%)

		Olamze			Soa		
Varieties	**Treatments**	**5 SAS**	**6 SAS**	**7 SAS**	**5 SAS**	**6 SAS**	**7SAS**
Varietie GLP 190-C (V1)	T0	33.33±5.24 a	53.33 ± 3,34 a	71.11 ± 7.69 a	40.00 ± 3.33 a	66.67 ± 3.55 a	100 ± 5.01 a
	T1	0 e	16.67 ± 1,92 de	21.11 ±3.58 ef	0 c	26.67 ± 3.04 d	36.67 ± 4.05 d
	T2	0 e	12.67 ± 1,33 e	15.55±2.53ghi	0 c	6.67 ± 1.92 g	23.33 ±2.50 fgh
	T3	0 e	16.67 ± 2,58 de	33.33 ± 4.87 d	0 c	13.33 ± 1.92 f	36.67 ± 4.50 d
	T4	12.22 ± 2.5 c	18.89 ± 2,70 d	66.67 ± 3.00 b	23.33 ± 2.50 b	46.67 ± 3.15 b	83.33 ± 2.50 b
	T5	0 e	6.67 ± 1,73 f	17.78±3.58fgh	0 c	10.00 ±2.50 fg	26.67±4.00 efgh
	T6	0 e	16.67 ± 1,15 de	21.11 ± 2.50ef	0 c	13.33 ±3.00 f	33.33 ± 3.58 def
	T7	0 e	6.67 ± 1,92 f	13.33 ±3.00 hi	0 c	6.67 ± 1.92 g	16.67 ± 2.50 hi
Varietie PNG (V2)	T0	24.44 ± 2.15 b	34.44 ± 3,92 b	66.67 ± 3.33 b	34.56 ± 1.84 a	46.67 ± 4.05 b	83.33 ± 5.58 b
	T1	0 e	12.67 ± 3,09 e	16.67±3.60fgh	0 c	18.89 ±1.92 ef	20.00 ± 2.29 gh
	T2	0 e	6.67 ± 1,73 f	12.67 ± 1.33 i	0 c	6.67 ± 3.04 g	23.33 ±2.50 fgh
	T3	0 e	16.67 ± 3,58 de	23.33 ± 2.50 e	0 c	10.00 ± 3.04fg	26.67±3.58 efg
	T4	6.67 ± 1.92 d	23.33 ± 2,50 c	46.67 ±3.20 c	23.33 ± 2.58 b	36.67 ± 2.50 c	46.67 ± 3.00 c
	T5	0 e	6.67 ± 1,92 f	12.67 ± 2.15 i	0 c	13.33 ± 2.76 f	26.67±3.58 efgh
	T6	0 e	6.67 ± 1,73 f	17.78±3.58fgh	0 c	6.67 ±1.92 g	23.33 ± 2.50 fgh
	T7	0 e	6.67 ± 1,92 f	12.67 ± 2.15 i	0 c	6.67 ± 0.19 g	10.00 ± 2.00 i
Pr (>F) V		**< 0.01***	**< 0.003***	**< 0.03***	**< 0.03***	**< 0.03***	**< 0.01***
Pr (>F) T		**< 0.0002*****	**< 0.0002*****	**< 0.0002*****	**< 0.0002*****	**< 0.0001*****	**< 0.0002*****
Pr (>F) VxT		**< 0.3828ns**	**< 0.0009*****	**< 0.0001*****	**0.2776 ns**	**0.4099 ns**	**0.2222 ns**

**: p < 0.05 (significant effect), **: p < 0.01 (highly significant effect), ***: p < 0.001 (very highly significant effect); ns: not significant; VxT : interaction; V : Varieties; V1 : GLP 195-C ; V2 : PNG ; T : Treatments; T0 : control, T1 : mycorrhizal treatment with Acaulospora tuberculata, T2 : mycorrhizal treatment with Gigaspora gigantea, T3 : mycorrhizal treatment with Entrophospora infrequens, T4 : chemical fungicide Terazeb, T5 : mycorrhizal treatment with a combination of Acaulospora tuberculata and Gigaspora gigantea, T6 : mycorrhizal treatment with a combination of Acaulospora tuberculata and Entrophospora infrequens, T7 : mycorrhizal treatment with a combination of Gigaspora gigantea and Entrophospora infrequens. WAS: Weeks After Sowing. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold*

Table 3. Influence of treatments and varieties on the severity of angular spot disease of common bean in pots

Severity (%)							
		Olamze			Soa		
Varieties	Treatments	5 SAS	6 SAS	7 SAS	5 SAS	6 SAS	7SAS
GLP 190 C (V1)	T0	15.50 ± 0.58 a	20.33 ± 3.27 a	30.89 ± 2.45 a	21.43 ± 2.61 a	26.67 ± 1.25 a	31.33 ± 1.53 a
	T1	0 d	5.83 ± 1.58 cde	11.83 ± 1.58 d	0 e	12.67 ± 1.05 cde	16.50 ± 0.87 c
	T2	0 d	3.16 ± 0.63 ef	7.83 ± 1.15 fg	0 e	5.33 ± 1.50 ij	10.16 ± 0.29 def
	T3	1.91 ± 0.52 c	4.83 ± 1.58 def	10.50 ± 1.86 de	0 e	12.50 ± 0.76 cde	12.67 ± 0.76 cde
	T4	5.50 ± 2.52 b	12.50 ± 2.58 b	15.17 ± 2.00 c	3.52 ± 0.58 d	14.83 ± 1.26 c	17.50 ± 2.05 c
	T5	0 d	3.70 ± 0.58 ef	9.17 ± 0.58 ef	0 e	10.83 ± 1.52 def	11.83 ± 0.57 def
	T6	1.33 ± 1.15 c	4.50 ± 1.50 def	10.50 ± 1.52 de	0 e	11.33±1.89 def	12.50 ± 0.87 de
	T7	0 d	2.33 ± 1.04 f	6.50 ± 1.00 gh	0 e	6.67 ±1.04 hi	9.83 ± 0.58 ef
PNG (V2)	T0	5.67 ± 2.70 b	11.67 ±1.04 b	21.5 ± 1.32 b	17.37 ± 1.03 b	21.33 ± 1.04 b	24.77 ± 5.15 b
	T1	0 d	5.16 ± 0.58 def	7.22 ± 0.63 fg	0 e	10.67 ± 2.25 ef	14.17 ± 1.52 cde
	T2	0 d	2.83 ± 0.58 f	5.83 ± 0.58 gh	0 e	4.83 ± 1.00 jk	9.67 ± 1.15 ef
	T3	0 d	3.50 ± 0.87 ef	6.91 ± 1.23 gh	0 e	6.50 ± 2.02 hi	10.17 ± 1.17 def
	T4	4.88 ± 0.76 bc	7.41 ± 1.59 c	8.50 ± 1.58 ef	7.33 ± 0.58 cd	11.67 ±1.75 def	15.83 ± 0.58 cd
	T5	0 d	3.83 ± 0.76 ef	6.50 ± 0.76 gh	0 e	6.33 ± 0.76 hi	10.17 ± 1.25 de
	T6	0 d	4.50 ± 1.00 def	6.50 ± 1.00 gh	0 e	7.67 ± 2.02 gh	11.83 ± 1.44 cde
	T7	0 d	2.75 ± 0.90 f	5.33 ± 1.52 h	0 e	3.50 ± 0.50 k	8.75 ± 1.00 f
Pr (>F) V		**< 0.003****	**< 0.001****	**< 0.01***	**< 0.01***	**< 0.04***	**0.295ns**
Pr (>F) T		**< 0.0005****	**< 0.0006*****	**< 0.0004*****	**< 0.0001*****	**< 0.0001*****	**< 0.0001*****
Pr (>F) VxT		**< 0.0001*****	**< 0.0001*****	**< 0.0001*****	**0.0915ns**	**0.4652ns**	**< 0.001****

**: p < 0.05 (significant effect), **: p < 0.01 (highly significant effect), ***: p < 0.001 (very highly significant effect); ns: not significant; VxT : interaction; V : Varieties; V1 : GLP 195-C ; V2 : PNG ; T : Treatments; T0 : control, T1 : mycorrhizal treatment with Acaulospora tuberculata, T2 : mycorrhizal treatment with Gigaspora gigantea, T3 : mycorrhizal treatment with Entrophospora infrequens, T4 : chemical fungicide Terazeb, T5 : mycorrhizal treatment with a combination of Acaulospora tuberculata and Gigaspora gigantea, T6 : mycorrhizal treatment with a combination of Acaulospora tuberculata and Entrophospora infrequens, T7 : mycorrhizal treatment with a combination of Gigaspora gigantea and Entrophospora infrequens. WAS: Weeks After Sowing. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold*

In pots containing sterilized Soa soils, the incidence of angular spot disease varied with treatments, varieties, and time. At 5, 6 and 7 SAS, significant differences were observed between treatments ($P < 0.0001$) and between varieties ($P < 0.01$). There is no significant effect between the variety-treatment interaction. At 7 SAS, the incidence of angular spot disease was higher for control T0 plants (100% and 83.33%) compared to plants treated with synthetic fungicides (83.33% and 36.67%) and composite mycorrhizal treatments T7 (16.67% and 10.00%), T5 (26.67% and 26.67%) and simple mycorrhizal treatments T2 (23.33% and 23.33%) in common bean varieties GLP 195-C and PNG. PNG (29.39%) had the lowest incidence compared to GLP 195-C (37.50%) (Table 2).

3.3.2 Influence of treatments and varieties on the severity of angular spot disease of common bean in pots

In pots containing Olamze sterilized soils, significant effects were observed between varieties ($P < 0.01$), between treatments ($P < 0.0001$) and between varieties x treatments ($P < 0.0001$) in the two varieties of the 5, 6 and 7 SAS common bean. At 7 SAS, the severity of angular spot disease was higher for control plants (30.89%) compared to plants treated with synthetic fungicides (15.17%) and mycorrhizal treatments T7 (6.50%), T2 (7.83%) and T5 (9.17%) in common bean variety GLP 195-C. For PNG, the severity of angular spot disease was higher in control plants (21.5%) compared to plants treated with synthetic fungicides (8.50%) and mycorrhizal treatments T7 (5.33%), T2 (5.83%) and T5 (6.50%). The PNG variety (12.55%) recorded the highest severity compared to the GLP 195-C variety (7.70%) (Table 3).

In pots containing sterilized Soa soils, significant effects were observed between varieties ($P < 0.05$) and between treatments ($P < 0.05$) at 5 and 6 SAS. There is no significant effect between variety-x-treatment interaction in the two varieties of the 5 and 6 SAS common bean. At 7 SAS, plants inoculated with T7 (9.83% and 8.75%), T2 (10.16% and 9.67%) and T5 (11.83% and 10.17%) mycorrhizae recorded the lowest severities compared to plants treated with T4 synthetic fungicides (17.50% and 15.83%) and control T0 (31.33% and 24.77%) in common bean varieties GLP 195-C and PNG. GLP 195-C (16.79%) was the most affected by angular spot disease compared to PNG (15.17%) (Table 3).

3.4 Influence of Treatments and Varieties on the Biochemical Parameters of Common Bean in Pots

3.4.1 Amino acid, proline and total phenol contents of fresh leaves

The results obtained on amino acid, proline and phenol content showed significant differences between treatments ($P < 0.01$) and varieties ($P < 0.0001$) at both sites. No significant effect was observed between variety-x-treatment interactions in the two varieties of the common bean (Fig. 5).

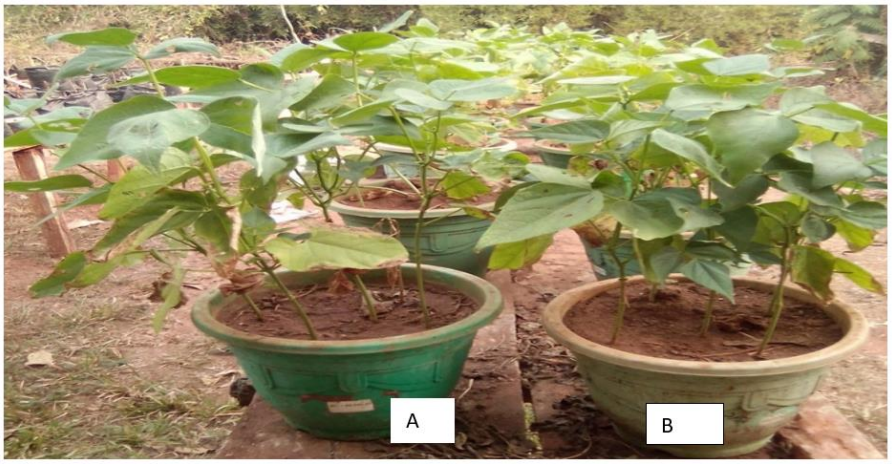


Fig. 4. Plants of common bean, A : without mycorrhizal, B : inoculated with mycorrhizal, Common bean leaves showing symptoms of foliar diseases attack with the severity lesions caused by *Pseudocercospora griseola*

In the pots containing the sterilized soil of Olamze, the highest amino acid content in the leaves was obtained by the simple mycorrhizal treatment T2 (4.05 mg/g) in the variety GLP 195-C. Concerning the PNG variety, the mycorrhizal treatment T7 (5.69 mg/g) obtained the highest amino acid content in the leaves compared to the synthetic fungicide treatment T4 (4.15 mg/g) and significantly ($P < 0.01$) in the control T0 (3.79 mg/g). Also, the highest proline content was registered by treatments T7 and T2 of GLP 195-C variety (6.89 mg/g and 6.92 mg/g) and PNG variety (15.49 mg/g and 15.19 mg/g) respectively. The highest phenol content was recorded in mycorrhizal treatments T2 of GLP 195-C variety (13.37 mg/g) and T7 of PNG variety (14.78 mg/g) (Fig. 5A).

In the pots containing the sterilized soil of Soa, the mycorrhizal treatments T7 (3.93 mg/g and 5.52 mg/g) and T2 (3.97 mg/g and 5.52 mg/g) obtained the highest amino acid concentrations in the leaves compared to the synthetic fungicide treatments T4 (3.46 mg/g and 4.49 mg/g) and significantly ($P <0.0001$) in the control T0 (2.31 mg/g and 3.60 mg/g) for the two varieties of common bean respectively (GLP 195-C and PNG). The highest proline and phenol content was registered in mycorrhizal treatments T7 for GLP 195-C variety (6.81 mg/g and 17.71 mg/g) and PNG variety (14.82 mg/g and 14.40 mg/g) respectively (Fig. 5B).

3.4.2 Phenolic content in fresh common bean leaves

The results obtained on the flavonoid and tannin content in the leaves showed significant differences between the treatments ($P < 0.0001$) and the varieties ($P < 0.0001$) in the two soils. In the pots containing the sterilized olamze soil, the mycorrhizal treatments T7, T2, T5, T6, T3 and T1 increased the flavonoid content by 32% to 83% and the tannin content from 124% to 242% compared to the control in GLP 195-C. For the PNG variety, the T7, T2, T5, T6, T1 and T3 mycorrhizal treatments increased the flavonoid content from 35% to 55% and the tannin content from 111% to 188% compared to the T0 control. The highest flavonoid and tannin content in the leaves was obtained by the PNG variety (10.21 mg/g and 2.86 mg/g) and the lowest were recorded by the GLP 195-C variety (10.21 mg/g and 2.68 mg/g) (Fig. 6A).

In the pots containing the sterilized Soa soil, the mycorrhizal treatments T7, T2, T5, T6, T3 and T1 increased the flavonoid content by 44% to 68% and the tannin content from 85% to 298% compared to the control T0 in GLP 195-C. For the PNG variety, the mycorrhizal treatments T7, T2, T5, T6, T1 and T3 increased the flavonoid content by 28% to 50% and the tannin content in the leaves by 60% to 184% compared to the T0 control. The highest flavonoid and tannin content in the leaves was obtained by the PNG variety (9.88 mg/g and 2.78 mg/g) and the lowest were recorded by the GLP 195-C variety (7.80 mg/g and 2.61 mg/g) (Fig. 6B).

3.4.3 Enzyme activity content of polyphenoloxidases and peroxidase in common bean leaves

Polyphenolxidase and peroxidase content varied significantly between treatments ($P < 0.0001$) in the two soils. There are no significant differences between varieties and between varieties x treatments interaction (Fig. 6). In the pots containing the sterilised olamze soil, the mycorrhizal treatments T2, T5, T7, T6, T1 and T3 increased the polyphenoloxidase content by 25% to 70 % and the peroxidase content in the leaves by 69% to 104% compared to control T0 in GLP 195-C. For the PNG variety, the mycorrhizal treatments T2, T5, T7, T6, T1 and T3 increased the polyphenoloxidase content by 31% to 92%, the peroxidase content in the leaves by 51 to 94% compared to the T0 control. The highest content of polyphenoloxidase and peroxidase enzyme activities in the leaves was obtained by the PNG variety and the lowest was recorded by the GLP 195-C variety (Fig. 7A).

In the pots containing the sterilised soil Soa, the mycorrhizal treatments T7, T5, T2 and T6 increased the polyphenoloxidase content by 23 to 65 % and the peroxidase content in the leaves by 72 to 109% compared to control T0 in GLP 195-C. For the PNG variety, the T2, T5, T7, T6, T1 and T3 mycorrhizal treatments increased the polyphenol oxidase content from 28% to 85%, the peroxidase content in the leaves from 51% to 94% compared to the T0 control. The highest content of enzymatic activities of polyphenoloxidases and peroxidases in the leaves was obtained by the PNG variety and the lowest was recorded by the GLP 195-C variety (Fig. 7B).

3.4.4 Protein content in the leaves of the common bean

Results on protein content in the leaves varied depending on the treatments and varieties in the

two soils. A significant effect was observed between treatments (P < 0.0001) and between variety x treatment interaction (P < 0.001). No significant effects were observed between varieties in the two varieties of common bean (Fig. 8).

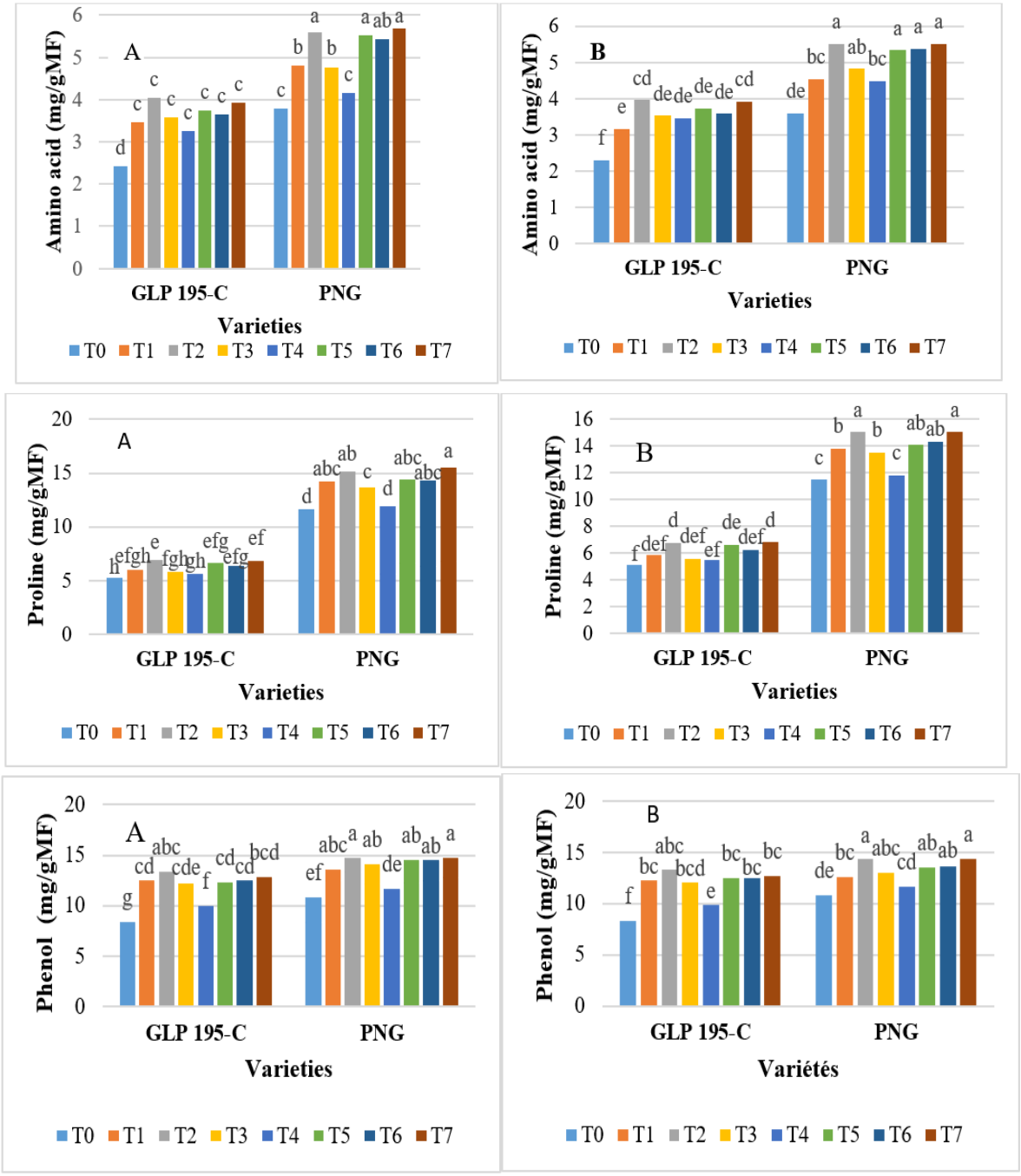


Fig. 5. Influence of treatments and varieties on the amino acid, proline and phenol content of common bean

A : Olamze, B : Soa. T0 : Control, T1 : Acaulospora tuberculata, T2 : Gigaspora gigantea, T3 : Entrophospora infrequens, T4 : chemical fungicide Terazeb, T5 : Acaulospora tuberculata and Gigaspora gigantea, T6 : Acaulospora tuberculata and Entrophospora infrequens, T7 : Gigaspora gigantea and Entrophospora infrequens. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold

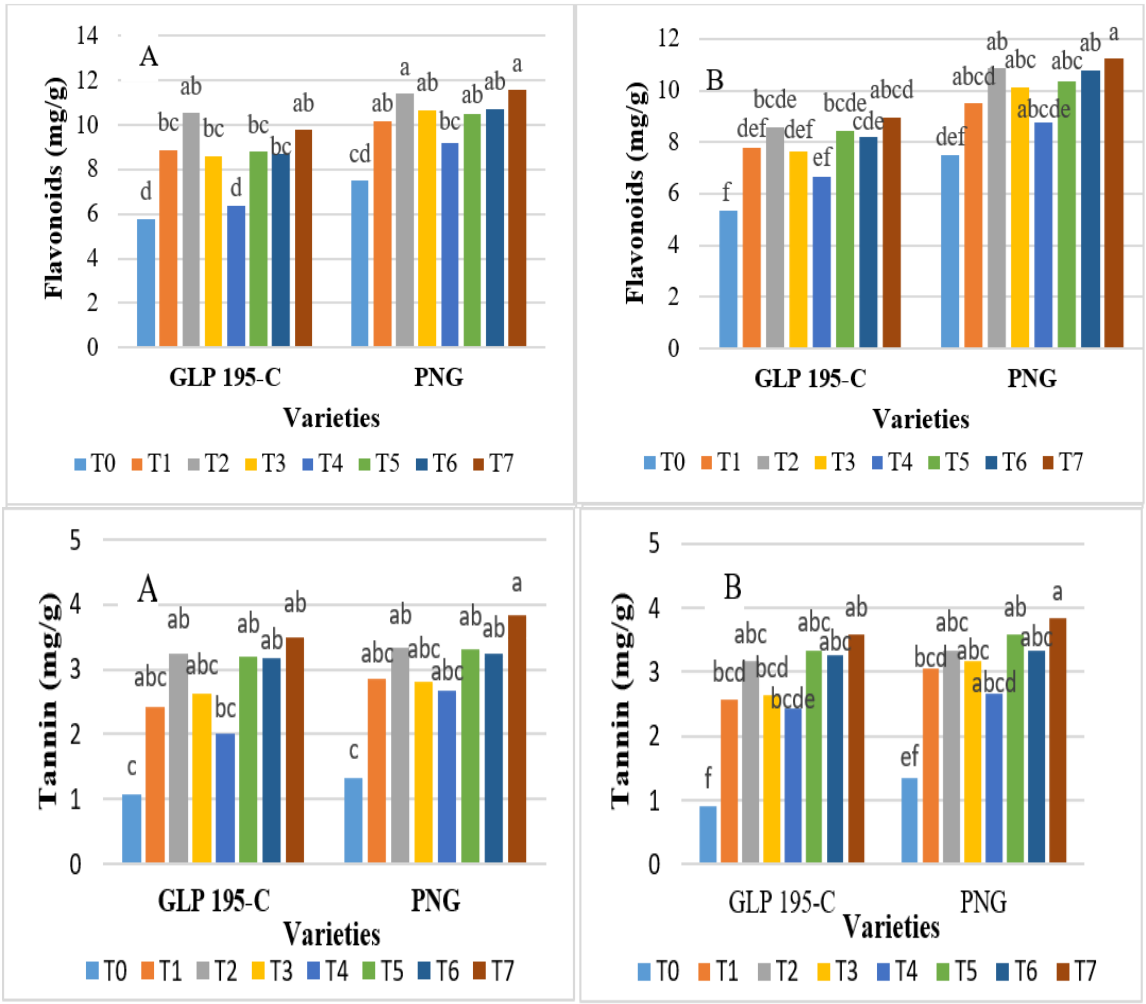
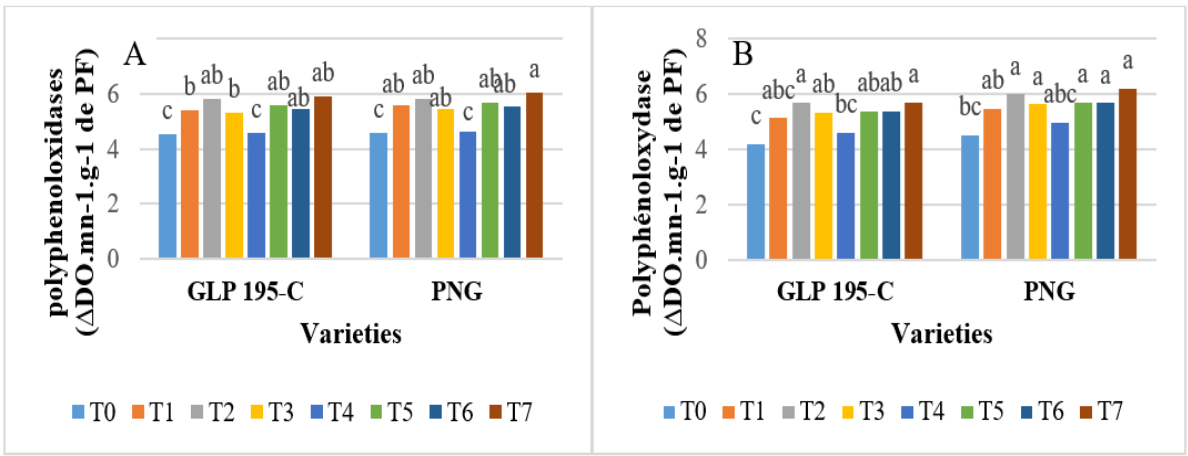


Fig. 6. Influence of AMF on flavonoid and tannin content in common bean leaves

A : Olamze, B : Soa. T0 : Control, T1 : Acaulospora tuberculata, T2 : Gigaspora gigantea, T3 : Entrophospora infrequens, T4 : chemical fungicide Terazeb, T5 : Acaulospora tuberculata and Gigaspora gigantea, T6 : Acaulospora tuberculata and Entrophospora infrequens, T7 : Gigaspora gigantea and Entrophospora infrequens. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold



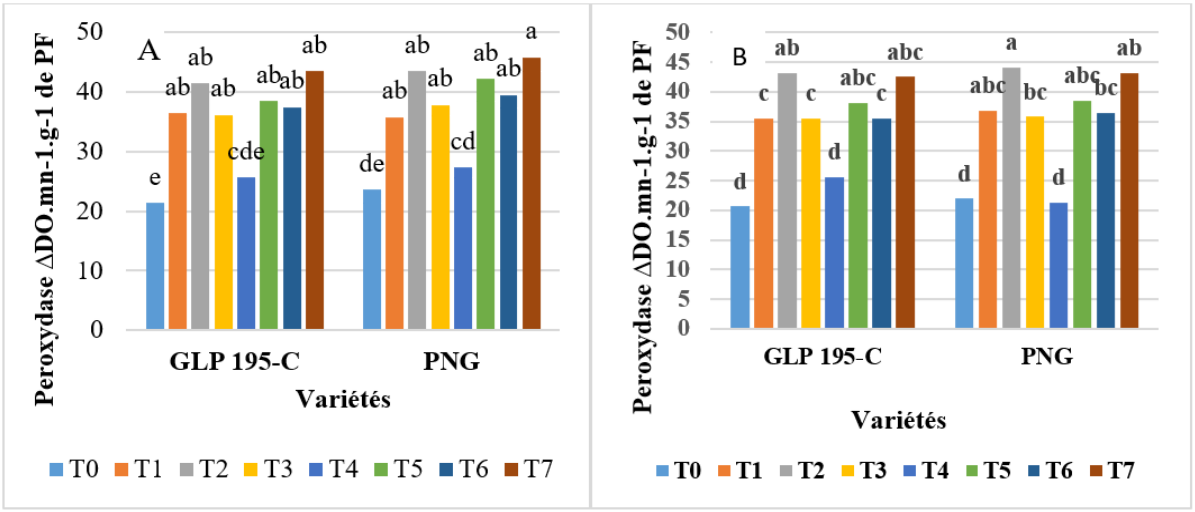


Fig. 7. Influence of AMF on the polyphenoloxidase and peroxidase content of common bean leaves

A : Olamze, B : Soa. T0 : Control, T1 : Acaulospora tuberculata, T2 : Gigaspora gigantea, T3 : Entrophospora infrequens, T4 : chemical fungicide Terazeb, T5 : Acaulospora tuberculata and Gigaspora gigantea, T6 : Acaulospora tuberculata and Entrophospora infrequens, T7 : Gigaspora gigantea and Entrophospora infrequens. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold

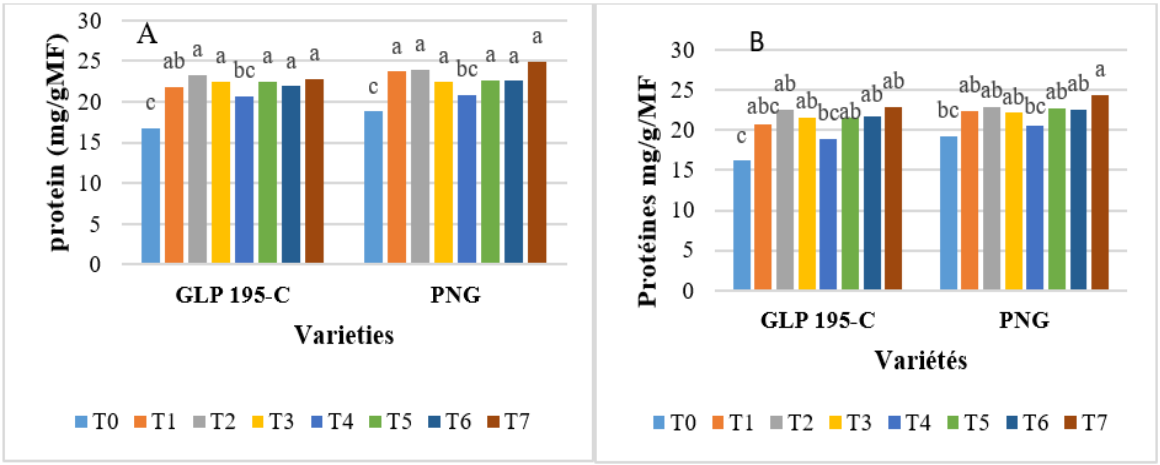


Fig. 8. Influence of AMF on the protein content of common bean

A: Olamze, B: Soa. A : Olamze, B : Soa. T0 : Control, T1 : Acaulospora tuberculata, T2 : Gigaspora gigantea, T3 : Entrophospora infrequens, T4 : chemical fungicide Terazeb, T5 : Acaulospora tuberculata and Gigaspora gigantea, T6 : Acaulospora tuberculata and Entrophospora infrequens, T7 : Gigaspora gigantea and Entrophospora infrequens. Means followed by the same letter for each variety are not significantly different according to Tukey's test at the 5% threshold

In pots containing Olamze sterilized soil, plants inoculated with T2, T7, T5, T6, T1 and T3 mycorrhizae increased leaf protein content by 30% to 39% and 19% to 32% compared to T0 control pots in GLP 195-C and PNG respectively (Fig.8A).

In pots containing sterilized Soa soil, plants inoculated with T7, T2, T5, T6, T1 and T3 mycorrhizae increased leaf protein content by 28% to 41% and 16% to 27% compared to control T0 pots in GLP 195-C and PNG, respectively. The highest protein content in the leaves was obtained by the PNG variety and the lowest was recorded by the GLP 195-C variety (Fig.8B).

4. DISCUSSION

Mycorrhizal fungi belonging to the phylum Glomeromycota form mutually beneficial symbionts with the majority of plant species in terrestrial ecosystems (over 90 %) (Brundrett, [32], Smith and Read, [8]. Mycorrhizal associations have facilitated plant colonization on land (Redecker et al*.,* 2000) by influencing plant physiology and soil structure [8,33]. Several studies have demonstrated that Arbuscular Mycorrhizal Fungi (AMF) enhance plant resistance against various fungal pathogens (Bi et al*.,* [20], Delavaux et al., 2017). Moreover, the induction of plant defenses during mycorrhization plays a vital role in mycorrhizal-enhanced resistance [34]. It is in this context that this study was investigated to induce natural defense of common bean against angular leaf spot disease through mycorrhization.

The results obtained on mycorrhizal root colonization of common bean showed that colonization rates ranged from 63.33% to 83.33% in the pots of mycorrhizal plants compared to non-mycorrhizal plants (00%) for both varieties of common bean. The absence of mycorrhizal hyphae in the pots of plants not inoculated with AMF shows that the pots were free of any contamination.

Mycorrhizal plants improved amino acid content from 44% to 70%, proline content from 20% to 33%, total phenol content from 36% to 60%, protein content from 16% to 41%, flavonoid content from 27% to 82%, tannin content from 60% to 298%, polyphenoloxidase content from 15% to 74% and peroxidase content from 31% to 109% compared to control in both varieties of common bean. Several hypotheses can be put forward to explain : AMF promoted the development of the plant root system, which allowed them to explore a larger volume of soil, to absorb greater amounts of phosphorus and nitrogen and consequently to increase primary and secondary metabolites. Indeed, phosphorus and nitrogen are constituents of amino acids, proteins, phenol and phenolic compounds. It is well established that after root colonization by arbuscular mycorrhizal fungi, plants adjust the expression of their physiological, biochemical, and molecular genes, resulting in the accumulation of important plant metabolites such as proline, terpenes, proteins, and phenols Tchameni et al*.* [35], Savioli et al*.,* [36]. Amino acid metabolism is affected following the establishment of colonization by endomycorrhizal fungi. These results demonstrate a systemic effect of mycorrhizae. A recent in vitro study demonstrated the ability of arbuscular mycorrhizal fungus hyphae to directly absorb amino acids from the culture medium and transfer them to the root of the plant (Whiteside et al*.,* 2012). The results obtained showed that inoculation of plants with AMF reduced the incidence of the order of 20 to 150% and the severity of the angular leaf spot disease of the common bean by the order of 15 to 100% compared to non-inoculated plants (synthetic fungicide treatment and the absolute control). This reduction in the incidence and severity of angular leaf spot disease could be attributed to AMF. AMF are able to regulate the types and amounts of secondary metabolites in the physiological metabolism of host plants, an important mechanism by which arbuscular mycorrhizal fungi induce plant resistance to disease [37]. AMF can induce the production of phytochemicals such as calloses, alkaloids, phenols, phenolic compounds (flavonoids and tannins) and enzymatic compounds (polyphenoloxidases and peroxidases) on the surface of the internal and external hyphae of the root, and these secondary metabolites are beneficial to plants, helping them to resist adverse conditions caused by diseases. The deposition of callose and pectins and the activation of the phenylpropanoid pathway resulting in the accumulation of lignin in mycorrhizal plants are thought to be involved in plant protection [6] (Lee et al*.,* 2005). *Glomus* and *Gigaspora sp* initiate cell wall defense related to protein production, enzyme activity, and increased phenolic compound production of common bean against *Ralstonia solani* [21]. The increased synthesis of total soluble amino acids, proline, total phenols, proteins, phenolic compounds and enzymatic activity allows plants inoculated with AMF to reduce their aggressiveness against *Phaeoisariopsis griseola*. Proline is an amino acid that accumulates in many plant species in response to environmental stress and is an important secondary metabolite for responses to abiotic and biotic stresses [38]. The family of phenolic substances includes various compounds such as flavonoids and phenolic carboxylic acids, which are secondary metabolites all related to signaling molecules and plant defense systems [39]. This considerable reduction in disease could also be attributed to the inoculation of mycorrhizal strains, which are thought to have promoted the induction of defence-related regulatory genes in common bean leaves and conferred resistance to the

pathogen [40]. Campos et al*.* [41] had already demonstrated this by working on the systemic induction of resistance genes by arbuscular mycorrhizal symbiosis in rice. The significant reduction in disease could also be attributed to AMF. AMF stimulate plant growth through better nutrition, improved plant health, and symbiotic compensation for damage caused by the plant pathogen [42,43] (Whipps, 2004 ; Dalpe, 2005 ; Wehner et al*.,* 2010). The morphological and architectural transformation of the root, which can alter the infectious dynamics of the pathogen, although evidence of a correlation has not been demonstrated to date. In addition, AMF induces the formation of thicker lateral roots [44]. Modification of the microflora and the increase in the level of organic matter in the soil. These changes can lead to the stimulation of compound production by microflora with antagonistic activity against certain root pathogens [19]. The induction or suppression of certain plant defense mechanisms, including enzymatic mechanisms [6]. Indeed, mycorrhization protection against root parasites has been associated with the accumulation of phenols, phytoalexins and the induction of the activity of specific isoforms of hydrolytic enzymes such as chitinases and -1,3 glucanases in mycorrhizal roots [17]. The protection conferred by the mycorrhizal association to the plant against Meloidogyne incognita was associated with the expression of a chitinase gene, VCH3, expressed throughout the root system [17]. Finally, the accumulation of defense proteins, in particular PR (Pathogenis Related) proteins and the involvement of the signaling pathways of jasmonic acid, ethylene and salicylic acid, known to play a major role in the regulation of plant defense mechanisms, seem to be at the origin of these protection processes (van Wees et al*.,* 2008). Many other results also indicate a bioprotective effect of AMF : a reduction or even inhibition of the negative effect of certain plant pests [45,28,46]. These results could be attributed, among other things, to the variety effect, since the PNG variety is apparently resistant, unlike the GLP 195-C variety which is tolerante [47-50].

5. CONCLUSION

The objective of this work was to evaluate the bioprotective potential of arbuscular mycorrhizal fungi against the development of common bean angular spot disease. Biochemical parameters, namely : content of amino acids, proline, total phenol, flavonoids, tannins, polyphenoloxidase, peroxidase and proteins. Epidemiological parameters, incidence and severity of angular spot disease were evaluated. From the results obtained, it emerges that : Simple mycorrhizal treatments and mycorrhizal combinations improved amino acid content from 44% to 70%, proline content from 20% to 33%, total phenol content from 36% to 60%, protein content from 16% to 41%, flavonoid content from 27% to 82%, tannin content from 60% to 298%, polyphenol oxidase content from 15% to 74% and peroxidase content from 31% to 109% compared to the control in both common bean varieties.

Analysis of the biosynthesis of secondary metabolites involved in resistance to angular spot disease revealed an increase in amino acid, proline, total phenol, protein, flavonoid, tannin, polyphenoloxidase and peroxidase and a reduction in foliar diseases in common bean plants inoculated with arbuscular mycorrhizal fungi compared to non-inoculated plants. Under conditions of low inputs from smallholder farmers in many parts of Africa, it has been envisioned that agricultural practices that can enable effective mobilization of arbuscular mycorrhizal fungi in soils will lead to improved bean yields. The findings call for an integrated approach involving disease-resistant germplasm, appropriate inputs that will support plants and protect them from *Phaeoisariopsis griseola* attacks.

The present study showed the potential for the development of a biological control measure to control the spread of *Phaeoisariopsis griseola* on African common bean varieties and allows for the development of a variety resistant to angular leaf spot disease that will help smallholder farmers increase crop yields.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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