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Effects of arbuscular mycorrhiza fungi on stimulation of nutrient content and induction of biochemical defense response in *Xanthosoma sagittifolium* plants against root rot disease caused by *Pythium myriotylum*

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ABSTRACT

This study was carried out to evaluate the effect of two selected arbuscular mycorrhizal (AM) fungi on cocoyam(*Xanthosoma sagittifolium*) plant growth, nutrition and bio protection against rot root. Cocoyam seedlings were inoculated with *Acaulospora tuberculata* and *Gigaspora margarita* in sterilize pot soil. Twelve weeks after planting, rhizosphere plant were infected with *Pythium myriotylum* and two weeks later, they were harvested for investigation of morphology, functioning and molecular changes related to plant defence response. The results showed that, the colonization of cocoyam plants with both AM fungal species significantly increase plant shoot, root dry matter, nutrients content and reduce disease incidence. Higher activities of defense related enzymes [phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO)], total phenol content and flavonone accumulation such as apigenin-pentosyl-hexoside, lutein-pentosyl-hexoside, caffeic acid and apigenin-dihexoside were obtained in mycorrhizal plants. This suggested the implication of these components in mycorrhizal cocoyam plants against *P. myriotylum.* AM fungi could be used as biological control agents to fight against Oomycete plant pathogens after assessments under field trials.

INTRODUCTION

Cocoyam (*Xanthosoma sagittifolium* L. Schott) is an intertropical crop cultivated for its high content of carbohydrates and vitamin C. In Cameroon, its annual production is about 1,700 000 tons and its plays an important role in a balance diet for nearly 13 million people (FAO, 2016). However, the major constraints which contributed to reduce the production, the nutritional and economic value of this crop, is rot root disease causing by an Oomycetes namely *Pythium myriotylum*. Under severe conditions, yield loss could be reach 50 to 80% (Nzietchueng, 1983; Zhang and Yang, 2000). To control this disease, farmers commonly used cultural practices, resistant cultivars and chemical fungicide.

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However, cultural practices are not always effective and resistant cultivars are not available. The repetitive use of chemical fungicides is harmful for Human and environment and as a result it may lead to fungicide resistance in the pathogen population. In addition, the pathogen could become more sensible and aggressive when the effects of fungicide diminish. Recently, researches are oriented to biocontrol of soil borne disease including *Pythium* by using arbuscular mycorrhizal (AM) fungi (Parniske, 2008). AM fungi are the most extensive symbiotic relations between certain soil fungi and most terrestrial roots plants. During the mutualism, AM fungi help host plant to growth by increase water and mineral nutrition and AM fungi obtain carbohydrate from plant (Parniske, 2008). Many studies have shown the role of AM fungi in plant resistance to biotic and abiotic attacks. In fact, different species of AM fungi have been effective in reducing plant disease causing by various species pathogens such as *Cylindrocladium, Fusarium, Macrophomina, Phytophthora, Rhizoctonia, Sclerotinium* and *Verticillium* on different host species (Harrier and Watson, 2004; Tchameni et al., 2012). Mechanisms used by AM fungi for disease control are competition for infection site, root damage competition, morphological change in the host root and change in the myco-rhizosphere microbial community. The deceasing in susceptibility in plant pathogen could be explained by modifications in plants physiology. These effects may characterize through changes in production of phenolic compounds and proteins enzymatic such as polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) (Zeng, 2006; El-Khallal, 2007). Phenolic compounds are aromatic components implicated into strength and rigidity of cell walls and for providing barriers during infection attacks by pathogens (Tan et al., 2004; Conaeicao et al., 2006). PPO is an oxidative enzyme involved in the conversion of polyphenols into quinons, which is known as antimicrobial component. It also plays a keys role during cell lignification and hyper-sensibility reaction during plant infection defense (Ray et al., 1998; Mohammadi and Karr, 2002). Additionally, PAL could regulate the biosynthesis of phenolic components during plant pathogenic infection (Wen et al., 2005).

This work aimed to investigate the effects of selected AM fungi, *Acaulospora tuberculata* and *Gigaspora margarita* on cocoyam plants development and to induce biochemical defense against *P. myriotylum*.

MATERIALS AND METHODS

Plant pathogen

P. myriotylum was used as plant pathogen. This pathogen was isolated from root of six month cocoyam plant showing typical symptom of rot root (Dhingra and Sinclair, 1985). Tissues segment (5-10 mm^2) from the infected root was sterilized in 1% sodium hypochlorite for 2-5 min and rinsed with sterile water. Tissues are seed aseptically on *Pythium* selective potatoes dextrose agar (PDA) medium supplemented with penicillin and ampicillin (250 mg/L). After 3-4 days of incubation at 28°C, the mycelia growth emerging from tissues were transferred to fresh selective PDA medium. The operation was done several times to obtain pure culture of the isolate. Identification of the *Pythium* isolate was based on macroscopic and microscopic criteria according to the method described by Johnson et al. (2002). Specific virulence of *P. myriotylum* isolate was evaluated. Pathogen inoculum was a stock suspension of mycelia prepared by inoculates fresh culture of *P. myriotylum* during 5 days in PDA medium and sequentially ground by a mixer for 3 min after mixed with 200 mL of sterile distilled water (Adiobo et al., 2007). The concentration of this inoculum (100 mycelia strands.mL^{-1}) was microscopically determined using a haemocytometer.

AM inoculum

The strains of AM fungi used in this study were *A. tuberculata* and *G. margarita*. The details relating to the identification of these strains were given by Tchameni (2014). These AM fungi were selected for their ability to enhance cocoa growth and protect it against *Phytophthora megakarya.* Each inoculum was a mixture of spores, mycelia and root fragments in sand.

Planting and growth conditions

The cocoyam plants used in this study were the white cultivar, highly susceptible to root rot (Perneel et al., 2007). These plants were obtained through micro-propagation and provided by International Institute of Tropical Agriculture (IITA), Cameroon.

The experiment was done in natural condition in the nursery of the Faculty of Science of the University of Douala (Cameroun). Polystyrene pots of 3 L (size: 25 cm × 30 cm) were filled with sterilized (autoclaved twice at 121°C during 1 h) substrate (2.5 kg/pot) made by the mixture of soil and sand at 2:1 w/w. One healthy plant of cocoyam was sown in the pots and each pot receives 100 g of AM inoculum (10 spores/g). All pots were watered when necessary.

Twelve weeks after AM inoculation, soil infestation was done by watering of rhizosphere of each plant with 20 mL of pathogen inoculum (100 mycelia strands.mL^{-1}) while negative control were treated with 20 mL of sterilized water. The treatment was design as follows: non infected plants with control (AMo), inoculation with *A. tuberculate* (AM_1), inoculation with *G. margarita* (AM_2); infected

plants with the same treatments (AMo, AM_1 and AM_2). Each treatment was made by 8 pots (one plant per pot) with three replicates, the plants grown during 14 weeks, and the experiment was repeated twice.

Disease assessment

Disease root rot due by *Pythium* was assessed two weeks after infected plants pot by using disease severity (DS) and disease index (DI). To evaluate DS, the symptoms that appear on the leaves were observed according to the scale used by Adiobo et al. (2007). This scale is a 0-4 rating defined as follows: 0 = no symptoms of the disease on the leaf (0 %); 1 = start of yellowing of the leaf (1–25%); 2 = pronounced and total yellowing of the entire leaf (26–50%); 3 = start of drying of the leaf (51–75%); 4 = total desiccation and death of the entire leaf (76–100%). DI was evaluated from these data as follows:

$$DI = \{(1 \times n_1 + 2 \times n_2 + 3 \times n_3 + 4 \times n_4) / (4 \times N)\} \times 100$$

Where N is total number of plants; n_1 is number of plants with scale 1; n_2 is number of plants with scale 2; n_3 is number of plants with scale 3; n_4 is number of plants with scale 4.

Plant sampling and analysis

Fourteen weeks after planting, five plants of each treatment were harvested and the shoots were separated from the roots. One part of fresh shoots was kept at -20°C until used and another part was lyophilized for phenols analyze. Five hundred milligram of root (1 cm piece) were removed and kept into ethanol (50%) for the evaluation of AM fungi colonization. The remaining roots, together with the shoots, were dried at 70°C for 72 h, ground and kept for dry matter and mineral analyze.

AM fungi root colonization

Assessment of AM fungi root colonization was performed by the method describe by Kormanik and McGraw (1982). Five hundred milligram of each root sample were cleared by 10% potassium hydroxide and stained with acid fuchsine. After observing under a light microscope, the percentage of root colonized by AM fungi was evaluated by the gridline intersect method. AM fungi colonization included the presence of vesicles or mycelia into the roots.

Estimation of total chlorophyll and mineral uptake

The quantification of total chlorophyll was performed following the methodology described by Lichtenthaler and Wellburn, (1987). Five hundred milligram of fresh leaves were ground with 5 ml of ethanol (90°). The extract was centrifuged at 5000 rpm for 10 min and the supernatant was used to measure absorbance at 664.1 and 648.6 nm wavelengths. Total chlorophyll content (chlorophyll a and b) was calculated and expressed as µg/g of leaf weight.

Three hundred milligram of dried shoot were mineralizing using sulphuric acid. Total nitrogen content was determined according to the method of Devani et al. (1989), while total phosphorus was performed using the ammonium-molybdate blue method (Okalebo et al., 1993). In all sample, the flame Photometer was used to estimate potassium content while the absorption spectrophotometer was done for calcium and magnesium content. The results were expressed as mg.100 g^{-1} dry weight.

Analysis of biochemical parameters

Enzymes assay

PPO assay: The activity of PPO was evaluated by the method of Mayer et al. (1965). One gram of fresh leaf was ground in mortar with 2 mL of ice cold 0.1 M of phosphate buffer pH 7.0. The crude extract was centrifuged at 10,000 rpm at 4°C during 15 min and the supernatant obtained was used as enzyme extract. The reaction mixture consisted of 1.5 mL of 0.1 M of phosphate buffer (pH 7.0) and 200 µL of 0.01 M catechol as substrate. The reaction was initiated by adding of 200 µL of enzyme extract and the mixture was incubated for 2 min at 30°C. The specific activity was expressed as a function of the change in absorbance at 420 nm/min/mg protein. The quantity of proteins was done in to the supernatant according to the method of Bradford (1976).

PAL assay: For the activity of PAL, one gram of fresh leaf was ground in mortar with 5 mL of 0.05 M phosphate buffer, pH 8.8 containing 0.1 g insoluble polyvinyl pyrrolidone at 4°C. The crude was centrifuged at 15,000 g for 30 min and supernatant was used for enzyme analysis. PAL activity was determined spectrophotometrically at 290 nm by the method described by Ross and Sederoff (1992). The mixture containing 100 µL of enzyme extract, 500 µL of 0.05 M phosphate buffer, pH 8.8 and 600 µL of phenylalanine was incubated at 30°C during 1 h. According to Bradford (1976) method, the supernatant was used to evaluate the quantity of proteins. The enzymatic activity was expressed as a rate of the change in the absorbance/h/mg of proteins.

Phenolic compounds analysis

Extracts preparation

Sixty mg of dried cocoyam leaves were ground in grinder.

Table 1. Effect of mycorrhizal fungi on growth parameters of cocoyam plants infected or not with *P. myriotilum*.

Parameters	Non infected			Infected		
Mycorrhizal status	**AM_0**	**AM_1**	**AM_2**	**AM_0**	**AM_1**	**AM_2**
Root colonization (%)	0.00 ± 0.00^{a}	66.66 ± 5.00^{b}	86.68 ± 5.77^{c}	0.00 ± 0.00^{a}	60.00 ± 10.00^{b}	63.33 ± 5.00^{a}
Shoot dry matter (g)	4.40 ± 0.40^{a}	13.30 ± 3.08^{b}	15.00 ± 0.35^{b}	4.50 ± 3.56^{a}	8.60 ± 2.85^{a}	8.53 ± 2.03^{a}
Root dry mater (g)	0.50 ± 0.000^{a}	0.73 ± 0.058^{cd}	1.33 ± 0.153^{e}	0.23 ± 0.058^{b}	0.67 ± 0.153^{ad}	0.50 ± 0.100^{a}

The data are presented as mean ± SD. For each treatment, in the same line, means with same letters are not significantly different. The Duncan test was used to make comparisons two by two. The significance threshold was set at p-value <0.05. **AM_0,**Without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates and the experiment was repeated 2 times.

After addition of 1 ml of methanol and sonification during 30 min into centrifuge tube, the samples were centrifuged (3000 rpm, 15 min) and the supernatants were collected. The extraction procedure was repeated twice and the whole supernatants were mixed to give about 2 mL of the final extract.

LC-DAD-ESI-MS analysis

The phenolic compounds from the methanolic extracts of the leaves of plant cocoyam were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 series with DAD detector and ESI interface) and mass spectrometry (Agilent 6130 LC/MS quadrupole) according to the method describe by Kusznierewicz et al (2012). The HPLC parameters were: PhenomenexKinetex XB-C18 100A column (150 × 4.6 mm, particle size 5 µm); elution solvent mixture of 0.1% aqueous formic acid solution (solvent A) and 0.1% methanolic formic acid solution (solvent B) at a flow rate of 0.8 ml/min; the injection volume of all the samples was 10 µL. The elution gradient profile used was 30 to 50% B in 15 min, 50 to 100% B in 5 min, followed by 10 min to 100% B. The column was previously equilibrated between injections for 5 min with the initial concentration in mobile phase. Absorption spectra were recorded between 190 and 700 nm every 2 s with a bandwidth of 4 nm, while chromatograms were monitored at 270 and 350 nm. The MS parameters were as follows: capillary voltage, 3000 V; fragmenteur, 120v; drying gas temperature, 350°C; gas flow (N_2), 12 L/min; Nebulizer pressure, 35 psig. The instrument operated in positive and negative ion modes, scanning between 100 and 1000 m/z. The phenolic compounds were identified by comparing, the retention times and ultraviolet (UV) and mass spectra with those containing into the literature. However, luteolin glycosides were quantitated as isoorientin and apigenin glycosides as isovitexin (at 325 nm). These standards components were purchased from LGC standards (Sigma).

Statistical analysis

The data were presented as mean ± standard deviation (SD) and were analyzed with SPSS (18.0). Analysis of variance (ANOVA) was used to analyze all multiple comparisons and the comparisons among means were made using Duncan's multiple test at P≤0.05. The relationship between DI and defense parameters was done using Pearson's correlation.

RESULTS

AM fungi root colonization and dry weight

Cocoyam plants grown in soil inoculated by AM fungi were successfully colonized while non-inoculated plants were none colonized. Infected cocoyam plants with *P. myriotylum* reduced the level of root colonization by AM fungi. In both infected and non-infected cocoyam plants, *G. margarita* (AM_2) had the best level of root colonization (Table 1).

In general, shoot and root dry matters were significantly reduced in cocoyam plants infected with *P. myriotylum*, compared to the non-infected ones. However, the two strains of AM fungi significantly enhanced shoot and root of the infected cocoyam plants. Furthermore, mycorrhizal treatment of the non-infected plants significantly increased shoot and root dry matter.

Total chlorophyll and mineral nutrient content

Results presented in Table 2 showed that, except the total nitrogen, total chlorophyll, phosphorus, potassium, calcium and magnesium content were significantly lower in infected cocoyam plants compared to uninfected ones. In infected treatments, AM fungisignificantly increased the total contents of all tested elements and chlorophyll. In the same way, plants treated with AM fungi significantly increased mineral nutrition and photosynthesis. A significant increase in nitrogen content was observed in plants inoculated with AM fungi and infected with *P. myriotylum* (25.80 and 26.60 mg/g dry weight respectively for *A. tuberculata* and *G. margarita*). On the other hand, the infection significantly decreased the total chlorophyll, phosphorus and calcium content in

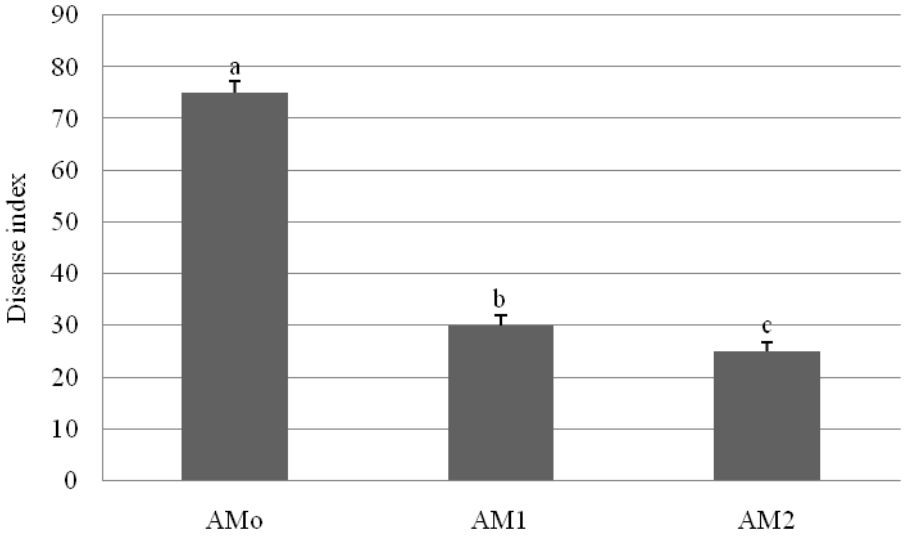

Figure 1. Effects of mycorrhizal fungi on rot root of cocoyam plants two weeks after infected with *P. myriotilum*. For each treatment, same letters are not significantly different. The Duncan test was used to make comparisons two by two. The significance threshold was set at p-value <0.05. **AM_0,**Without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates and the experiment was repeated 2 times.

Table 2. Effect of mycorrhizal colonization on total chlorophyll and mineral uptake of cocoyam plants infected or not with *P. myriotilum*.

Parameters	**Non infected**			**Infected**		
Mycorrhizal status	**AM_0**	**AM_1**	**AM_2**	**AM_0**	**AM_1**	**AM_2**
Total chlorophyll (µg/ml)	199.5 ± 1.2^{a}	217.1 ± 2.1^{c}	207.6 ± 1.5^{e}	162.1 ± 6.4^{b}	129.1 ± 3.0^{d}	182.4 ± 2.7^{f}
Nitrogen (mg/100 g)	2460.0 ± 0.0^{a}	2350.0 ± 0.0^{c}	2400.2 ± 0.1^{e}	2710.0 ± 0.0^{b}	2580.1 ± 0,0^{d}	2660.0 ± 0.0^{f}
Phosphorus (mg/100 g)	214.8 ±1.6^{bc}	246.7 ± 6.4^{a}	247.6 ±0.2^{a}	219.4 ±0.9^{b}	206.9 ± 0.1^{c}	214.4 ±1.9^{bc}
Potassium (mg/100 g)	2305.0±1.0^{a}	2323.0±1.7^{c}	2025.0± 1.0^{e}	2383.0±3.6^{b}	2390.0±2.0^{d}	2021.7±2.1^{e}
Calcium (mg/100 g)	336.0 ± 1.0^{a}	456.0 ± 2.0^{c}	576.0 ± 2.0^{e}	316.0 ± 0.0^{b}	450.0 ± 1.0^{d}	570.7 ± 1.2^{f}
Magnesium (mg/100 g)	122.6 ± 0.3^{a}	186.5 ± 6.0^{b}	152.8 ± 2.0^{c}	127.7 ± 3.1^{a}	190.2 ± 2.0^{b}	150.7 ± 4.0^{c}

The data are presented as mean ± SD. For each treatment, in the same line, means with same letters are not significantly different. The Duncan test was used to make comparisons two by two. The significance threshold was set at p-value <0.05. **AM_0,**Without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates and the experiment was repeated 2 times.

both control and inoculated plants.

Rot root disease assessment

No symptoms of root rot disease were observed in non-infected cocoyam plants (treated with distilled water). Inoculation with AM fungi significantly reduced the incidence of root rot disease (Figure 1). This reduction was 66.7 and 60.0%, respectively, in plants inoculated with *G. margarita* and *A. tuberculata*. Statistical analysis revealed that significant and negative correlations were observed between cocoyam root colonization with AM fungi and root rot index (r = -0.78, p = 0.001 in plants inoculated with *A. tuberculata* and r = -0.88, p = 0.001 in plants inoculated with *G. margarita*).

Analysis biochemical parameters

Enzymatic activities

The effect of two select mycorrhizal fungi (*A. tuberculate* and *G. margarita*) on defense related enzyme activities in cocoyam rot root infected with *P. myriotylum* were presented in Figure 2 and 3. The results indicated that

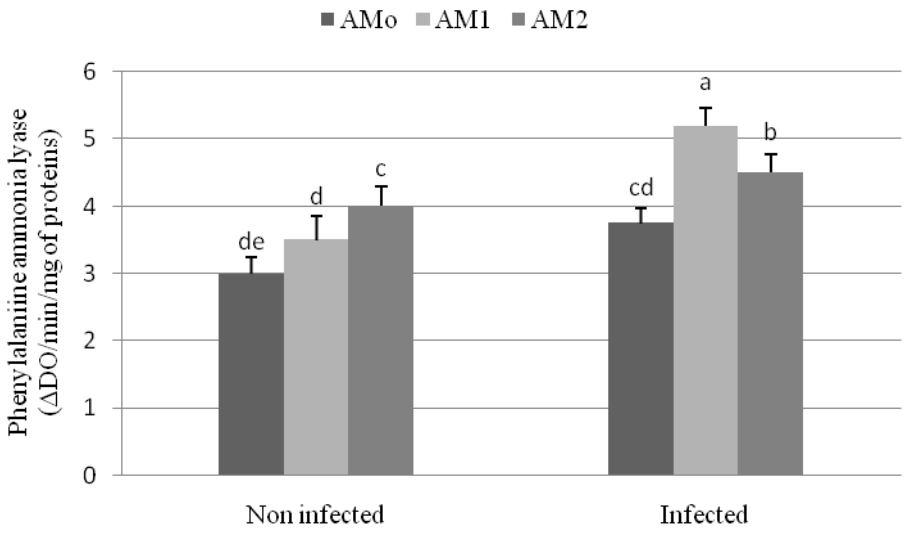

Figure 2. Effects of mycorrhizal fungi on PAL activities of cocoyam plants infected or not with *P. myriotilum*.For each treatment, the same letters are not significantly different. The Duncan test was used to make comparisons two by two. The significance threshold was set at p-value <0.05. **AM_0,**Without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates and the experiment was repeated 2 times.

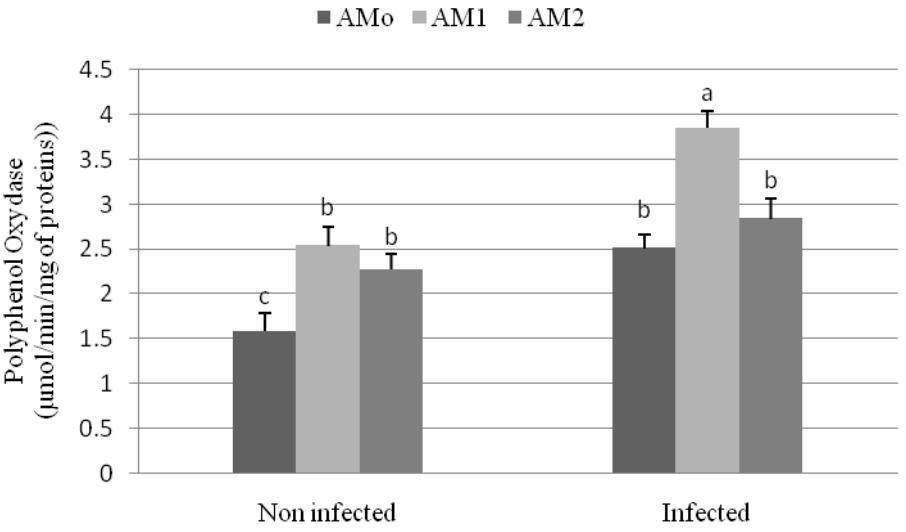

Figure 3. Effects of mycorrhizal fungi on PPO activities of cocoyam plants infected or not with *P. myriotilum*.For each treatment, the same letters are not significantly different. The Duncan test was used to make comparisons two by two. The significance threshold was set at p-value <0.05. **AM_0,**Without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates and the experiment was repeated 2 times.

the activities of PAL and PPO were significantly increased by the infection with *P. myriotylum* when compared with uninfected plants. Moreover, both enzyme activities of the infected plants treated with the two strains of AM fungi were significantly higher than those of non-mycorrhizal in uninfected and infected plants. The highest activity of PAL and PPO enzyme was obtained in infected plants treated with *A. tuberculata*. A significantly negative

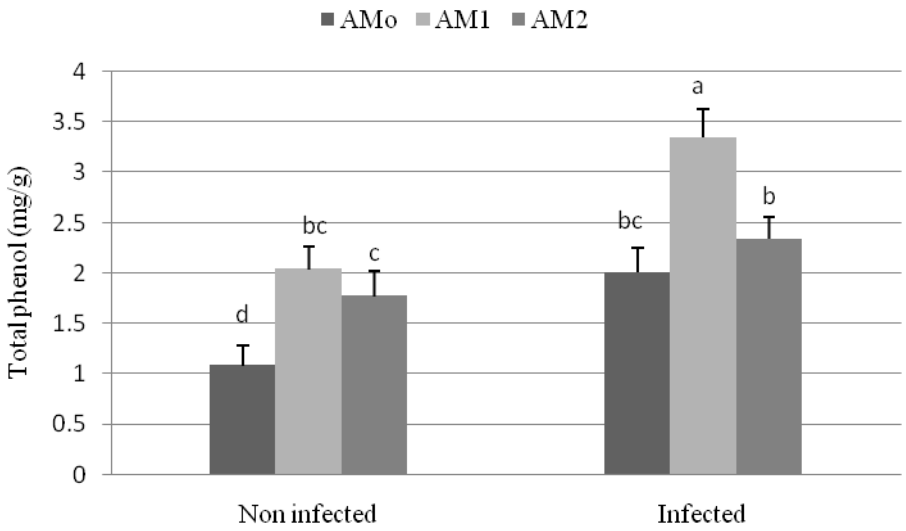

Figure 4. Effects of mycorrhizal fungi on total phenol content of cocoyam plants infected or not with *P. myriotilum*. For each treatment, the same letters are not significantly different. The Duncan test was used to make comparisons two by two. The significance threshold was set at p-value <0.05. **AM_0,**Without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates and the experiment was repeated 2 times.

correlation was found between DI of cocoyam rot root and PAL activity (P = 0.015; r = -0.675 and P = 0.010; r = -0.757 respectively, for *A. tuberculate* and *G. margarita*) and between DI and PPO activity (P = 0.002; r = -0.761 and P = 0.001; r = -0.866 respectively, for *A. tuberculate* and *G. margarita*).

Total phenol content

In general, the total phenol content of non-infected plant was significantly lower than that of the infected plants (Figure 3). In the order hand, the total phenol content of the infected plants treated with both AM fungi was significantly higher than that of non-mycorrhizal. Inoculation of cocoyam plants with both AM fungi significantly increases the total phenol content compared to non-mycorrhizal in uninfected and infected plants. The highest total phenol content was achieved in infected plants previously inoculated with *A. tuberculate* (Figure 4). There was a significantly negative correlation between DI and total phenol content (P = 0.001; r = 0.897 and P = 0.011; r = 0.856 respectively, for *A. tuberculate* and *G. margarita* treatments).

HPLC of total phenol

Analysis of the phenolic compounds shows the presence of 10 different compounds belonging to the group of flavones (Table 3 and Figure 5). The profiles of these compounds show that the concentration is variable depending on the treatment.

In general, there is significant increase in the concentration of different compounds in healthy plants, compared to infected plants, with the exception of the non-mycorrhizal control, where there is a significant decrease in the content of these compounds.

In uninfected plants, AM fungi did not significantly affect the profile of these components, compared to the control. On the other hand, in the mycorrhizal and infected plants, a high significant increase of these compounds is observed. The increase varies from 10 to more than 500 times. The most abundant compound in the control and infected plants is apigenin-pentosyl-hexoside followed by lutein-pentosyl-hexoside, caffeic acid and apigenin-dihexoside. These compounds decreased after infection in non-mycorrhizal plants and increased significantly with infection. It ranges from 0.1 to 142 µg/g of shoot dry matter.

DISCUSSION

This finding shows that the first assessment of AM fungi to increase growth and resistance of cocoyam plants against *P. myriotylum*, the causative against of rot root in Cameroon. It was highlighted that the plant defense responses induced by AM fungi against this disease. The results indicated that, colonization of cocoyam plants with

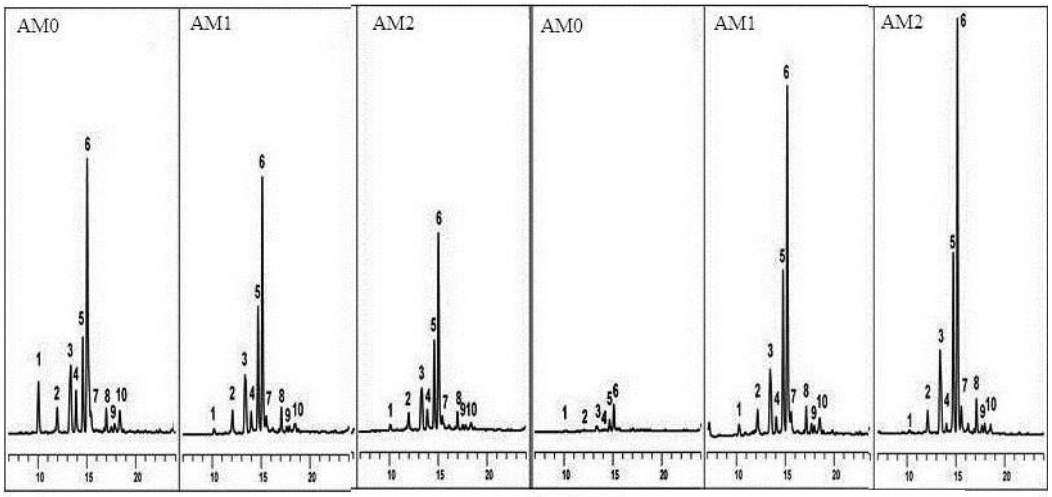

Figure 5. Profile of major phenolics determined in cocoyam leaves samples during LC-DAD-MS analyses. AM_0: without mycorrhiza fungi; AM_1: inoculation with *A. tuberculata*; AM_2: inoculation with *G. margarita*. Each treatment was made by 8 pots with 3 replicates. The number of a peak corresponds to compounds presented in Table 3.

Table 3. The qualitative and quantitative data on major phenolics determined in cocoyam leaves samples during LC-DAD-MS analyses.

Peak no	Compound	t_R [min]	Mass data		Phenolic content (µg/g of shoot dry weight)					
					Non infected			Infected		
			MS (-)	MS (+)	AM_0	AM_1	AM_2	AM_0	AM_1	AM_2
1	Caffeicacid	9.4	179	-	163.9±1.6	19.9±2.9	22.1±0.7	1.9±0.0	42.4±3.1	13.6±1.2
2	Apigenin-dihexoside	11.9	595	595	208.5±4.5	116.8±1.6	114.4±12.9	25.4±1.5	202.6±1.9	158.3±1.5
3	Lutein-pentosyl-hexoside	13.2	579	581	651.4±28.4	521.8±22.0	375.5±13.6	42.1±8.1	557.9±29.3	666.7±14.8
4	Lutein-pentosyl-hexoside	13.9	579	581	349.8±18.4	182.5±14.3	223.7±8.5	67.6±7.4	207.4±10.7	121.0±3.9
5	Apigenin-pentosyl-hexoside	14.6	563	565	435.1±19.8	575.6±8.2	406.2±4.6	61.5±2.8	733.8±54.8	825.0±20.4
6	Apigenin-pentosyl-hexoside	15.0	563	565	1373.5±55.1	1174.6±24.5	953.3±11.5	116.6±12.8	1549.4±64.3	1785.0±54.8
7	Apigenin-pentosyl-hexoside	15.4	563	565	127.0±7.2	106.5±1.7	109.5±6.0	37.6±8.4	101.4±6.8	162.1±0.3
8	Apigenin-pentosyl-hexoside	16.9	563	565	119.8±7.8	137.5±14.4	75.6±6.1	0.1±0.0	127.4±3.1	143.2±0.7
9	Apigenin-hexoside	17.5	431	433	82.6±1.2	37.3±6.1	25.2±5.4	0.1±0.0	71.6±2.6	33.1±4.0
10	Apigeninrutinosideisomer	17.8	577	579	74.5±6.0	38.8±7.3	27.5±8.6	0.1±0.0	69.5±8.2	53.0±5.1

The number of a peak corresponds to peaks on chromatograms presented in Figure 1. **AM_0,** without mycorrhiza fungi; **AM_1,** inoculation with *A. tuberculata*; **AM_2,** inoculation with *G. margarita*.

selected AM fungi (*A. tuberculate* and *G. margarita*) significantly decrease the effects of *P. myriotylum* on growth, photosynthesis, mineral content and DI. These results are in accordance with studies of Harrier and Watson (2004) who found that the vigor of plants significantly increased with different species of AM fungi inoculation. However, according of Zeng (2006), the efficacy of the symbiosis is host specific depending on the density of the pathogen and conditions of the environment during the interaction and the species of mycorrhizal fungus. One of the most positive effects of colonization by AM fungi on the host plant is the increase of phosphorus nutrition which is important in the higher tolerance of mycorrhizal plants to pathogens (Jia et al., 2004).

The results of this study show that the AM fungi used significantly increased the chlorophyll content and the uptake of phosphorus, calcium and magnesium. These observations could be due to the hormonal change in whole cocoyam plants inoculated with AM fungi, which would lead to improved photosynthesis and translocation of nutrients. The main consequence is better growth and resistance of the plant to attack by pathogens (Shaul-Keinan et al., 2002; Abdel-Fattah et al., 2011).

In the present study, the DI of root rot was significantly reduced in plants inoculated with AM fungi. These results is in accordance with the study of many authors who have shown that the inoculation of economically important tropical plants (beans cucumber, cocoa, etc.) with AM fungi has significantly reduced the severity of the disease caused by soil borne pathogens (Chandanie et al., 2006; Martinez et al., 2011; Tchameni et al., 2012; Eke et al., 2016). During the evolution, plants have developed many strategies to defend themselves against biotic stress. Among them, systemic acquired resistance (SAR) plays an important role in the plants defense against pathogens. SAR occurs in plants in response to colonization of AM fungi (Pozo et al., 2002). Moreover, some biochemical and physiological changes has been associated with AM colonization. These changes are characterized by the production of antimicrobial compo-nents such as phenolic components and oxidative enzymes. The synthesis of defense metabolites, such as phenolic compounds and oxidative enzymes are the most common mechanisms used by the plants in response to the infection with inducing agents. This phenomenon was obtained in this work by increasing the activities of the defense enzymes PAL and PPO and total phenol content in infected plants colonized by AM fungi. In response to the infection, plants synthesize phenolic compounds called phytoalexins (Heath, 1996). This reaction, also called immune response, is characterized by a thickening of the wall by deposit of lignin (polymer of aromatic compounds) and consequently limiting the process of the invasion of pathogenic agents (Abdel-Fattah et al., 2011). During microbial invasion, PPO catalyzed the conversion ofpoplyphenols into quinones, compounds implicated into lignification of cell wall and have antimicrobial activities (Mayer, 2006). Also, PAL enzyme catalyzes the first committed step of the core pathway of general phenyl propanoid metabolism. Branch pathways lead to the synthesis of compounds that have diverse functions in plants, notably in defense (Hammerschmidt, 2005). The increase of the activities of PAL and PPO in cocoyam plants could be suggested that, these enzymes are implicated in defense mechanism induced by AM colonization against *P. myriotylum* infection.

Our HPLC phenol results showed the accumulation of Apigenin-pentosyl-hexoside followed by Lutein-pentosyl-hexoside, caffeic acid and Apigenin-hexoside in infected mycorrhizal cocoyam plants. Although these compounds are the compounds which are synthesised during the normal development of plant tissue, their biosynthesis could be modified under the influence of several types of stress (Treutter, 2006). In this study, these compounds significantly decrease after infection of plant cocoyam with *P. myriotylum* and significantly increase in plant infected and previously treated with AM fungi. The results shows that, the infected cocoyam plants treated with AM fungi, accumulation of flavanone such as caffeine acid, apigenin and lutein have been noticed. These compounds could inhibited lytic enzymes such as cellulase, pectinases and xylanases produced by the pathogen and chelates the metals necessary for enzyme activity. There are also contributed to the formation of a hard and almost crystalline structure as a physical barrier against *Pythium* attack (Treutter, 2006)

Conclusion

From the results above, we can concluded that, the application of AM fungi namely *G. margarita* and *A. tuberculata* as bioprotective agent played a key role in enhancement of growth of cocoyam plants, mineral nutrition and resistance against *P. myriotylum.* These protective effects could be related by accumulation of defense enzymes (PAL and PPO) and phenol content in mycorrhizal infected plants.

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