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Mycorrhization of three cultivars of *Colocasia esculenta* L. Schott and evaluation of their resistance to mildew caused by *Phytophthora colocasiae*

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Abstract

The objective of this work was to evaluate the effect of arbuscular mycorrhizal fungi on the development of taro leaf blight during the interaction of *Colocasia esculenta* - *Phytophthora colocasiae*. Three cultivars of *C. esculenta* were used with local names, Ekwanfre, Macoumba, and Banlah. The experimental device was a complete randomized block with two repetitions. Four treatments were applied (Control, Mycorrhizal, Infected, and Mycorrhizal+infected). After one month of growth, an artificial infection was carried out with a solution of spores of *P. colocasiae* of $5x10^4$ sporangia/ml. Disease severity was assessed every 5 days for 20 days. On D_{20}, cytological analysis, mycorrhizal status, metabolite content, and leaf antioxidant enzyme activity were determined. The results showed that the incidence of the disease is 100% from D_{10} for the treatment infected with the Macoumba cultivar. Mycorrhization inhibited the action of the pathogen. The incidence and severity of the disease are low and less than 50%, in the Mycorrhizal+infected treatment plants compared to the Infected treatment. Cytological analysis revealed fungal, and mycorrhizal structures in the leaves and roots. The maximum frequency and intensity of mycorrhization were 73.33±02.51 and 12.83±04.75% in the mycorrhizal plants of the Macoumba treatment. The total chlorophyll content is less than 1 $mg.g^{-1}$ of WF, in the three cultivars in infection condition. The contents of total soluble proteins, amino acids, and proline are higher in the plants of the infected treatment compared to mycorrhizal and Mycorrhizal+infected. The H_2O_2 produced was scavenged by APX and G-POD compared to CAT. These results make it possible to note that the AMFs used contribute to the protection of the *C. esculenta* plants against the harmful effects linked to the presence of *P. colocasiae*.

Keywords: *Colocasia esculenta* L. Schott, taro leaf blight, *Phytophthora colocasiae* Racib, mycorrhizae, secondary metabolite, and antioxidant enzymes.
Abbreviations: Hydrogen peroxide (H_2O_2), Catalase (CAT) Ascorbate peroxidase (APX), and Guaiacol peroxidases (G-POD)

Introduction

Colocasia esculenta (L.) Schott is a monocot in the Araceae family. It is an important subsistence crop enjoyed by millions of people in developing countries (Mishra et al., 2008). In view of the diversity of nutritional attributes that this plant offers in this case with its richness in carbohydrates (60-90%) and starch (73-80%) (Jane et al., 1992), as well as nutrients, including minerals such as calcium, phosphorus, iron, and vitamins (vitamin C, thiamine, riboflavin, and niacin), it is noted that the consumption of *C. esculenta* is becoming important nowadays in the prevention of malnutrition in some producing countries (Otekunrin et al., 2021). Studies are increasingly highlighting antimicrobial, anti-hepatotoxic, anti-tumor, anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic, and anti-cancer properties in the latter (Islam et al., 2018; Ribeiro et al., 2021; Liang et al., 2021). The plant of *C. esculenta* is also a highly valued food source for its richness in bioactive compounds such as antioxidant, anti-obesity, anti-diabetic, immunomodulatory, and anti-metastatic activities involved in the prevention of oxidative stress, with a strong capacity to modulate the pro-inflammatory status, thus controlling the metabolic dysfunction of the individual (Davis and Ross, 2019; Ribeiro et al., 2021). In spite of this importance, the disruption of *C. esculenta* cultivation in several producing countries remains the attack by *Phytophthora colocasiae*, the causal agent of taro leaf blight. In Cameroon, farmers first recorded the disease in 2009 (MINADER, 2010). The attack of *C. esculenta* leaves by this fungus is the reason for the scarcity of large monoculture taro plantations throughout the country. The expression of the disease during the whole year is explained by the fact that the soils have become foci of infection because of their abundance of spores. Moreover, it should be noted that the aggression of the leaf of *C. esculenta* by *P.*

colocasiae, is an important brake for the setting in tuberization in plants. The dysfunction of photosynthesis induced by the disease would result in the absence or reduction of storage, hence the decrease in production and the scarcity of tubers available for consumers on the markets. This destruction of the leaves by *C. esculenta* by *P. colocasiae* is at the origin of the weakening of the plant's defense system and consequently, would explain the presence of attacks of other pathogens thus observed at the level of the rhizosphere following the example of *Pythium myriotylum* (Kemekong, 2015). To control the attacks of *P. colocasiae*, studies carried out show that some authors present the uses of plant extracts or essential oils (Zhang et al., 2021; Muhammad et al., 2022). While the majority resort to the use of chemicals, this includes the conventional use of synthetic copper and phosphorus fungicides (Jackson, 1999; Omane et al., 2020; Omeje et al., 2022). In spite of their sometimes proven effectiveness, the impact of these chemical treatments remains in the long run an enormous risk in the pollution of the environment, whose consequence will be the development of resistance of the pathogen. However, in a context where climate change is sounding an alarm and also recurrent observations of the after-effects of the use of these chemical plant protection products on human and animal health are a worrying situation. It would be interesting today to propose and explore other methods of biological control such as the use of arbuscular mycorrhizal fungi. Studies present the role of arbuscular mycorrhizal fungi (AMF) in soil health, plant health, and food security (Bonfante and Genre, 2010; Fokom et al., 2023; Boutasknit et al., 2023). Arbuscular mycorrhizal fungi are soil microorganisms that form symbioses with the roots of the majority of higher plant species (Nelly et al., 2019). Their extraradical mycelium plays an important role as an extension of plant roots and can reach beyond the root exhaustion zone to better explore the soil for better water and nutrient uptake (Hohnjec et al., 2005). They contribute to nutrition and growth enhancement in plants and likewise, it is noted that they influence soil enzyme activities while promoting microbial activity (Abdel, 2011). In the context of plant protection against pathogens, the presence of AMFs in the rhizosphere would be at the origin of the induction of the systemic expression of proteins and genes involved in the defense at the level of root and leaf tissues (Campos-Soriano et al., 2010). They would also facilitate the accumulation of secondary metabolites in the plant during the interaction, such as phenolic, vitamins, and sugars (Rouphael et al., 2017; Avio et al., 2020) while causing the attenuation of the expression of oxidative stress caused by the pathogen (Wu and Zou, 2009). Furthermore, mycorrhization is reported to contribute to the increase of peroxidase activity in plants inoculated and infected with *Dianthus caryophyllus* (Atakan and Ozkaya, 2021). The presence of arbuscular mycorrhizal fungi in the rhizosphere of the plant is also thought to be responsible for the production of several compounds such as phenols, chitinase enzymes, and -1,3 glucanase enzymes suspected to control *Ganoderma boninense* attacks in oil palm (Nurzannah et al., 2022). However, what would be the influence of arbuscular mycorrhizal fungi on taro leaf blight attacks caused by *Phytophthora colocasiae*? It has been demonstrated that arbuscular mycorrhizal fungi have the potential to stimulate growth in *X. sagittifolium* and similarly in *C. esculenta* (Djeuani, 2018; Djeuani et al., 2021). Their use in the improvement of these two plants is therefore significant. However, their impact on the control of taro leaf blight caused by *Phytophthora colocasiae* in *C. esculenta* would be an asset. It is in this perspective that, the objective of this work was to evaluate the effect of arbuscular mycorrhizal fungi during the *C. esculenta* - *P. colocasiae* interaction. More specifically, we aimed to determine their impact on the evolution of taro leaf blight while highlighting their effect on the content of some metabolites and on the activity of antioxidant enzymes.

Results

Evaluation parameter of disease expression in the conditions of infection

Disease incidence and severity

The incidence of the disease evaluated in the plants of the Infected and Mycorrhizal+infected treatment varies and increases significantly over time in the three cultivars of *C. esculenta* (Figures 1A, B, and C). It can be seen that the progression of the disease is very high in the plants of the Macoumba cultivar of the infected treatment compared to the Banlah and Ekwanfre cultivars, with a maximum incidence of 100% of the disease observed from D_{10} (Figure 1B). This incidence is very low in the Ekwanfre cultivar at D_{20} (88%) (Figure 1C). For the Mycorrhizal+infected treatment, the plants were less attacked. The presence of mycorrhizae delayed the expression of the disease in *C esculenta* plants of Banlah and Ekwanfre cultivars until D_{10} (Figures 1A and B). However, this incidence appears constant between D_{15} and D_{20} in the plants of the Mycorrhizal+infected treatment of the Macoumba cultivar (48%) (Figure 1B). The progression of downy mildew varied significantly on the Student Newman and Keuls test ($P<0.05$) between the infected and Mycorrhizal+infected treatment on D_{20} (Table 1). Mycorrhization has an inhibitory effect of 100% on the severity of the disease during the first 5 days of infection in the plants of the Mycorrhizal+infected treatment of Banlah and Macoumba, and during the first 10 days, in the plants of the Mycorrhizal+infected treatment at Ekwanfre. The inhibitory effect related to mycorrhization is 50.25, 48.27, and 55.69% respectively in the plants of Banlah, Macoumba, and Ekwanfre Mycorrhizal+infected on D_{20} (Table 1).

Microscopic appearance of healthy and diseased leaves

The results of microscopic observations highlighted distinctions between healthy and diseased *C. esculenta* leaves (Figures 2A and B). In healthy plants from the Control and Mycorrhizal treatments, the microscopic analyzes make it possible to distinguish plant cells differentiated by their contours from the cytoplasmic membrane of the cytoplasm and the nucleus, which are green in color (Figure 2A). However, for the infected and Mycorrhizal+infected treatments, a degradation of the chlorophyll present in the plant cells of the leaves of *C. esculenta* is observed (Figures 2B and C). Sporangia containing spores were also identified in diseased leaves of treatment of the cultivar Macoumba infected (Figures 2D and E).

Mycorrhizal status of roots of mycorrhizal plants

The cytological analyzes of the absorbent hairs of the roots of the plants carried out on D20, made it possible to observe an absence of mycorrhizal structures, in the plants of the control treatment (Figures 3A, E, and I) and infected (Figures 3C, G, and K), in three *C. esculenta* cultivars used. However, in the plants treated with mycorrhizal (Figures 3B, F, and J) and Mycorrhizal+infected (Figures 3.D, H, and L), observations revealed endophytic structures such as intra

and inter-hyphae characteristic roots of endomycorrhizae, crossing the root cortex on either side. Arbuscules and vesicles were also observed in the roots of plants from Macoumba (Figures 4A, B, and D), Banlah (Figure 4C), and Ekwanfre (Figure 4E). The mycorrhizal status of the roots of the mycorrhizal *C. esculenta* plants determined shows that the frequency of mycorrhization is significantly different in the Student Newman and Keuls test ($P<0.05$), in all the cultivars used for the mycorrhizal and Mycorrhizal+infected treatment (Figure 5). The maximum value of this mycorrhization frequency is 73.33±02.51%, recorded in the Macoumba plants of the mycorrhizal treatment. In the Mycorrhizal+infected treatment plants, 50.00±03.46% and 50.00±02.00% are recorded in the Macoumba and Ekwanfre cultivars respectively. However, the intensity of mycorrhization appears very significant ($P<0.05$) for the plants of the Macoumba cultivar (12.83±04.75%), compared to the Banlah and Ekwanfre cultivars (Figure 6). This intensity is very low in all the *C. esculenta* plants of the Mycorrhizal+infected treatment, with values of 02.57±00.84, 03.63±00.41 and 03.42±00.65%, less than 5%, respectively for the plants of Banlah, Macoumba and Ekwanfre (Figure 6).

Evaluation of some biochemical parameters in the leaves of C. esculenta according to the treatments applied

Total chlorophyll content

The total chlorophyll in the leaves varies in the three cultivars of *C. esculenta* according to the treatments applied at D_{20} (Figure 7). In the control, this content does not vary significantly. It is 02.27±00.05; 02.83±00.00 and 02.39±00.01 $mg.g^{-1}$ of PMF, respectively for the Banlah, Macoumba, and Ekwanfre cultivars. Mycorrhization almost doubles this total Chl content in the mycorrhizal treatment plants compared to the control plants, in all the cultivars. The maximum peak is 04.74±00.03 $mg.g^{-1}$ of PMF, in the plants of the Banlah cultivar of the mycorrhizal treatment. For the infected and Mycorrhizal+infected treatments, the total Chl contents are very low compared to the control plants. In the plants of the infected treatment, it is less than 1 $mg.g^{-1}$ of PMF. The results show, 00.59±00.02; 00.68±00.01 and 00.58±00.01 $mg.g^{-1}$ of PMF, respectively for the Banlah, Macoumba, and Ekwanfre cultivars.

Secondary metabolites evaluated in leaves following treatments

The content of the different secondary metabolites evaluated in the leaves of *C. esculenta* varied according to the treatments in the three cultivars used (Figure 8). The control treatment plants have a high total soluble sugar content in the Macoumba cultivar compared to the two other cultivars with 20.65±00.32 $mg.g^{-1}$ of PMF. This content of total soluble sugars is significant in all mycorrhizal plants, with a high value of 26.70±00.08 $mg.g^{-1}$ of PMF for the Ekwanfre cultivar (Figure 8A), and appears very low in all infected plants. Total soluble protein content was high in infected treatment plants compared to control, mycorrhizal and Mycorrhizal+infected treatments for all cultivars. The maxima are 77.27±02.93 and 71.62±01.95 $mg.g^{-1}$ of PMF, respectively the infected treatment of the Macoumba and Banlah cultivars (Figure 8B). Total amino acid and proline contents in *C. esculenta* leaves were higher in all cultivars under infection conditions. The results show a maximum of 73.24±00.24 $mg.g^{-1}$ of PMF in the Banlah plants of the infected treatment (Figure 8C). As for the proline content, it is very significant ($P<0.05$), in the Macoumba plants of the infected treatment (00.06±00.00 $mg.g^{-1}$ of PMF), compared to the control, mycorrhizal and Mycorrhizal+infected treatments for all cultivars. This proline content is almost very low in all infected plants. For phenolic compounds, the high levels are 121.64±01.43 and 129.61±00.44 $mg.g^{-1}$ of PMF, in the plants of Macoumba and Ekwanfre, for the mycorrhizal and Mycorrhizal+infected treatments (Figure 8E).

Antioxidant enzyme activities in leaves following treatments

The H_2O_2 content and the activities of CAT, G-POD, and APX evaluated varied significantly according to the treatments applied (Figures 9A, B, C, and D). They appear very low in the plants of the control treatment of the three cultivars. On D_{20}, the presence of *P. colocasiae* in the leaves stimulates the synthesis of H_2O_2 in the *C. esculenta* plants of the infected and Mycorrhizal+infected treatments, in all three cultivars. The high H_2O_2 contents are 3.62±0.25 and 4.08±0.16 $mM.min^{-1}.g^{-1}$ of FW, in the plants of Macoumba and Banlah, mycorrhizal and Mycorrhizal+infected treatments (Figure 9A). This production of H_2O_2 significantly influenced the activities of CAT, G-POD, and APX evaluated (Figures 9B, C and D). These antioxidant enzymes are similarly significantly higher in the *C. esculenta* plants of the infected treatment in all three cultivars compared to the plants of the control, mycorrhizal and Mycorrhizal+infected treatments. The CAT activities determined were 14805.55±00.66 $mM.min^{-1}.g^{-1}$ of FW, in the Macoumba plants of the infected treatment (Figure 9B). This CAT activity is not significant in the plants of the infected and Mycorrhizal+infected treatment in the Banlah cultivar. The activities of G-POD appear weaker compared to those of AXP and CAT (Figure 9C). It remains very significant in the Macoumba plants of the infected treatment (6932.33±02.66$mM.min^{-1}.g^{-1}$ of FW) compared to the Control, Mycorrhizal, and Mycorrhizal+infected treatments. Unlike CAT and G-POD evaluated, APX was synthesized the most (Figure 9D). Its activity is very high and is 63071.42±00.28; 67071.42±00.19 and 65785.71±00.01$mM.min^{-1}.g^{-1}$ of FW, respectively in the plants of Banlah, Macoumba, and Ekwanfre of the infected treatment (Figure 9D).

Discussion

The objective of this work was to evaluate the effect of arbuscular mycorrhizal fungi on the evolution of taro downy mildew during the *C. esculenta – P. colocasiae* interaction. The results obtained show that the incidence and severity of the disease evaluated in the plants of the infected and Mycorrhizal+infected treatment vary and increase significantly over time in the three cultivars of *C. esculenta*. The progression of the disease is very high in the plants of the Macoumba cultivar of the infected treatment compared to the Banlah and Ekwanfre cultivars, with maxima of 100% of the incidence of the disease from D_{10}. Similarly, the severity of the disease evaluated was very high in the Macoumba cultivar at D_{20}. These results reflect the sensitivity of the Macoumba cultivar to attacks by *P. colocasiae*. Studies by Kemekong, 2015, also showed that in the field, downy mildew expression appears higher in plants of the same cultivar, compared to the other two cultivars. However, the *C. esculenta* plants that were used in this experiment were 4 months old and therefore would be in their first growth phase.

Table 1. Effect of mycorrhization on disease severity in the three cultivars of *C. esculenta* infected with *Phytophthora colocasiae*.

Varieties	Treatment	Time (Days)									
		D_0		D_5		D_{10}		D_{15}		D_{20}	
		Disease severity (%)	Effect (%)	Disease severity (%)	Effect (%)	Disease severity (%)	Effect (%)	Disease severity(%)	Effect (%)	Disease severity (%)	Effect (%)
Banlah	Inf.	00.00 [a]	-	29.60 [c]	-	42.30 [e]	-	62.36 [g]	-	70.20 [h]	-
	Myco. + Inf.	00.00 [a]	00.00	00.00 [a]	100.00	08.00 [b]	81.08	26.00 [c]	58.30	34.92 [d]	50.25
Macoumba	Inf.	00.00 [a]	-	31.08 [d]	-	54.20 [f]	-	77.60 [h]	-	90.20 [i]	-
	Myco. + Inf.	00.00 [a]	00.00	00.00 [a]	100.00	25.33 [c]	53.26	38.75 [d]	50.06	46.66 [e]	48.27
Ekwanfre	Inf.	00.00 [a]	-	07.50 [b]	-	13.05 [b]	-	31.91 [d]	-	45.59 [e]	-
	Myco. + Inf.	00.00 [a]	00.00	00.00 [a]	100.00	00.00 [a]	100.00	12.25 [b]	61.61	20.20 [c]	55.69

Average following by different letters in the same column are significantly different (P<0.05) according to Student Newman and Keuls test. Inf: infected plants, Myco+Inf: mycorrhizal then infected plants, D: day

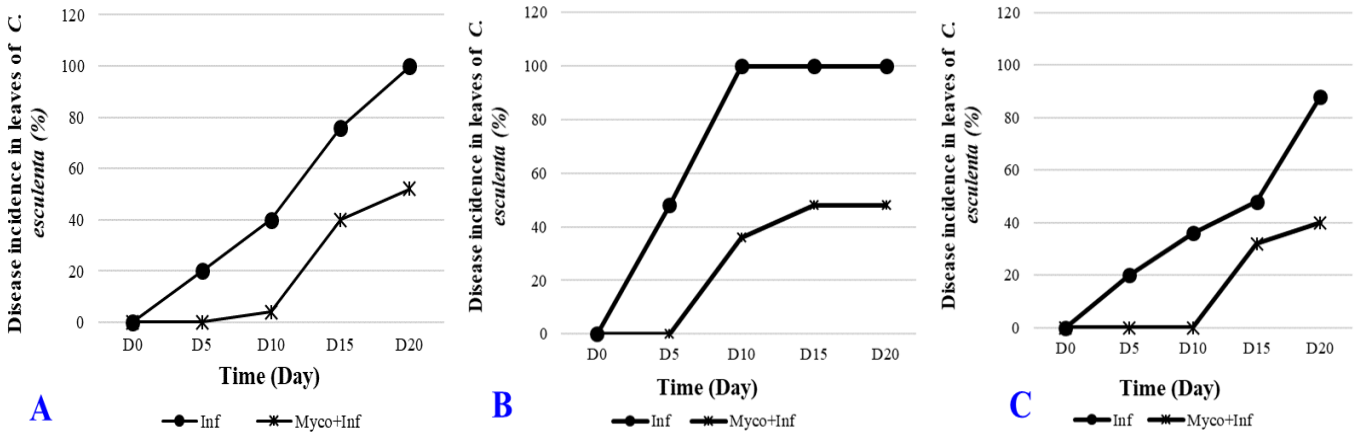

Figure 1. Evolution of disease incidence in the three cultivars of *C. esculenta* over time. Treatment infected (Inf) and Mycorrhizal+infected (Myco+Inf). varieties: Banlah (A), Macoumba (B), and Ekwanfre (C). D: day

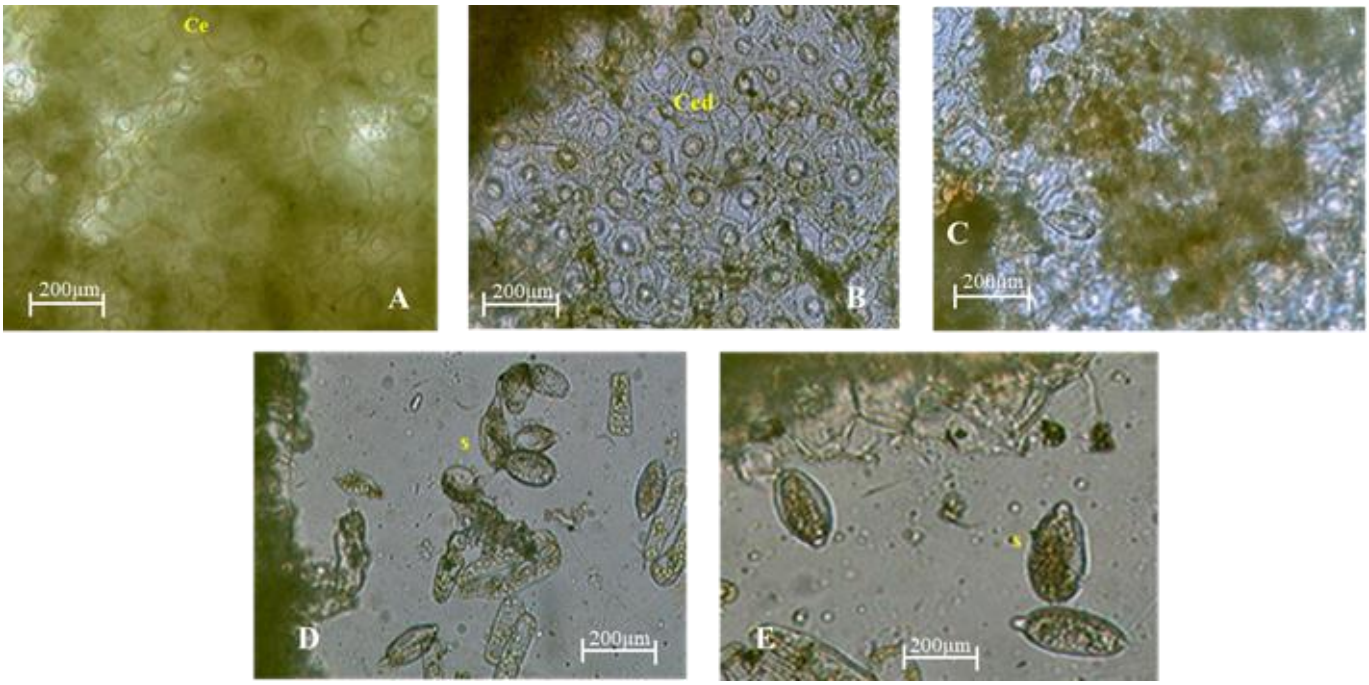

Figure 2. Cytological section of healthy and attacked leaves of *C. esculenta* by *Phytophthora colocasiae* observed with a Leica optical microscope. Diaplan (ORSTOM) at 200µm. Healthy leaves rich in chlorophyll (A), diseased leaves with degraded chlorophyll appearance (B), diseased leaves with degraded chlorophyll appearance with a sporangium (C) and sporangia observed in diseased leaves (D and E). Normal plant cell (Ce), pathogen-degraded plant cell (Ced), sporangia (s).

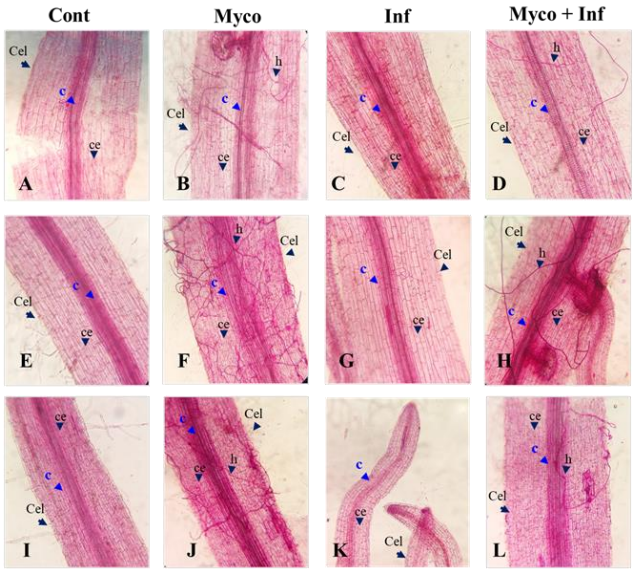

Figure 3. Aspect of the root of *Colocasia esculenta* plant stained with Fuchsin acid and observed at 400X. Roots of Banlah (A, B, C and D), Macoumba (E, F, G and H) and Ekwanfre (I, J, K and L). Treatment: Control (A, E and I), Mycorrhizal plants (B, F and J), Infected plants (C, G and K) and mycorrhizal+infected plants (D, H and L). Plant cells of the hypodermis (Ce), plant cells of the epidermis (cel), cortex (C), and hyphae (h).

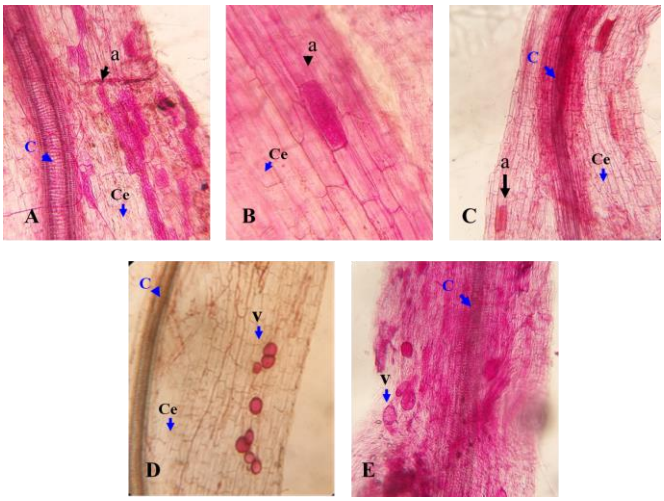

Figure 4. Appearance of arbuscules and vesicles inside the root of different varieties of *Colocasia esculenta* plant stained with Fuchsin acid and observed at 400X. Roots of Macoumba (A, B and D), Banlah (C) and Ekwanfre (E). Arbuscules (a), plant cells of the hypodermis (Ce), plant cells of the epidermis (cel), cortex (C), hyphae (h), and vesicles (v).

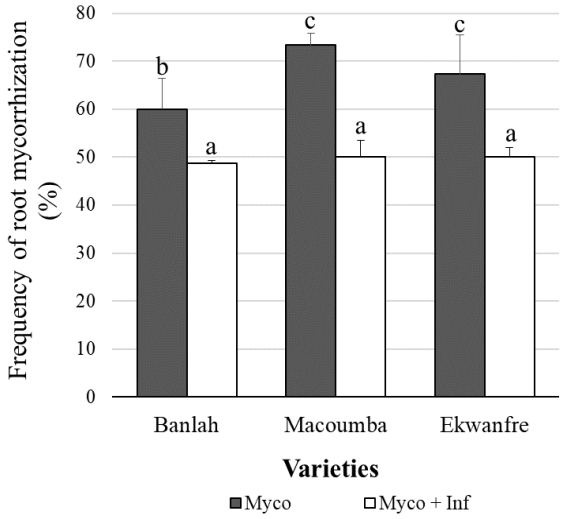

Figure 5. Frequency of mycorrhization of roots of the three cultivars of *Colocasia esculenta*, in the absence and presence of *Phytophthora colocasiae*. Mycorrhizal plants (Myco) and Mycorrhizal+infected plants (Myco + Inf).

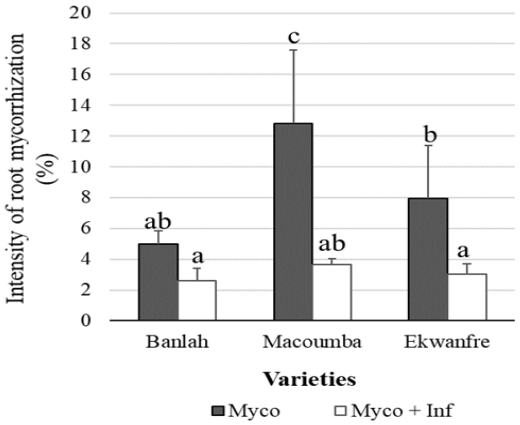

Figure 6. Intensity of mycorrhization in the roots of the three cultivars of *Colocasia esculenta*, in the absence and presence of *Phytophthora colocasiae* at D_{20}. Mycorrhized plants (Myco) and mycorrhized and then infected plants (Myco+Inf).

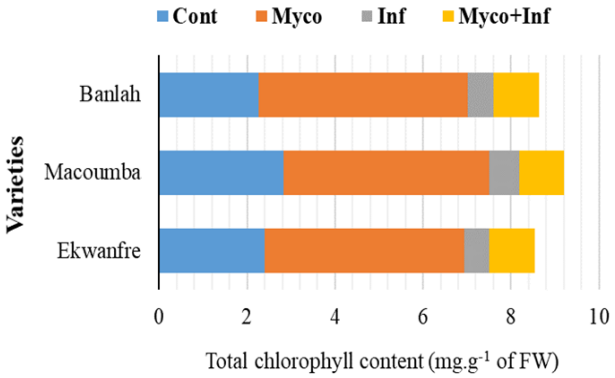

Figure 7. Evaluation of total chlorophyll content in leaves of three cultivars of *C. esculenta* at D_{20} following treatments. Treatments: Control (Cont), Mycorrhizal (Myco), Infected (Inf) and Mycorrhizal+infected (Myco+Inf).

In the first phases of growth, the damage caused by pathogens appears more severe in certain plants, but this sensitivity of the Macoumba cultivar could be attributed in part to the genetic difference that exists between the three cultivars on the one hand, but also, would perhaps be linked to the absence or low synthesis of specific defense enzymes in response to attacks by *P. colocasiae*. This would also explain the rapid degradation of the leaves of this cultivar thus observed during the interaction with the pathogen. In all the plants of the Mycorrhizal+infected treatment, the aggressiveness of P. colocasiae was less. Mycorrhization would contribute to protecting the plants of the three cultivars and inhibit the expression and progression of downy mildew. The inoculated arbuscular mycorrhizal fungi (*Gigaspora margarita* + *Acaulospora tuberculata*) would contribute to the activation of the synthesis of enzymes and/or metabolites involved in the inhibition and/or modification of the metabolic activities of *C. esculenta* playing a role significant in protection against *P. colocasiae*. Sanmartín et al., (2020), for example, concluded that mycorrhizal tomato plants inoculated with *Rhizoglomus irregular* synthesized callose as a barrier tool against *Botrytis cinerea* infection. Similarly, results of reducing the severity of carbonaceous root rot caused by *Macrophomina phaseolina* were recorded by Spagnoletti et al. (2020), in Glycine max plants, inoculated with *R. intraradices*. Biological controls from the use of arbuscular mycorrhizal fungi are promising avenues for reducing the damage caused by pathogen attacks (Liu et al., 2007; Campos-Soriano et al., 2010; Wang et al., 2022; Weng et al., 2022). The plants of the three cultivars of *C. esculenta* of the Mycorrhizal+infected treatment show a drop in the parameters of the mycorrhizal status evaluated. This could be explained by the fact that the attacks of downy mildew cause the destruction of leaves in *C. esculenta*, consequently leading to the limitation of the carbon source. This limitation of the carbon source does not allow the plants of the Mycorrhizal+infected treatment to be able to respond favorably in terms of the cost of carbohydrates vis-à-vis the pre-established symbiosis. The hyphae thus observed in the roots of the *C. esculenta* plants of the Mycorrhizal+infected treatment used are solely the result of the establishment of symbiosis before infection. All the mycorrhizal structures observed in all the plants of *C. esculenta* in the three cultivars reflect the presence of a good establishment of symbiosis. The very high frequency of root colonization in the plants of the mycorrhizal treatment compared to the Mycorrhizal+infected treatment, makes it possible to note that the destruction of the leaves by the *P. colocasiae* is a limiting factor for the establishment of the symbiosis in *C. esculenta*.

The total chlorophyll content evaluated varied significantly according to the treatments applied to the three cultivars of *C. esculenta*. The drop in this chlorophyll content in infected plants would be the direct consequence of the aggressiveness of *P. colocasiae* responsible for leaf destruction. *P. colocasiae*, by destroying the leaves, would certainly use the carbohydrates present in the plastids, for its own growth and also for the establishment of the synthesis of its lytic enzymes. Mycorrhization almost doubles this chlorophyll content in mycorrhizal plants compared to control plants, in all cultivars. This increase in the total chlorophyll content would be the result of the improvement in hydromineral nutrition, thus leading to an improvement in photosynthesis to meet, in terms of carbon cost, the needs of symbiosis in the three cultivars of *C. esculenta*. Host plants can allocate up to 20% of their produced carbohydrate to their symbionts (AMF) (Douds et al., 2000; Soudzilovskaia et al., 2015). Therefore, the improvement of photosynthesis would therefore contribute to the increase in the production of sugars necessary for symbiosis. Sugars are the products of photosynthesis. They play a crucial role in the regulation of cellular functions (Pago et al., 2000). The photosynthates produced are used by AMFs to build mycorrhizal structures inside and outside the cells of their host plants. Moreover, Drain et al., (2016), report that the colonization of roots by AMF induces the appearance of mycorrhizal structures like arbuscules. These arbuscules are units at which exchanges between the host and the fungus take place. Their continuous formation in the roots has the effect of increasing the demand for sugars by AMFs. The plants of the three cultivars of the infected treatment showed the lowest total soluble sugar content. The presence

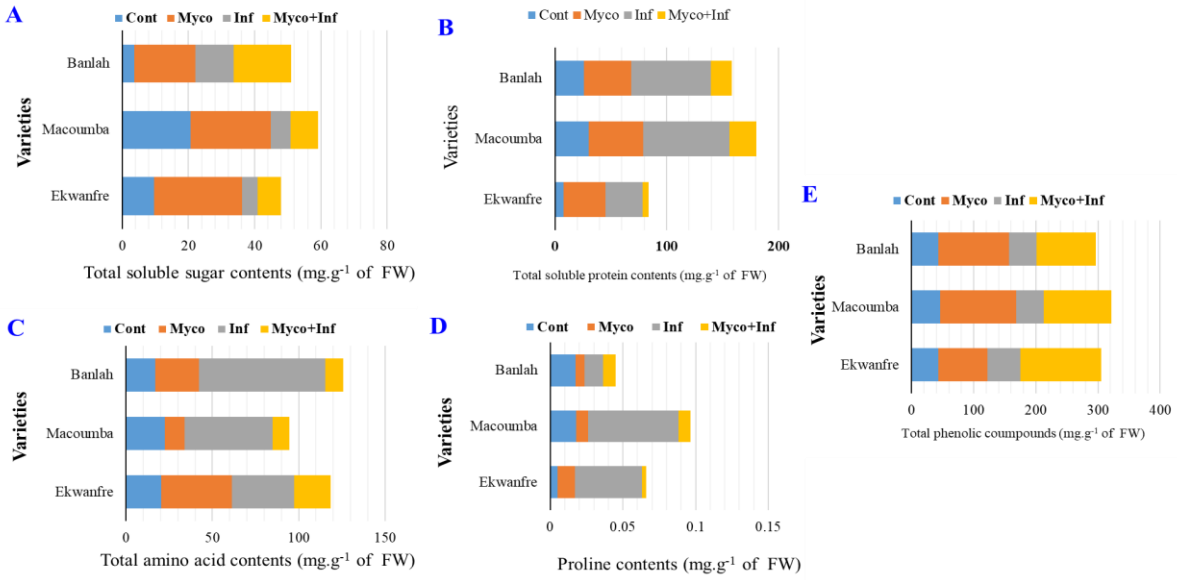

Figure 8. Variation of some metabolites in the leaves of *C. esculenta* following treatments at D_{20}, in the three cultivars used. Treatments: Control (Cont), Mycorrhizal (Myco), Infected (Inf) and Mycorrhizal+infected (Myco+Inf).

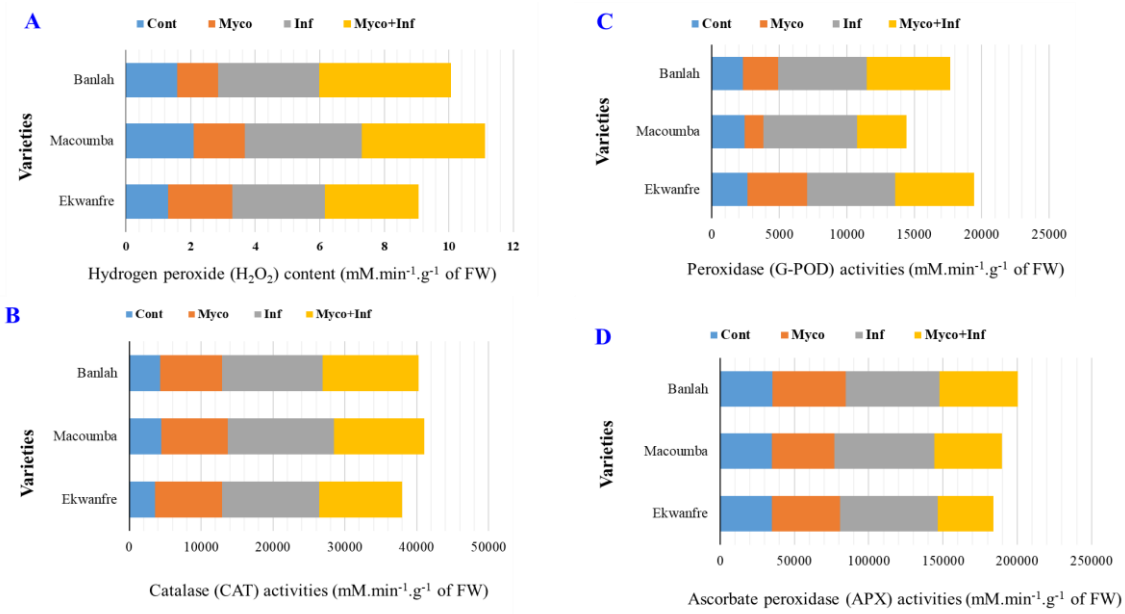

Figure 9. Variation in H_2O_2 content and oxidative enzyme activities at D_{20} in *C. esculenta* leaves according to treatments applied. Treatments: Control (Cont), Mycorrhizal (Myco), Infected (Inf) and Mycorrhizal+infected (Myco+Inf).

of low levels of chlorophylls in these infected plants explains this low synthesis of sugars.

The more the green plant undergoes the degradation of its chlorophyll, the less it realizes the synthesis of sugars. In the plants of the mycorrhizal treatment, the content of total soluble sugars evaluated was very high compared to the control and infected plants. These high values are the result of symbiosis. According to Zouine and El Hadrami, (2004), the sugars resulting from photosynthesis are involved in two metabolic pathways: the pathway of amino acids precursors of the biosynthesis of phenols and proteins and the pathway of phenylalanine ammonia-lyase (PAL). The results show that there would be a negative and non-significant correlation between the contents of total soluble sugars and that of total amino acids. The amino acid contents were higher compared to that of sugars. This suggests that the sugars produced would be directly involved in the amino acid synthesis pathway. These amino acid and proline contents in the leaves are higher in all cultivars under infection conditions. They present significant values in the Macoumba cultivar compared to Banlah and Ekwanfre. Of the three cultivars, Macoumba is more susceptible to *P. colocasiae*. Amino acids play a key role in the defense of plants against biotic stress. This important amino acid synthesis in infected plants, in response to attacks by *P. colocasiae*, would help activate the immune system in all three cultivars, since amino acids are known to serve as precursors to many primary and secondary metabolites in defense in plants. Moormann et al., (2022), also point out that amino acid metabolism is crucial for immune signaling within the plant when establishing a systemic immune response. The high proline levels would explain their involvement in helping *C. esculenta* plants to tolerate stress related to *P. colocasiae*. In addition to being associated with defense in plants, amino acids are also building blocks for protein synthesis. The results showed that total soluble protein levels were

significantly elevated in *C. esculenta* plants of the infected treatment in all three cultivars. This positive correlation suggests that the amino acids produced would be directly used in part in the biosynthesis pathway for defense-related proteins. Similar results of increased protein synthesis under infection conditions were obtained by El-Sharkawy and Abdelrazik (2022), in the leaves of four squash cultivars, previously inoculated with a mycorrhizal complex of the genus *Glomus* (*Glomus intraradices, G. monosporum* and *G. etunicatu*), after infection with *Fusarium solani*. Nevertheless, it appears that the levels of phenolic compounds evaluated are very low in infection conditions. However, the pathways for the biosynthesis of proteins and phenolic compounds all belong to the shikimate pathway (Hermann, 1995). So these low levels observed show that there would be a negative correlation between the levels of total soluble proteins evaluated and that of total phenolic compounds and that during infection in plants of *C. esculenta*, it is the pathway of biosynthesis of protein that was the most preferred. The results also show that on D_{20} that the presence of *P. colocasiae* stimulates the synthesis of H_2O_2 in the *C. esculenta* plants of the infected and Mycorrhizal+infected treatment, for all three cultivars. This production of H_2O_2 significantly influenced the CAT, G-POD, and APX evaluated. The role of CAT is the detoxification of cells by the neutralization of oxidized radicals accumulated in the cytoplasm during the plant-pathogen interaction like H_2O_2. CAT concentrations were also higher in *C. esculenta* plants from the infected and Mycorrhizal+infected treatment, for all three cultivars. It should be noted that the values of G-POD and APX evaluated are similarly significantly higher in the plants of *C. esculenta* in infection conditions in all three cultivars compared to the control plants, mycorrhizal and mycorrhizal+ infected. This would reflect the vulnerability of *C. esculenta* plants to the infected treatment compared to the Mycorrhizal+infected treatment. In addition, the free radicals produced would first be scavenged by the CAT. Davey et al., (2000), show that peroxidases are classified among the antioxidants which protect plants against oxidative stress. So in this work, these antioxidant enzymes would surely help *C. esculenta* plants not only to trap H_2O_2, products due to the presence of the pathogen but also to recover superoxides, reactive oxygen species (ROS), and the free oxygen radicals (O-) produced, responsible for damage to nucleic acids, lipids, membranes and proteins (Srivali et al., 2003). Results of increased antioxidant enzyme (POD) activities were also observed by Atakan and Ozkaya (2021), in caranation plants inoculated and then infected with *Fusarium oxysporum*. These high CAT, G-PPO, and APX activities would play a significant role in the induction of resistance in *Colocasia esculenta* against *P. colocasiae*.

Materials and methods

Plant material and effect of arbuscular mycorrhizal fungi on the development of taro leaf blight

The three cultivars of *C. esculenta* cultivated in Cameroon, with local names Banlah, Macoumba, and Ekwanfre, were used. The *in vitro* plants used were produced by apex culture according to the method of Omokolo et al., (1995) and Djeuani et al., (2014). The experimental device was a complete randomized block with two repetitions. Four treatments were applied (Control, Mycorrhizal, Infected, and Mycorrhizal+infected). 300 *in vitro* plants aged 4 months were used, with 75 plants/treatment applied. The inoculation of plants was carried out according to the method of Djeuani et al., (2018). Applied arbuscular mycorrhizal fungi consisted of 30g of *Gigaspora margarita + Acaulospora tuberculata* per inoculated plant. These plants were acclimatized for a month. Infection consisted of spraying healthy *C. esculenta* leaves with 1 ml of spore suspension quantified at $5x10^4$ sporangia/ml according to Zhu et al., (2001), to induce the disease. The incidence and severity of the disease in plants treated with Infected and Mycorrhizal+infected were estimated using the method of Tchoumakov and Zahanova (1990). Disease progression was assessed every 5 days for 20 days. For the severity, the degree of leaf scorch of *C. esculenta* was evaluated according to the scale of Susan et al., (2012).

Microscopic observation of healthy and diseased leaves and determination of mycorrhization indices

Leaf fragments were taken from *C. esculenta* plants following the four treatments applied on D_{20}. The preparation and observation of these sheets were carried out according to the modified method of (Bärlocher, 1991). As for the observation of the roots in the Mycorrhizal and Mycorrhizal+infected treatment on D_{20}, root fragments were taken from the three cultivar plants. The staining of these roots was carried out according to the protocol of Phillips and Hayman (1970). The frequency and intensity were calculated according to the calculation method of Trouvelot et al. (1986).

Evaluation of the content of some metabolites and the activity of antioxidant enzymes during the interaction in the leaves

In the three cultivars of *C. esculenta*, leaves were sampled following the treatments applied on D_{20}. The contents of total chlorophyll (Arnon, 1949), total soluble sugars (Babu et al., 2002), total amino acids (Yemm and Cocking, 1995), proline (Trolls and Lindsey (1955), modified by Rascio et al., 1987), total soluble protein (Esma and Gulnur, 2016; Bradford, 1976), total phenol (Marigo, 1973), and hydrogen peroxide (H2O2) (Velikova and Loreto, 2005), and Similarly, the activities of catalase (CAT) (Athar et al., 2008), ascorbate peroxidase (APX) (Karyotou and Donaldson, 2007), Guaiacol peroxidases (G-POD) (Thorpe et al., 1978) have been determined.

Statistical analysis of data

The results obtained from this study were subject to a descriptive analysis (Mean±standard deviation). The results are represented in the form of graphs and tables (Microsoft Excel 2016 software). The IBM SPSS Version 22.0 software was used to perform statistical analyses and compare the means by analyzing variance (ANOVA) using the Student-Newman-Keuls test at the 5% threshold.

Conclusion

The results show that, among the three cultivars of *C. esculenta* used, the plants of the Mycorrhizal+infected treatment resist the most *P. colocasiae* attacks during the interaction. The Macoumba cultivar appears the most susceptible to taro leaf blight. The low levels of total Chl in the plants of the infected treatment of the three cultivars reflect the aggressiveness of the pathogen on the leaves. The contents of metabolites evaluated varied according to the treatments depending on the cultivars. Activities of antioxidant enzymes linked to the presence of inoculated AMF are recorded. This approach to the control of *P.*

colocasiae by application of AMF is a promising way to better explore and understand.

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