



Effect of the Hydric Factor and Arbuscular Mycorrhizal Fungi (AMF) on the Severity of *Phytophthora colocasiae*

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Abstract: *Colocasia esculenta* (L.) Schott is an important food for millions of people in countries of Africa, Asia and Central America. The cultivation of taro suffers from an epidemic disease, the taro mildew, caused by *P. colocasiae*, which is a disease that manifests itself as oily and circular spots on tubers, stems, petioles and on taro leaves causing losses of more than 50% in less than ten days. This study was carried out to evaluate the impact of this pathogen on the leaf area parameter in two varieties of taro (Ibo coco and Atangana) at different water levels, then evaluate the incidence of the disease severity on the leaves at different water levels and study the behavior of this pathogen on plants previously inoculated with arbuscular mycorrhizal fungi (AMF) of the genus *Gigaspora magarrita* and *Acaullospora tuberculata*. The plants were separated into three batches (control batch not infected, batch infected with pathogen and batch not only infected but also inoculated with AMF at different water contents (500 ml, 370 ml, 250 ml and 125 ml), the batch infected with the pathogen alone showed a reduction in the growth parameters compared to the control, and the inoculated and infected batch also showed a reduction in growth parameters but closer to the control. In addition, the speed of propagation of the lesion in the inoculated batch is reduced compared to that of the uninoculated batch, and this speed also decreases with the water content, as a result of which the mildew of taro develops easily. Moreover, the addition of AMF makes it possible to minimize its propagation.

Keywords: *Colocasia esculenta*, *Phytophthora colocasiae*, Arbuscular Mycorrhizal Fungi, Water Content

1. Introduction

Colocasia esculenta (L.) Schott (taro), a perennial and herbaceous monocotyledonous plant from the *araceae* family, about two meters tall, is of Asian origin [1]. This tuber crop is an important staple or subsistence food for millions of people in countries in Africa, Asia and Central America [2, 3]. It is valued for the organoleptic and nutritional qualities of its leaves and tubers, thus occupying an important place during the traditional ceremonies of certain African and Asian populations [4]. In 2011, its global production was estimated at 12 million tons for a cultivated area of 2 million hectares [5, 6]. According to

IITA [7], 77% of the world's taro production comes from sub-Saharan Africa. In 2012, its world production was estimated at 10 million tons. It is the fourteenth most consumed tuber in the world [8]. Cameroon was the fourth largest producer in the world and third in Africa after Ghana and Nigeria [9, 10, 5, 11].

In Cameroon, taro is grown in all regions for its leaves and tubers, which have good nutritional qualities because it contains a very digestible starch and vitamin C. Tubers and leaves also have medicinal properties against tuberculosis, ulcers, pulmonary congestion and fungal infections [12, 13]. The highly digestible tuber starch makes taro an excellent food for diabetics [14]. Taro has proteins that are rich in

essential amino acids, but with a low isoleucine content of tryptophan and methionine [15]. Despite the economic importance of food and socio-cultural, the taro culture in Cameroon has suffered from an epidemic disease since 2010; the mildew caused by *P. colocasiae* [16]. This disease that affects the leaves and tubers can completely destroy the cultivars in less than 10 days and cause yield losses of the order of 50 to 100% [17, 18, 19, 20]. The disease perpetuates devastation and has a considerable impact on peasant incomes [21]. Its life cycle depends on genotypic factors [22] and environmental factors (rainfall, humidity and temperature). For the latter case, the neutral pH and an average temperature of 27°C are the optimum growth conditions of the pathogen [21] and high humidity [23].

The use of metalaxyl-based chemical fungicides has been advocated by some authors [24, 25] in the control of *Phytophthora* spp. However, due to the problems of residues, phytotoxicity, development of resistance in the target organism, high cost, danger to humans and the environment, alternative control methods are increasingly being considered. At present, considerable efforts are directed towards methods that minimize these risks. In this sense, [21] we evaluated the antifungal activity of plant extracts against *P. colocasiae*. The determination of optimum hydric conditions and the search for arbuscular mycorrhizal fungi capable of reducing or even stopping the development of the disease are necessary. These techniques have the advantage that they are not only accessible to all farmers but also pose no danger to man and the environment [26, 27].

Mycorrhizal biofertilizers are manifested not only in plant mineral nutrition, but also in their adaptation to water stress, salt stress and resistance to pathogens [28].

2. Material and Methods

2.1. Material

The cultivars used consisted of two local varieties of taro (Ibo coco, whose morphological characteristics are similar to those of *Xanthosoma sagittifolium* and Atangana, generally filiform). The seeds were taken from the fields of the local producers of Douala city. These seeds were derived from the associated cropping system. Both cultivars were selected because of their easy accessibility in the Cameroonian markets.

Pure strains of *P. colocasiae* were obtained by isolation on contaminated taro leaves from the study site and grown on PDA (Apple Dextrose Agar) in a Petri dish.

The inoculum consists of a mixture of spores of arbuscular mycorrhizal fungi (AMF) genus *Gigaspora margarita* and *Acospora tuberculata*.

2.2. Methods

There was talk of preparing the substrate, and putting the plants in a greenhouse in not inoculated condition, inoculated and inoculated treated. Subsequently take the growth parameters for six weeks.

2.2.1. Study Site

The experimentation took place in the greenhouse of the Laboratory of Biology and Physiology of Plant Organisms of the University of Douala. It was conducted from 12 November 2016 to 12 January 2017 (8 weeks).

2.2.2. Substrate Preparation

The sand was washed about five times, then sterilized with 2% chloridic acid, and thoroughly rinsed with sterile distilled water twice while controlling pH to stabilization at 7. After drying, the sand was used to fill the nursery bags with about 3kg of sand per sachet and all transported in greenhouse.

2.2.3. Isolation and Purification of *P. colocasiae*

The leaves infected with the mildew of the taro were collected in a taro patch at the application research farm of the University of Douala (Cameroon) and taken to the laboratory. They were cut into fragments of about 2 mm² at the growth front of the pathogen before being superficially disinfected in a 5% sodium hypochlorite solution for 2 mn. After three rinses with sterile distilled water, the fragments were dried on hydrophilic paper and then deposited at a rate of four fragments per petri dish on a gelled V8 culture medium supplemented with a solution of antibiotics composed of penicillin (250mg / l), ampicillin (250mg / l) and nystatin (20mg / l) [29, 30]. After three days of laboratory incubation at a temperature of 23 ± 1°C, colonies of the pathogen, visible around the fragments, were removed and transplanted into new Petri dishes containing the PDA culture medium. This process was repeated several times until pure cultures of *P. colocasiae* were obtained, and then identified under the ordinary microscope on the basis of the morphological characteristics of the mycelium (unseeded) and fructification (sporangia as described by Brooks [20] and Scot *et al.*, [6].

2.2.4. Preparation and Inoculation of AMF

The inoculum consisted of a mixture of spores of AMF from the Applied Microbiology Laboratory of IRAD Nkolbisson (Yaounde). For 200g of inoculum there are about 200 spores. The inoculated plants each received 100 g of inoculum, ie about 100 spores. Inoculation was by direct contact of the inoculum with the roots of the plants.

2.2.5. Production of Greenhouse Seedlings

a) Experimental design

The experiment was carried out according to an experimental system in simple blocks and divided into three batches, the control (Figure 1), the batch infected with *P. colocasiae* only and the batch in addition to infection with *P. colocasiae* and AMF, all subjected to four types of water treatment (100%, 75%, 50%, 25%).

b) Preparation of plant material

The healthy rhizomes of two varieties of taro (Ibo coco, Atangana) previously washed were sterilized with sodium hypochlorite (2%) as a result of alcohol at 95° and then seeded in sachets on the basis of one rhizome per Sachet themselves contained in the basins to avoid water losses

during the experiment.

The monitoring of the watering was carried out throughout the experiment following an arithmetic sequence $U_n + 1 = U_n + r$ of reason $r = 25\%$ and the first term $U_0 = 25\%$ for all treatments. The negative control batch was formed as before.

The positive control batch was constituted by the example of the control batch supplemented with the *P. colocaseae* inoculum. Finally, the test batch, in addition to the treatment of the positive control batch, was supplemented with a complex of AMF.

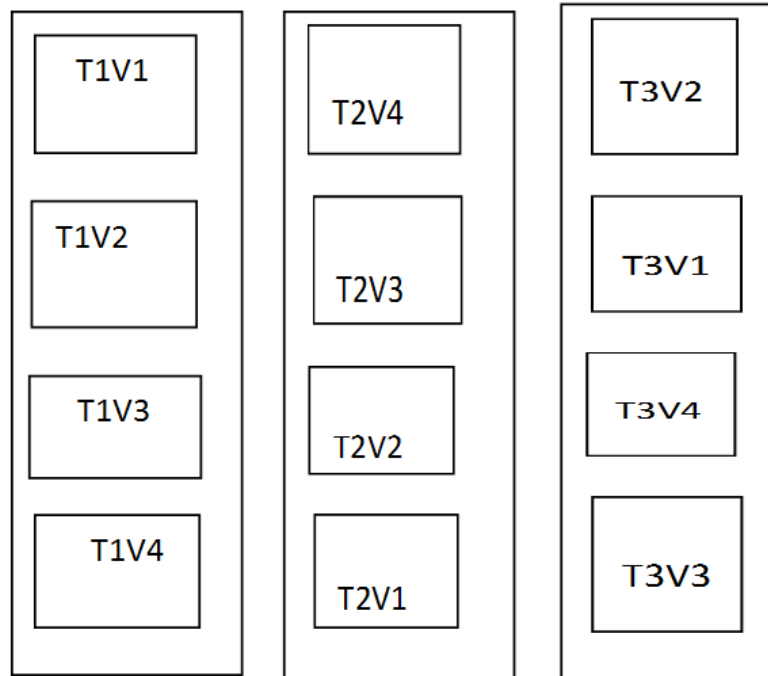


Figure 1. Experimental design; T1-plants without any infection; T2-infected plants with *P. colocasiae*; T3-plants infected with *P. colocasiae* +AMF; V - volumes of water; V1- 100% = 500 ml; V2- 75% = 370ml; V3- 50% = 250 mL V4- 25% = 125 mL.

2.2.6. Evaluation of Leaf Area

The leaf area was evaluated using a transparent graduated scale. The results obtained are recorded for the calculation of the leaf area using the Raunkier formula according to which $S \text{ (cm}^2\text{)} = \frac{2}{3} L \times l$

With S - leaf area; L - the longest length; l - the widest width.

2.2.7. Incidence of Disease

The incidence of disease was also evaluated by the method Bandy opadhyay *et al.*, [31] which counted the number of plants that showed the disease after infection on the total number of plants that had been infected.

$$I = (N_{ppl} / N_{pi}) \times 100$$

I-Incidence; N_{ppl} - Number of plants with lesions; N_{pi} - Number of infected plants according to [22].

2.2.8. Study of Severity

The diameter of disease-induced lesions was evaluated from a transparent graduated rule of 30 centimeters after the first symptoms appeared and the severity was assessed by the Bandyopadhyay *et al.*, [31] method which consists of dividing the area infected by the total area of the leaf multiplied by 100.

$$S = (SI) / (St) \times 100$$

S – Severity; SI- Area of lesions; St - Total area according to Adomako *et al.*, [22].

2.3. Statistical Analysis

The data was entered in an Excel sheet (Microsoft Office 2007) and analyzed with SPSS software version 20.0. The descriptive statistics concerned the presentation of the data as a percentage and mean \pm standard deviation for the qualitative and quantitative variables respectively. The ordered ANOVA was used to compare averages between groups. DUNKAN's nonparametric tests were used to compare the mean values of the parameters between two and more groups.

3. Results

3.1. Water Preferences: Effect of *P. colocasiae* on Leaf Area Treated at Different Proportions in Water

Overall, there was a significant (Figure 2) decrease in the leaf area of these two varieties (Atangana and Ibo coco) infected with *P. colocasiae* with 100% and 75% water treatment compared to the control. Furthermore, no significant differences were observed with the 50% and 25% water treatments in both the infected batches and the control batches.

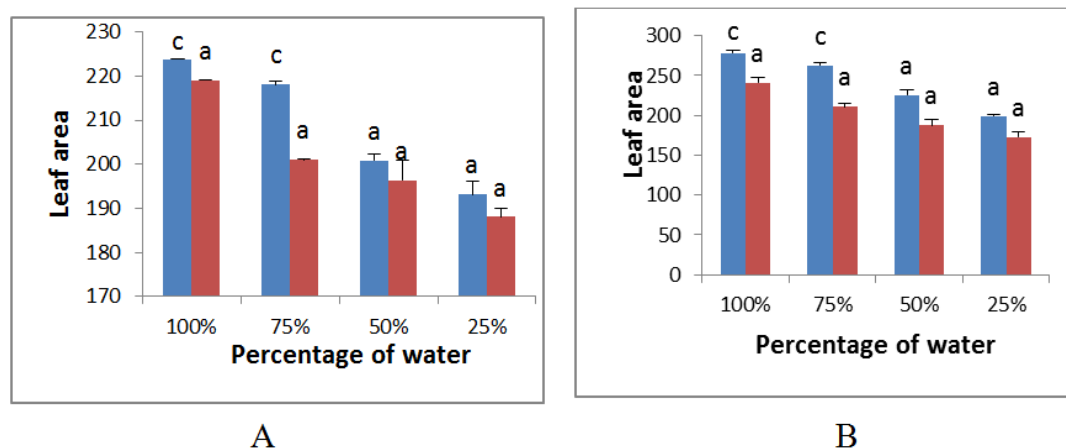


Figure 2. Effect of *P. Colocasiae* on the leaf area of the Atangana (A) and Ibo coco (B) cultivars treated with different proportions of water. The bars bearing the same letters are not significantly different from the threshold of significance $p < 0,05$ (blue color- control; Red color –infected plants).

3.2. Incidence and Severity of Disease

3.2.1. Incidence

All 20 plants of each infected taro cultivar showed lesions regardless of water content. Hence I (Incidence) = 100%.

3.2.2. Severity

The results of tables 2 and 3 show that, higher water content the greater severity of the two cultivars. More, the severity is greater in Ibo coco than in Atangana for the same water contents.

Table 1. Severity of disease on Atangana subjected to different water treatment (Wt-Water treatment; La-Leaf area; Al-Area of lesions due to disease).

Wt	Single test, <i>P. colocasiae</i>		
	La	Al	Severity(%)
100%	219,08	121,2	55
75%	201,04	101,001	50
50%	196,38	85,3	43
25%	188,04	67,1	35

Table 2. Severity of disease on Ibo coco subjected to different water treatment (Wt-Water treatment; La-Leaf area; Al-Area of lesions due to disease).

Wt	Single test, <i>P. colocasiae</i>		
	La	Al	Severity(%)
100%	241,26	157,3	65
75%	211,09	133,2	63
50%	187,8	97,3	52
25%	172,6	62,1	36

3.3. Influence of AMF

3.3.1. Influence of AMF on Leaf Area

The application of AMF to the infected plants leads to a significant increase in the leaf area in these two varieties with water frequencies between 100% and 75%, unlike batches that have received nothing. However, no significant difference was observed when the watering frequencies were between 50% and 25% water.

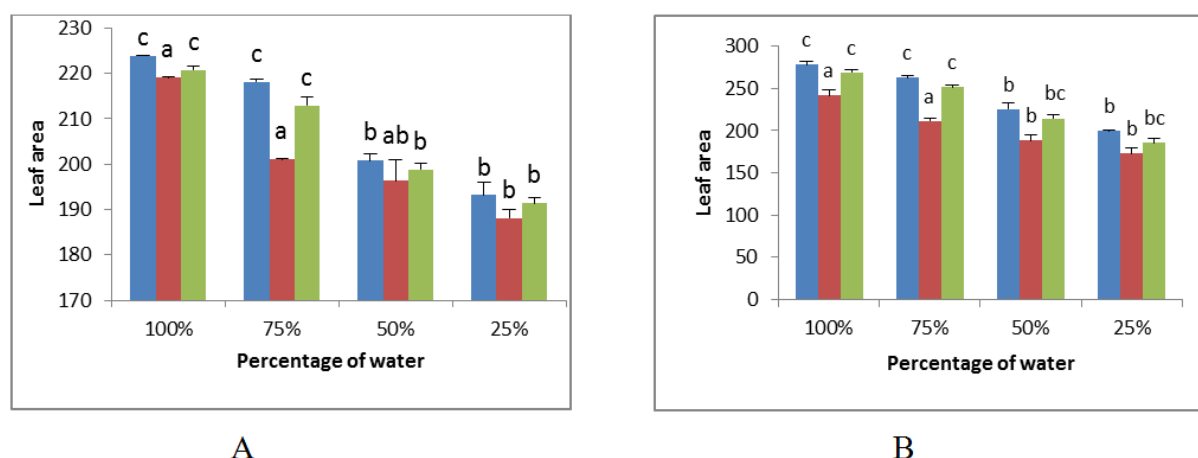


Figure 3. Effect of AMF on the leaf area of Atangana (A) and Ibo coco (B) cultivars showing Infected and subjected to different water treatments. The bars with the same letters are significantly different from the threshold of significance $p < 0,05$ (blue color- control; Red color –infected plants; Green color- infected plants + AMF).

3.3.2. Influence of AMF on Incidence

All 40 plants (20 plants infected with *P. colocasiae* and 20 plants infected with *P. colocasiae* and treated with AMF) of each variety of taro showed symptoms of taro mildew. Hence I (Incidence) = 100%

3.3.3. Influence of AMF on Severity

The severity of the disease increases proportionally with

the increase in water content when the plants have not received the AMF (Table 3 and 4). However, this severity generally decreases when AMF are applied to infected plants of both varieties of taro (Figure 4). Moreover, in the Atangana variety, the severity was lower (39%) compared to the Ibo coco variety (53%) at the same frequency of watering (100%).

Table 3. Severity of the disease on the Atangana cultivar on the tests (single test, *P. colocasiae* and combined test, *P. colocasiae* and AMF) and subjected to different water treatments. (Wt-Water treatment; La-Leaf area; Al-Area of lesions due to disease).

Single test				Combined test			
Wt	La	Al	Severity (%)	Wt	La	Al	Severity (%)
100%	219,08	121,2	55	100%	222,59	88,5	39
75%	201,04	101,001	50	75%	214,08	83,2	38
50%	196,38	85,3	43	50%	202,26	43,4	21
25%	188,04	67,1	35	25%	192,55	40,01	20

Table 4. Severity of the disease on the Ibo coco cultivar on the tests (single test, *P. colocasiae* and combined test, *P. colocasiae* and AMF) and subjected to different water treatments. (Wt-Water treatment; La-Leaf area; Al-Area of lesions due to disease).

Single test				Combined test			
Wt	La	Al	Severity (%)	Wt	La	Al	Severity (%)
100%	241,26	157,3	65	100%	276,89	145,4	53
75%	211,09	133,2	63	75%	262,96	135	51
50%	187,8	97,3	52	50%	226,32	76,3	34
25%	172,6	62,1	36	25%	187,24	48,1	26



Figure 4. Effect of AMF on the severity disease: A-Plants infected with *P. colocasiae* after three days, B- Plants infected with *P. colocasiae* and treated with AMF after three days.

4. Discussion

The present study shows the degree of virulence of *P. colocasiae* on *C. esculenta* and the influence of wet on its development. The growth parameters of infected plants are considerably reduced when the plant is subjected to a water treatment of 370 to 500 ml of water per day. On the other hand, no significant difference was observed in the contribution to the control when subjected to a water treatment less than 250 ml of water per day. Indeed *P. colocasiae* induced the lesions on all the plants of the two varieties Ibo coco and Atangana. These results are consistent with those of Adomako *et al.* [22] obtained by studying the incidence of downy mildew on 12 varieties of taro.

The severity of the disease was all the greater when the water treatment was considerable; 50% / 370ml, 55% / 500ml for the Atangana variety and 63% / 370ml, 65% / 500ml for the Ibo coco variety compared to other treatments where no significant difference was observed with the controls. These

results are consistent with those of Asseng *et al.* [23] who claim that the frequency of watering increases the severity of *P. colocasiae* on taro. This severity was more pronounced in the Ibo coco variety, which appears to be more susceptible to attack by this pathogen than Atangana. These results are similar to those of authors like Tsopmbeng *et al.* [32] who claim that Ibo coco manifests more lesions in wet condition. Nevertheless, they are contradictory to those obtained by Asseng *et al.* [23], which demonstrated that *Xanthosoma sagittifolium* was less susceptible to *P. colocasiae* than *C. esculenta* when it is known that Ibo coco is morphologically closer to *Xanthosoma sagittifolium* than *C. esculenta*.

The plants in the test batch (including the AMF) of the two varieties showed a considerable increase in leaf area. These results could be explained by the fact that AMF inhibit the development of pathogens either by helping the plant in the secretion of antimicrobial substances or by increasing the nutritive qualities of the latter. Several authors [33, 34, 35] agree with this finding that plants inoculated with arbuscular

mycorrhizal fungi are more resistant to pathogenic fungi and exposure of soil toxins.

Although there is addition of AMF, *P. colocasiae* still induces lesions although with more severity. The presence of AMF did not prevent the development of the disease in both Atangana and Ibo coco. These results are contrary to those of Adomako *et al.* [22] who showed that AMF allowed a reduction in the incidence of disease in 12 varieties of *C. esculenta* while inoculating the pathogen at the rhizome level. On the other hand, in the presence of water, the severity is reduced in plants inoculated with AMF (arbuscular mycorrhizal fungi) at high water contents (65 to 53% for Atangana with 100% water and 55 to 39% for Ibo coco with 100%). However, it is better (35 to 20%) and (36 to 26%) in these two varieties respectively Atangana and Ibo coco when the water contents decrease up to 25%. This variation in severity may be due to the ability of AMF to induce and boost the mechanisms of resistance of the plant. These results are consistent with those of Meddich *et al.* [36] who have shown that mycorrhization by *Aoufous* isolates or *Glomus mosseae* allowed the modification of the water parameters of clover subjected to severe water stress.

In the Atangana variety, the severity is reduced by 39 to 20% respectively when the frequency of watering increases from 100 to 25%. Conversely, in Ibo coco, this severity increases from 53 to 26% for water contents of 100 and 25% respectively. This difference can be explained globally by the action of AMF which allow the plant to better resist diseases by changes in physiological activities in the root. Plants attacked by a pathogen react by producing antibiotic substances against these organisms [37] although this phenomenon is more pronounced in Atangana than in Ibo coco. According to Smith and Read, [38]; Gonzales-guerrero [39], AMF improves the rooting of plants by the production of phytohormones. The external mycelium of AMF is known for its beneficial effect on soil structure, through the production of a glycoprotein called Glomaline, which promotes the aggregation of soil particles by its chemical characteristics. The AMF protects the plant against biotic (pathogenic) and abiotic stresses (salinity, drought, deficiency or excess nutrients, excess of heavy metals, soil degradation). They promote the diversity of plant communities and plant succession, as each plant shows a high level of compatibility with some AMF ecotypes, hence the importance of conserving the diversity of these fungi to benefit diversity and to the succession of plants.

5. Conclusion

At the end of this study, the general objective was to find ways to stop the spread of taro mildew by combined water and AMF action on two varieties of taro grown in Cameroon (Atangana and Ibo Coco). The disease spreads more rapidly in Ibo Coco than in Atangana and the evolution of the disease in two varieties is proportional to the water content (100%, 75%, 50% and 25%). In the presence of AMF, the spread of lesions caused by the disease is not only reduced by 11 to

17% and 10 to 14% respectively for the Atangana and Ibo Coco varieties but also evolves proportionally according to the water contents.

On the basis of these data, it's obvious that *P. colocasiae* appreciates high levels of water and AMF can be used as an alternative for the control of this pathogen. To this end emerges the eternal problem of choice the seeding period in sub-Saharan Africa. Agriculture in these countries is practiced exclusively during the rainy seasons when it is known that these periods are conducive to the development of insect pests and pathogens. Intensive agriculture can be practiced during dry seasons using irrigation methods.

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