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Study of Symbiotic Pathogenic Microorganisms Associated with *Phytophthora colocasiae* Causal Agent of Taro Mildew (*Colocasia esculenta* (L.) Schott)

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Abstract: Symbiotic pathogenic microorganisms associated with *P. colocasiae* leading to the manifestation of taro mildew have been identified and studied. The experiment was carried out in an experimental set-up consisting of completely randomized blocks with three treatments (infection with the pure strain, with the unpurified strain and a co-infection with both strains). Isolation and purification of the microorganisms was carried out in the PDA medium and the identification on the analysis of the macroscopic and microscopic morphological characters. The results of the trapping revealed among several a single microorganism which has a high affinity with *P. colocasiae*, this microorganism named *Vorticella* is identified as the agent of the amplification of the severity of the downy mildew. Greenhouse studies of taro plants showed that the unpurified strain caused enough damage with 96.67% disease incidence and a severity of 70.18% compared to plants inoculated with pure strain which had an incidence of 40% and a severity of 10.48%. All these results show that *P. colocasiae* is the causal agent of the mildew of the taro in the infection and the development of the disease; it is accompanied by other microorganisms with which it forms a biofilm. In this biofilm there is a microorganism which plays a very important role in the spread of the disease and it is thanks to it that all parts of the plant are attacked.

Keywords: *P. colocasiae*, *C. esculenta*, Symbiotic Microorganisms, Vorticella

1. Introduction

Taro is an important staple or subsistence food for millions of people in countries in Africa, Asia and Central America [1, 2]. Its global production is estimated at 12 million tons on a cultivated area of 2 million hectares [3, 4]. According to IITA [5], 77% of the world's taro production comes from sub-Saharan Africa. It is the fourteenth most consumed tuber in the world [6]. In 2012, its world production was estimated at 10 million tons. Cameroon was the fourth largest producer of taro in the world, and the third largest producer in Africa after Nigeria and Ghana with a production of 1.6 million out of the 7 million tons for all of Africa [7].

Taro is grown in all five agro-ecological zones of Cameroon for its leaves and tubers which possess good nutritive qualities (very digestible starches, vitamin C). It occupies an important place during traditional ceremonies in some African and Asian populations [8, 9].

Tubers and taro leaves also have medicinal properties against tuberculosis, ulcers, pulmonary congestion and fungal infections [10, 11]. The highly digestible tuber starch makes

taro an excellent food for diabetics [12].

Despite its economic, food and socio-cultural importance, taro cultivation in Cameroon has suffered from an epidemic disease since 2010; the mildew caused by a pathogen called *P. colocasiae* [13]. However, this pathogen associated with other microorganisms interacts to cause the disease which results in a decline of nearly 80% of taro production, hence its absence on the shelves in the Cameroonian markets. The disease mainly affects leaves but can completely destroy sensitive cultivars in less than 10 days and cause yield losses of about 50% [14, 15]. Although the impact of this disease on the population remains to be assessed, experience has shown that it will have a remarkable impact on farmers' incomes and food security in Cameroon.

The taro mildew is one of the main factors limiting the cultivation of taro in the world. The traditional methods of management of this disease which are hitherto widely used in agriculture require the use of phytosanitary products in very large quantities. At the same time, measures are taken worldwide to encourage the agricultural world to limit its use. Prophylactic control and sanitation are modes that can be appreciated by farmers [10].

P. colocasiae is the causal fungus of taro mildew, the severity of which depends on a symbiosis with other microorganisms. Knowledge of these symbiotic microorganisms could be a possible solution to reduce or even eradicate this fungus in order to produce healthy taro cultivars. However, understanding the dynamics of microorganisms and their capacity to evolve requires a study of the problem in its global dimension and must take into account the complexity of the interactions between three main determinants: the characteristics of the pathogen, the sensitivity of Host and the environment. The overall objective of this study is to identify the pathogenic microorganisms associated with *P. colocasiae* leading to the manifestation of the taro mildew of its invasion and its destruction. Specifically, it will be necessary to isolate and purify *P. colocasiae* on the infected plant, to inventorize the associated pathogenic microorganisms and to evaluate the synergy between these microorganisms and the pathogen by compatibility tests.

2. Material and Methods

2.1. Material

The cultivars used consisted only of a local variety of taro (Ibo coco). The seeds were taken from the fields of local producers in the city of Douala. These seeds were derived from the associated cropping system.

Pure strains of *P. colocasiae* were obtained by isolation on contaminated taro leaves from the study site (Figure 1) and grown on PDA (apple dextrose agar) in a Petri dish.

Figure 1. *Beginning of infection by appearance of first spots (A), total destruction of leaf (B) by P. Colocasiae.*

2.2. Methods

2.2.1. Isolation and Purification of the Microorganism

a) Preparation of the Culture Medium

The culture medium used is Apple Dextrose Agar (PDA). For the preparation of 1 liter of medium, 200 g of apple are weighed, peeled and then cut into small pieces and baked in a volume of water (250 ml) for 15 minutes. The apple juice obtained is filtered and adjusted to 500 ml of distilled water. 20 g of sucrose and 12 g of agar are weighed and dissolved in this juice and then the volume is supplemented to 1 liter. The mixture is boiled for 5 minutes for complete dissolution then autoclaved at 121°C for 15 minutes. After cooling, the medium is supplemented with antibiotics 250 mg / l (ampicillin and penicillin) (Figure 2) [17].

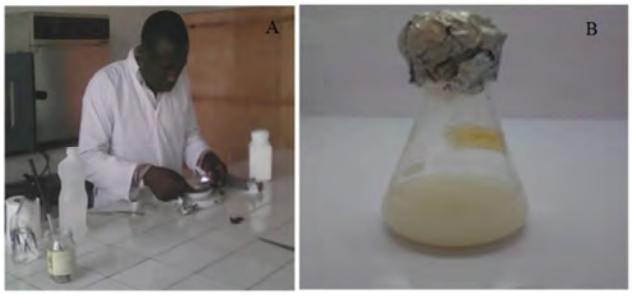


Figure 2. *Preparation of the PDA medium (A), ready-to-use medium (B).*

b) Isolation of the Microorganism

Infected leaves were previously washed with tap water and then subjected to a series of disinfection with 95% ethanol for 30 seconds in 5% sodium hypochlorite for 2 minutes and then in ethanol 75% for 2 minutes, so as to remove the microorganisms present. Leaves are then rinsed three times with sterile distilled water to remove traces of disinfectant. The sampling zones are selected and the 5 to 7 mm foliar plates are cut with a sterile scalpel (Figure 3). The fragments taken are cultured on the selective medium contained in Petri dishes. Incubation is done in the dark in an oven at 26°C. for seven days for fungi. The observation is carried out daily until the mycelium characteristic of the pathogen appears [18].

Figure 3. *Cutting of the foliar plates from 5 to 7 mm.*

c) Purification of the Microorganism

Seven days after the culture of the microorganism in the culture medium (PDA) supplemented with antibiotics, a series of two to three subcultures is carried out, followed by culture in an agar medium containing only agar without a nutritional supplement. Three to four days after its growth, a series of two subcultures are again made in order to obtain the pure strain, and then this strain is transplanted into the PDA medium supplemented with antibiotics and incubated in the dark in the oven at 26°C for five to seven days; to have many boxes containing the pure strain.

2.2.2. Pathogenicity Test and Maintenance of the Pathogenicity of the Microorganism

a) Pathogenicity Test

Pathogenicity of the isolates collected is checked on detached leaflets and the tubers of taro. It is confirmed by the postulate of Koch [19].

b) Inoculation and Incubation

Inoculation of loose leaves: Leaflets or healthy leaves are washed with tap water. They are then disinfected with 1% sodium hypochlorite for 2 minutes and then washed with sterile distilled water. A sporangial suspension (5×10^4 sporocysts ml-1) of each isolate was prepared from a 10-day mycelial culture. An amount of 40 to 60 µl of the inoculum is deposited on the underside of the leaflets [20]. The latter are incubated in boxes moistened at 20°C. and at a 16 h photoperiod. After 24 hours, the upper side is placed on top. Inoculated foliage tissue is examined after 4 to 7 days of incubation.

-*Inoculation of the tubers:* After washing and disinfecting the healthy tubers of taro, holes with a diameter of 10 mm and a depth of 5 mm are produced using a scalpel, One side of the tubercle. The inoculation is carried out by placing 50-100 µl of the sporangial suspension of each isolate (5×10^4 sporocysts, M1-1) previously prepared. The inoculated tubers are incubated at 20°C in the dark in moistened cans. The inoculated tubers are examined daily until the 7th day of incubation.

c) Maintenance of the Pathogenicity

In order to maintain the pathogenicity of the isolates, these isolates are stored for short periods in test tubes containing 10 ml of pea medium. Before transplanting for subsequent storage, the isolate is cultured for inoculation to previously disinfected leaflets or tuber fragments. After the appearance of the lesion, the isolation of the isolate is carried out according to the above technique. The fungi is then stored in test tubes for later use. This manipulation is repeated every 2 to 4 months to maintain the pathogenicity of the isolates. This re-isolation is also carried out before each characterization test.

2.2.3. Screening for Species Associated with P. Colocasiae Affecting Taro Mildew

The predisposed strategy for identifying associated species is summarized in three steps:

Step 1: Building the community through the use of the pathogen as a trap for associated microorganisms in a natural habitat.

Step 2: The selection of micro-organisms on the basis of their ability to grow in the vicinity of the pathogen.

Step 3: Identification of the organisms affecting the mildew of taro.

For the first stage, biofilms (mixedspecies) are formed from representative samples of the natural ecosystem (infected leaf plates and rhizosphere). The second step will consist in incubating the microcolonies of *P. colocasiae* on the one hand and the leaves on the other hand for at least 72 hours at 25°C in the humidity. And finally, select the microorganisms that have survived and grown in the vicinity of the pathogen for identification.

The biofilms (Mixed-species) will be dissociated and plated on agar plates containing an extract of P. colocasiae and without main nutritional supplement. Therefore, in the biofilm P. colocasiae secretes cyclic AMP (cAMP) and a large number of microorganisms must be in close proximity to the biofilm in response to cAMP as a (positive) chemotactic signal. Therefore, this step will be carried out with the aim of targeting the microorganisms that interact with P. colocasiae and able to develop on the biofilm matrix. Colony-forming microorganisms will be isolated with an antibiotic (chloramphenicol, for example) to remove bacteria. At the end, each isolate of the identified microorganisms will be co-incubated (Hyphae and spores) with P. colocasiae in vitro and co-infected with healthy plants to identify isolates that have an effect on taro downy mildew.

2.2.4. Experimental Design

Three treatments, each containing 30 taro plants, were put in place. After washing with a 1% sodium hypochlorite solution, rinse and dry, the sand was put in the sachets, holes about 10 cm deep were dug inside which the taro seeds were planted.

a) Layout and Principle of Blocks

After the first leaves were lifted, the inoculation was carried out in the different blocks as follows (Figure 4): block 1; inoculation with pure strain, block 2; inoculation with the unpurified strain and block 3; Inoculation with the pure strain and the unpurified strain.

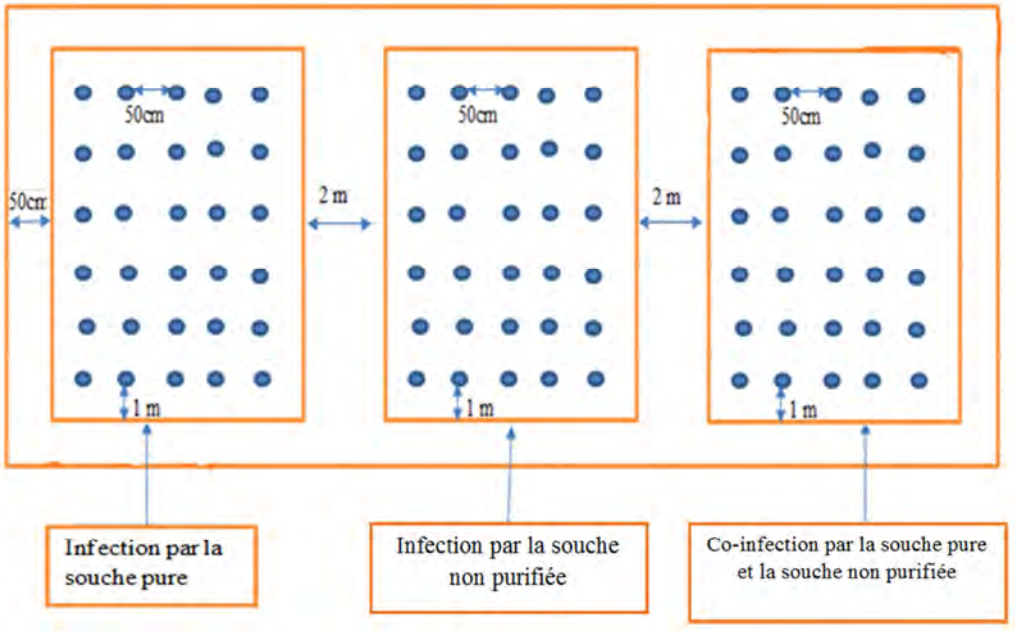


Figure 4. *Experimental design.*

b) Method of Inoculation of Plants

The sick taro (mildewed) leaves were collected from the peri-urban plantations of the Douala city. The friction between these diseased leaves and the young leaves of the taro plants and the use of a pure strain of *P. colocasiae* which we isolated from the diseased leaves allowed the contamination of our test.

c) Inoculation with Pure Strain

The inoculation was carried out by depositing the pure strain of *P. colocaciae* (aged two days because the young strains are more virulent than the vigils) with a few drops of water on the upper parts of the young leaves previously rubbed with sand sterilized. Then we took the parameters of the evolution of the disease every two days, from the onset of the first symptoms until the death of the leaf.

d) Inoculation with the Unpurified Strain

Inoculation was made on contact between the pieces of leaves affected by the disease and the leaves of the young plants, injured at the level of their upper surface. These diseased leaves were harvested from plants with downy mildew, coming from the periurban plantations of the city of Douala. After the deposit of the piece of contaminated leaf, a little water was poured to allow the zone to be wet, because it is thanks to this moisture that the pathogen will induce the disease. Subsequently we took the parameters of the course of the disease every two days, from the onset of the first symptoms until the death of the leaf.

e) Severity of Disease

Severity of disease is defined as the ratio of leaf areas affected regions by the disease on the total leaf area before infection and multiplied by 100. Severity is a determining factor in the study impact of a disease in a region or even a country. This leaf area was measured using non-destructive methods as described by Lu *et al.*, [21], which is calculated according to the following formula:

$$\text{Leaf area} = \text{LPA} \times \text{WP}$$

LPA: length of the leaf from the apex to the area that connects the petiole with the leaf WP: the width of the sheet from one end to the other.

To calculate the severity, we used the formulas described by Chaube and Pundhir [22]. This formula is as follows:

$$\text{Severity of disease (\%)} = (\text{Surfaces of leaf attacked areas}) / (\text{Total leaf area before infection}) \times 100$$

Omeje *et al.* [23] described a 5-point scale (0-4) that was used to deduce the type of infection and describe the range of disease severity described in Table 1.

Table 1. *Sickness Severity Scale.*

Scale	Range of severity (%)	Deduction
0	<1	No infection
1	1-25	Low infection
2	26-50	Moderate infection
3	51-75	High infection
4	>75	Very high infection

f) Incidence of Disease

Incidence of disease is defined as the quotient of the number of taro plants infected by the total number of taro plants multiplied by 100. This incidence is one of the very important factors for the study of a disease in a locality, a region a country, a continent or even the whole world. To calculate the incidence of the disease, the formulas described by Chaube and Pundhir [22] were used. This formula is as follows:

Incidence of disease (%) = (number of infected plants) / (total number of plants) X 100

2.2.5. Method of Diagnosis

Diagnosis was made through observations made on the leaves and stem. The presence of the fungus on the plant was recognized by dark brown or green spots present on the leaf, leaf rot and stem rot (Figure 5). The number of plants with these symptoms was then recorded each day and totaled at the end of each week and then at the end of the test.

Figure 5. *Diagnosis of the disease through the appearance of spots.*

Identification of Microorganisms

Identification of fungi was made on the analysis of macroscopic and microscopic morphological characters. The appearance of the vegetative apparatus was observed during the macroscopic analysis of the colonies obtained after culture, as well as the rate of colony growth and the development temperature. During the microscopic analysis of the colonies, organ structures such as the vegetative apparatus, fruiting organs and spores were observed as described by Aurelie [24].

2.2.6. Statistical Analyzes

Statistical analyzes were carried out using the software R. The matched test made it possible to check the evolution of the diameter of the spot of the disease and the linear correlation test with growth in pairs was done in order to observe the similarities between the various parameters.

Whenever there was a significant difference between the averages, the least significant difference method (LSD) was used to separate them.

3. Results

3.1. Isolation and Purification of P. Colocasiae

In relation to the isolation of *P. colocasiae*, the results obtained in Petri dishes (Figure 6A) show the appearance of the mycelia after 7 days of incubation. The pathogenicity test carried out allowed us to confirm its presence after inoculation of the leaves discs. We thus observed the symptoms characteristic of mildew indicating its actual presence (Figure 6B). The pure strain was obtained after several series of subcultures (Figure 6C) followed by a second pathogenicity test in order to check its pathogenicity.

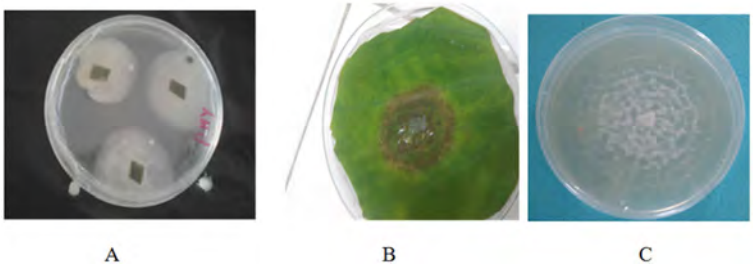


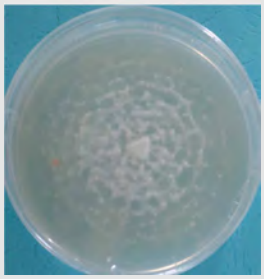
Figure 6. *A-Isolation of P. colocasiae, B-Pathogenicity test, C-Pure strain of P. colocasiae.*

3.2. Identification of P. Colocasiae

3.2.1. Macroscopic Identification

The macroscopic description of the pure strain of *P. colocasiae* obtained in a Petri dish was made using well-defined criteria (Table 2).

Table 2. *Macroscopic description of P. Colocasiae.*

	Critères	**Description**
	Texture: Duvet	Aerial mycelia are short, sharp, pale, diffuse and concentric
	Topography	Raised
	Color	White
	Growth rate	Rapid growth (≥3cm)

3.2.2. Microscopic Identification

The microscopic observation (Gx 400) of the pure strain obtained in a Petri dish shows the hyphae which are non-septated and broad; the sporangia are ellipsoidal, have a prominent papilla and carried a short pedicel of variable length of characteristic of *P. colocasiae* (Figure 7)

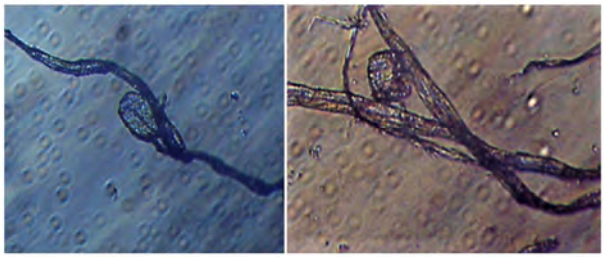


Figure 7. *Microscopic observation of P. colocasiae (x 400).*

3.3. Determination of Symbiotic Microorganisms

3.3.1. Trapping of Microorganisms Associated with P. Colocasiae

The study of diversity within symbiotic microorganisms which can contribute to the emergence and severity of *P. colocasiae* has revealed several categories of microorganisms (Figure 8). Among these one, was found to have high symbiotic potential with *P. colocasiae*.

Figure 8. *Trapping of microorganisms associated with P. colocasiae.*

3.3.2. Identification of the Symbiotic Microorganism Associated with P. Colocasiae

(i). Macroscopic Identification

The trapping results revealed among several a single microorganism that had a high affinity with *P. colocasiae*. The culture was carried out in petri dishes and then purified. The macroscopic observation on PDA medium was carried out using well-defined criteria (Table 3).

Table 3. *Macroscopic description of a symbiotic microorganism associated with P. Colocasiae.*

Criteria	Description
Texture: powder	Aerial mycelia producing numerous conidia creating a powdery appearance surface similar to sugar or flour
Topography	Plane
Color	White and milky white
Growth rate	Moderate growth (between 1 and 3 cm)

(ii). Microscopic Identification

Microscopic observation (Gx 400) of this microorganism isolated on PDA medium shows a ciliate of about 120 to 150 µm in length, with a contractile stem which is associated with a zone that is a feeder dome (Figure. 9)

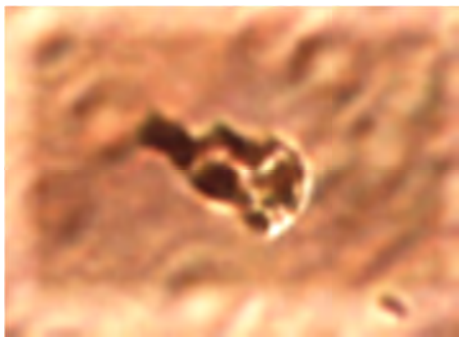


Figure 9. *Microscopic observation (Gx400).*

3.4. Comparative Study of Inoculation Methods on the Incidence of the Disease

3.4.1. Inoculation with Pure P. Colocasiae Strain

Variation of average diameter of leaf spot obtained on the different days is grouped in Table 4. Its growth rate increases with the number of days. However, rapid growth was observed until the 6th day after inoculation (1.11 cm / day). From then on, it begins to diminish and eventually stops at the 14th day.

Table 4. *Evolution of mean diameter of leaf spot of disease as a function of the number of days after inoculation (DAI) with the pure strain of P. colocasiae.*

Number of DAI	2	4	6	8	10	12	14
Mean diameter (cm)	1,01	2,01	4,23	6,04	7,74	8,74	8,84

3.4.2. Inoculation with the Unpurified Strain

In view of the results recorded in table 5, diameter of leaf

spot of disease increases considerably with time until the total leaf invasion.

Table 5. *Evolution of mean diameter of disease as a function of the number of days after the infection with the unpurified strain of P. colocasiae.*

Number of DAI	2	4	6	8	10	12	14
Mean diameter (cm)	1,48	3,58	6,08	9,58	13,59	17,78	-

Overall, the two strains of *P. colocasiae* infested the plants of *C. esculenta* after 2 days of inoculation. But the unpurified strain is more virulent than the purified one (Figure 10). The growth rate of the leaf diameter of leaf spot of diseased leaves with the pure strain is lower than that inoculated with the unpurified strain regardless of the period. However, after 14 DAI the leaves inoculated with the unpurified strain die. The statistical studies showed a positive correlation ($p = 0.007$), with the critical threshold of ($p \leq 0.05$) on the variation of the diameters.

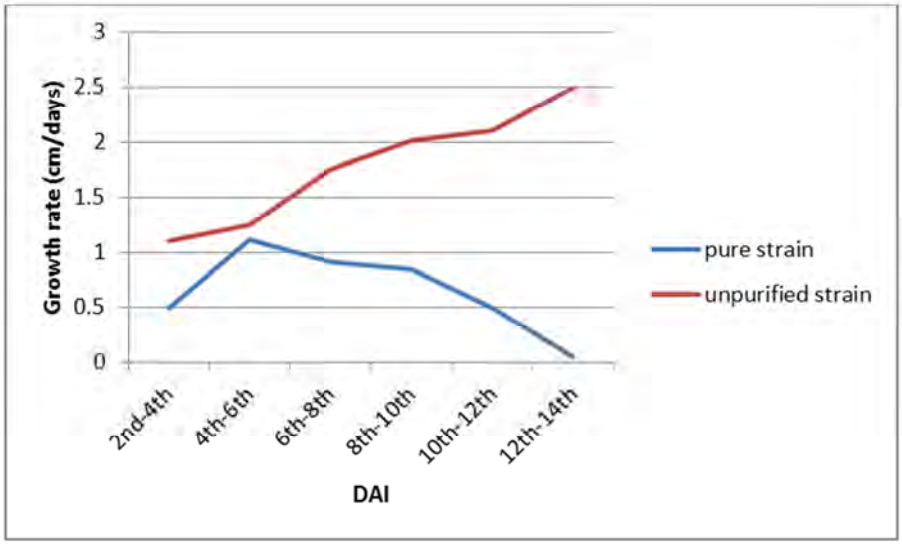


Figure 10. *Growth rate of mean diameter of leaf spot.*

3.5. Determination of the Severity and Incidence of Disease Caused by the Pure Strain of P. Colocasiae

3.5.1. Determination of Severity

However, we got a severity of 10.48%. According to the severity scale, the pure strain induces a low infection because its severity is between 1 and 25%.

3.5.2. Determination of the Incidence

The pure strain of *P. colocasiae* commits less damage because the incidence of the disease caused by this strain is not very high 40%. In addition the infection was not complete on all the plants.

3.6. Determination of the Severity and Incidence of Disease Caused by the Unpurified P. Colocasiae Strain

3.6.1. Determination of Severity

The severity of disease caused by unpurified strain is calculated from the formulas given to the methodology. However, we obtained a severity of 70.18 %. According to the severity scale, the unpurified strain induces a high infection because its severity is between 51 and 75%.

3.6.2. Determination of the Incidence

Unpurified strain was tested on 30 taro plants on which 22 could be contaminated. This allowed us to calculate the incidence of the disease which is 96.67%. The unpurified *P. colocasiae* strain is quite damaging because the incidence of the disease caused by this strain is high. In addition the infection was effective on more than half of infected plants or even three-quarters.

4. Discussion

4.1. Isolation and Purification of P. Colocasiae Infected Plant

Pathogenicity test was carried out in order to confirm the effective presence of *P. colocasiae* after isolation on PDA medium followed by a series of transplants for purification [25]. It is made by contact of the inoculum with the leaf disks of healthy plants. The appearance of symptoms characteristic of downy mildew proves the virulence of the pathogen [26]. The growth of this pathogen is slower on the PDA medium than on the V8 medium. This result corroborates that of Tsopmbeng *et al*. [27] on the evaluation of different culture media for the growth and sporulation of *P. colocasiae*. This allows us to state that the V8 medium is suitable for the isolation of *P. colocasiae* so much so that the PDA medium is suitable for its purification.

Morphological observation of purified *P. colocasiae* shows a striped and petaloid striped facies with a scalloped growth front, that is to say the downy colony whose short aerial mycelia are sharp, pale in color, diffuse and concentric, with a white backhand and rapid growth rate [28, 29].

Microscopic observations of purified *P. colocasiae* on

PDA show hyphae that are non-septated and broad, sporangia are ellipsoidal, have a prominent papilla and carry a short pedicel of variable length [15, 4]. This result corroborates that of Coulibaly *et al.* [30] on the characterization of *Phytophthora* ssp isolates.

4.2. Determination of Symbiotic Microorganisms

Study of diversity within symbiotic microorganisms that can contribute to the emergence and severity of *P. colocasiae* has revealed several categories of microorganisms. Among these, one of its microorganisms has been found to have a high symbiotic potential with *P. colocasiae*. These results are in agreement with those obtained by Galiana *et al*., [31] on the screening approach of ecosystems of associated pathogenic microorganisms causing diseases to host plants. According to Wolinska and King [32], this community is capable of inducing a touching disease and exerting pressure on pathogen and host selection. The results of the trapping revealed among several a single microorganism which has a high affinity with *P. colocasiae.* Macroscopic observation of this microorganism purified on PDA medium was carried out using well-defined criteria. It reveals a powdery texture, a raised topography whose color on the inner side is white and its back is milky white, and its growth rate is rapid [28, 29]. These results are in line with those of Galiana *et al.* [31].

Microscopic observation (Gx 400) of this microorganism isolated and purified on PDA medium shows a ciliate about 120 to 150 µm in length, with a contractile stem that is associated with a zone that is a feeder dome [15, 4]. These results are in agreement with those obtained by Galiana *et al*. [31] on the screening approach of ecosystems of associated pathogenic microorganisms causing diseases to host plants. According to Ravva *et al.* [33], once rooted in the biofilm temporarily, the ciliate probably feeds on bacteria, small protozoa, or organic food. It leaves the biofilm by carrying away at the end of its stem the material of the oomycete by swimming; this allows the dissemination of it with a speed of 100 m / s [31]. This ciliate is a protist named *Vorticella*.

4.3. Comparative Study of Inoculation Methods on the Incidence of the Disease

The growth rate of the diameter of the leaf spot which appeared after inoculation with the pure strain is less than that inoculated with the unpurified strain. The statistical studies showed a positive correlation ($p = 0.007$), at the critical threshold ($p \leq 0.05$) for the change in diameters [34]. However, after 14 days leaves are invaded almost in its totality and eventually die. With the pure strain, a hypersensitivity of the plant is observed. The growth rate increases until the 6th day (0.91cm /day), then begins to decrease until the 14th day and finally cancels out. This is a very classic case of active resistance on the part of the host plant [20].

4.4. Determination of Severity and Incidence

According to the severity scale, the pure strain induces a low infection (10.48%) because its severity is between 1 and 25%. It commits less damage because the incidence of the disease caused by this strain is not very high 40%. In addition the infection was not complete on all the plants. Moreover, the incidence of the disease is a parameter taken according to the conditions prevailing in the environment. These results are in agreement with those of Adinde *et al.* [35] who have been working on the incidence and severity of P. colocasiae in the Iwollo region of south-eastern Nigeria. According to Adomako *et al.* [20], incidence and severity of *P. colocasiae* is due to the presence of disease-promoting factors such as cultivar sensitivity and environmental conditions.

According to the severity scale, the unpurified strain induces a high infection (70, 18) because its severity is between 51 and 75%. It was tested on 30 taro plants on which 22 could be contaminated and this allowed us to calculate the incidence of disease which is 96.67%. The unpurified *P. colocasiae* strain is quite damaging because incidence of disease caused by this strain is high. In addition the infection was effective on more than half of infected plants or even three-quarters. These results corroborate those of Adomako *et al.* [20] who worked on the prevalence rate of *P. colocasiae* causal agent of taro mildew in the Semi: deciduous forest zone in Ghana. According to Asseng *et al.* [36], the frequency of watering significantly affects the number of infected taro plants. In view of these results the unpurified strain commits more damage than the pure strain. The disease caused by the unpurified strain quickly destroys the leaves of the taro plant, as that caused by the pure strain. This is because the unpurified strain is a collection of microorganisms that interact to cause disease and amplify its development while the pure strain is a microorganism that acts alone to cause the disease and its development and spread are Slow, whose destruction of the leaves is less rapid compared to that of the unpurified strain.

5. Conclusion

At the end of this study, the aim was to study the symbiotic pathogenic microorganisms associated with *P. colocasiae*, the causative agent of taro mildew. It appears that *P. colocasiae* is a fungus whose morphology varies, depending on the culture medium used for its isolation and purification. Its microscopic observation reveals that it is a false mushroom because these hyphae are unbound. The trapping allowed us to obtain the microorganisms associated with *Phytophthora*. We were able to identify one of these microorganisms that form the biofilm with *Phytophthora*, playing a very important role in the spread of the disease because it is *Vorticella* that carries the spores of *P. colocasiae* while swimming with a speed Rapid to spread the spores away from the biofilm and spread the disease throughout the plant.

The evolution of the diameter of the disease caused by the pure strain is increasing as a function of the days, the spot reaches half of the leaf and no longer progresses where the pure strain does not completely destroy the leaf while the

unpurified strain destroys totally the leaf and the evolution of its diameter is fast. The pure strain induces a low infection because its incidence is about 40% and its severity is about 10.48% whereas the unpurified strain induces a high infection because its severity is about 70.18% and its incidence of about 96.67%.

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