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Study of Antagonistic Beneficial Microorganisms to *Phytophtora colocasiae*, Causal Agent of Taro Mildew (*Colocasia esculenta* (L.) Schott)

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Abstract: The cultivation of taro is of great economic and social importance on a global scale. The current orientation towards agricultural production and the serious consequences of the mildew of taro to crops prompts to find alternatives to chemical control. This study aims at selecting in the taro habitat (leaves, rhizosphere) the microorganisms with high antagonistic potential capable of ensuring the biological control of *P. colocasiae.* Fungus isolated from the taro-infected leaves of the cultivar "Macumba or Ibo coco" from the V8-Agar medium, was kept in pure culture. The different antagonists were obtained by two trapping techniques using *P. colocasiae* as bait for the associated microorganisms and by the decimal dilution technique. The results reveal fourteen antagonist isolates, including five fungi and two bacteria isolated from the leaves; Against 4 bacteria and 3 fungi at ground level. Identification of the latter identified the presence of *Penicillium Sp, Trichoderma Sp, Aspergillus Sp, Pythium Sp., Bacillus Sp,* Rhizobium, Streptomyces and seven other unidentified isolates (Ni). The different *in vitro* tests showed that *Rhizobium* and Ni4 showed the strongest inhibitions (91.66 and 90.69%). The greenhouse tests showed the high-inhibitory effect of *Trichoderma Sp*. and *Rhizobium*, which showed very low foliar alteration percentages (9.65 and 1.86%). These antagonists would be of particular benefit to farmers in the development of biological pesticides.

Keywords: Taro Mildew, *Phytophtora colocasiae*, Biological Control, Antagonist Microorganisms

1. Introduction

The cultivation of taro has been widely extended in Cameroon thanks to many virtues that make it a giant among the world's food crops. A high-quality commodity for many human and animal populations, taro is an essential element in agricultural production. Its global production is estimated at 12 million tons on a cultivated area of 2 million hectares [1, 2]. Sub-Saharan Africa occupies an important place with 77% of total production [3]. Cameroon is the fourth largest producer in the world and third in Africa after Nigeria and Ghana, producing 1.6 million of the 7 million tons produced by the African continent [4, 5]. The importance given to this plant is explained by its aesthetic, nutritional and medicinal value of its leaves and tubers. It is an ornamental plant with good digestibility, rich in vitamin C and B6, and used for the treatment of diseases such as tuberculosis, ulcer, pulmonary congestion and fungal infections [6-8]. This importance makes taro a plant

that deserves to be studied for possible agro industrial developments.

However, although it has all the above-mentioned qualities and its socio-economic and cultural importance, taro culture has been threatened in Cameroon since 2010 by an epidemic disease called mildew, which is the main obstacle to the rational development of its production. This pathology is characterized by stunting of the aerial parts, foliar alterations, wilting; leaf rots, sometimes leading to plant death [9, 10]. The pathogen of this disease is an isolated oomycete identified as *P. colocasiae* [11]. Its dormant zoospores develop during saprophytic life and represent the inoculum of the disease. In the presence of excessive moisture, an average temperature of 27°C, and a neutral pH, these zoospores germinate in response to root exudates and penetrate the roots even in the absence of any wounds with very rapid propagation [12]. The parasite develops and colonizes the entire plant and then remains localized within the conducting vessels causing their obstruction and consequently the wilting of the infected plant.

The taro downy caused by P. colocasiae caused heavy losses in productivity of the cultivated plots estimated at 80% of national production [11, 13, 14]. Approximately 413.051 t of taro tubers were lost, estimated at $ 122 million US [15]; causing farmers to abandon the crop in add the rumor that the disease could be transmitted to humans [16]. This has led to higher prices on the market. Similarly, prices of some food items such as yam (Dioscorea Spp), cocoyam (Xanthosoma sagittifolium), sweet potato (Ipomoea batatas) and banana (Musa spp.) also increased by 33, 100, 40 and 140% respectively [17]. Reduced plantation yields seem to explain the decline in world production from 12 million tons in 2010 to 10 million tons in 2012 [12]. This scourge continues to cause psychosis in agricultural production and Cameroonian populations, which requires emergency measures to eradicate it in order to preserve this endangered genetic resource in this country [16, 17].

The preventive measures used by crop rotations, the use of improved disease-resistant varieties and fungicides remain a serious problem for farmers to eradicate the disease; because they are not only costly but have drawbacks for humans and the environment, to which is added the risk of the appearance of new resistant pathotypes. A palliative measure was therefore envisaged by the study of microorganisms with high inhibitory potential, isolated from the taro habitat in order to biologically control the action of the pathogen. These organisms offer the advantage of not only being available at low cost to farmers, which are non-toxic to the plant and the environment, but also to increase yield by directly removing the inoculum from the pathogen and/or by inducing Plant resistance [18, 19]. This resistance currently represents a new strategy for the defense of plants against pathogen aggressions.

2. Material and Methods

2.1. Presentation of the Sampling Site

The district Bonanguèlè is a town located in the district of Dibombari, Moungo division (Cameroun), agro-ecological zone n°4, located 18km from Douala city. This district extends on 4°11’ north latitude, 9°39’ east longitude, for an area of 15000 hectares.

2.2. Material

The infected leaves and the taro rhizospheric soil were harvested in the field in the Bonanguèlè district (Dibombari) and the healthy corms of taro plant bought at the Dakar (Douala) market (Figure 1).

Figure 1. *Biological samples. A: infected taro leaf, B: rhizospheric soil, C: corms of taro plant.*

2.3. Methods

Rhizospheric soil samples were collected in field on two plots of 42m^2 area each to serve as isolation of soil antagonists. The samples were preceded by a cleaning of the soil surface of the plant to remove dead leaves and debris from other plants. The soil samples were then taken in the surface layers (Figure 2), in a 30 to 40 cm thick horizon according to the method of Davet and Rouxel [20]. This sampling was carried out on two plots: one infected with mildew and the other with no aspect of the disease.

2.3.1. Isolation and Purification of P. colocasiae

The samples of the leaves infected with taro mildew of the three cultivars "Macoumba, Atangana and Country" were harvested at 6 am in one of the plots of taro grown in the

locality previously described and then transported to the Laboratory of Biology and Physiology of Plant Organisms (LBPPO) for further work. These sheets were washed and then rinsed with sterilized distilled water. They were then cut into fragments of about 2 mm^2 at the growth front of the pathogen [12] before being superficially disinfected in a 5% sodium hypochlorite solution for 2 minutes to remove bacteria and fungi present on the surface. After three rinses with sterilized distilled water, the fragments were dried on hydrophilic paper. Isolation of the *P. colocasiae* strain was by deposition of infected leaf fragments in dishes containing V8 + chloramphenicol medium followed by incubation in the dark at 25°C. (Figure 3a). Transplanting for purification of the strain was done on Potatos Dextrose Agar (PDA) medium (Figure 3b). Microorganisms were identified by macroscopic analysis (Figure 3c) (appearance of colonies, size, color of their lap, growth rate, temperature), microscopic (Figure 3d) (appearance of mycelium, spores, phialides, conidiophores, etc.) according to Brooks [10] and Scot *et al.,* [2], and using an identification key according to Pitt and Honcking [21].

Figure 2. *Sampling of the rhizospheric sample.*

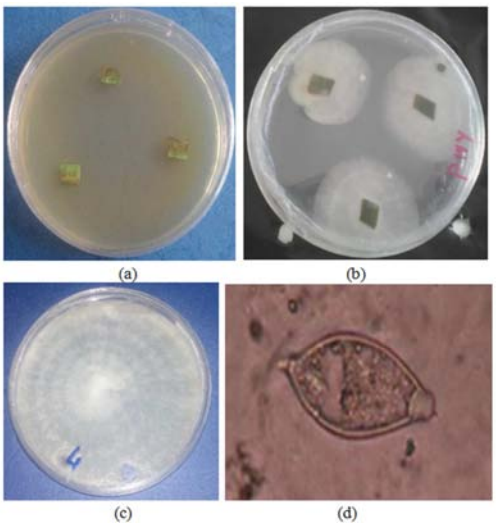

Figure 3. *Isolation and identification of P. colocasiae; (a) and (b) Isolation; (c) pure strain; (d) microscopic observation of asexual sporangia.*

2.3.2. Screening for P. colocasiae Antagonist Species

(i). Sterilization of Leaves

The surface of the infected leaves was previously washed with tap water and then subjected to a series of 95% ethanol disinfection for 30 seconds, 10% sodium hypochlorite for 2 minutes, and then 75% ethanol for 2 minutes so as to remove the microorganisms present on the surface. The leaves are then rinsed three times with sterilized distilled water to remove traces of disinfectant [22, 23]; then sectioned in 2 mm^2 dimension. The obtaining of the microorganisms representative of the two natural habitats (leaf plates and rhizosphere) was carried out by trapping thanks to the pathogen.

(ii). From Infected Leaf Plates

The methodology used here was based on the assumption that among microorganisms capable of coexisting within a biofilm with at least one given species of phytopathogenic oomycete there would be a particular which would have the effect of preventing development of an infection by said oomycete species. More specifically, this strain of microorganism would make it possible to control the growth of said phytopathogen. Growth control is achieved by at least partially inhibiting the growth of the phytopathogenic oomycete. In petri dishes containing the agar nutrient medium, the leached and sterilized leaf fragments are cultured at the ends of the petri dishes (in a proportion of two to three fragments per dish) each carrying at its center a mycelial suspension of purified strain of *P. colocasiae*. The incubation was carried out in the dark at 26°C. for 3 days. Hence, *P. colocasiae* secretes cyclic adenosine monophosphate (cAMP), a chemo-attracting substance that attracts and interacts with microorganisms capable of growing in its vicinity (forming a biofilm) (Figure 4). Each colony of microorganism formed is thus removed and transplanted into new petri dishes containing the PDA medium (for fungi) and the YEMA medium (for bacteria). Successive subcultures are carried out until the pure strains are obtained. The microorganisms isolated in the vicinity of the pathogen and purified were thus confronted with *P. colocasiae* in order to select the different antagonists.

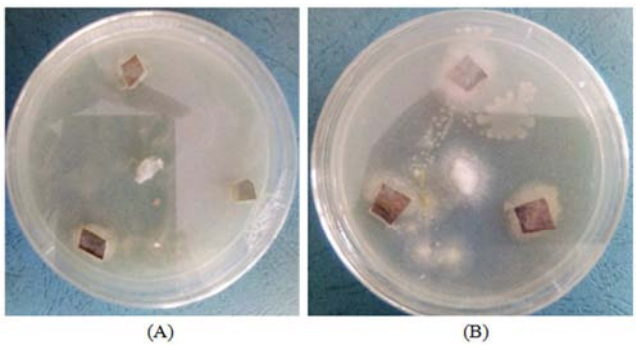

Figure 4. *Leaf antagonist trapping.*

(iii). From the Rhizospheric Soil

The rhizosphere is defined as the soil zone in which the

soil microflora is subjected to root influences [24, 25].

The soil samples (infected and uninfected taro rhizosphere) described above were dried and sieved separately; an amount of 10 L of infected soil was placed in two 20 x 40 cm aluminum pails and covered with 4 L of sterile distilled water so as to obtain just a film of water on the surface of the soil (Figure 5). Healthy leaflets of the "Macoumba" taro variety are washed and disinfected by soaking in a 1% sodium hypochlorite solution for 1 minute and then rinsed with sterile distilled water and cut into 2 mm^2 size. These fragments are directly deposited in buckets previously moistened for 50 fragments per bucket to serve as bait for *P. colocasiae* antagonists. Incubation was carried out at 20°C in the dark for 3 days. The leaf fragments were then rinsed twice with sterile distilled water and transferred to sterile 90 mm Petri dishes containing the agar nutrient medium. These dishes were incubated at 25°C. and in the dark for 72 h. The colonies observed were transplanted in the PDA and YEMA media (respectively for fungi and bacteria) until the pure strains were obtained.

Figure 5. *Trapping from the soil of the rhizosphere.*

Uninfected soil samples were thoroughly ground in an enameled porcelain mortar. Thus, 5 g of the ground product is transferred into 45 ml of sterile distilled water contained in an Erlenmeyer flask. The mixture was then stirred for 30 minutes to obtain good particle disintegration. To achieve a good variable concentration of propagules and to facilitate colony counting, a dilution series is carried out from the stock solution, the concentration of which is 10-1 [26]. To obtain a 10-2 solution, 1 ml of the stock solution is transferred into 9 ml of sterile distilled water. Thus, a decimal dilution series ranging from 10-2 to 10-7 was performed in haemolysis tubes. One hundred (100) microliters of each dilution was plated onto non-agar agar culture media contained in petri dishes. For each dilution and for each culture medium 6 petri dishes were seeded. The incubation was conducted in the dark in an oven at 26°C. for 2 days for the bacteria and 7 days for the fungi. Each microorganism obtained was purified and identified in order to select the different antagonists.

2.3.3. Confrontation Between Each Strain of Microorganisms Obtained and P. colocasiae

The comparison tests were carried out in a petri dish on solid culture media (PDA) for fungi and YEMA (Yeast Extract Mannitol Agar) for bacteria, according to the method described by Vincent *et al.,* (1991). The isolates of fungi and bacteria obtained (isolated from the leaves or rhizosphere) are placed or spread on half a half or at one end of an agar box (for fungi) or containing YEMA medium (for bacteria), while a fungal disc of *P. colocasiae*. (Hyphe or spore) of 8 mm from a 7-day culture is deposited on the opposite half. The latter is placed along a diametrical axis equidistant from the isolates so that the distance separating them is about 4 cm (Figure 6). This *P. colocasiae* / bacterial or *P. colocasiae* / fungus confrontation is followed for 7 days at ambient temperature, the distance between the end of the colonies and that of the fungal mycelium is then measured from 48 hours.

Figure 6. *Confrontation P colocasiae / fungi.*

Estimation of the antagonistic effect: The antagonist activity is thus evaluated by percentage of inhibition of the diametrical growth (PIDG) of the pathogen facing the antagonist compared to that of the control containing only the fungal mycelium. The percent inhibition is calculated according to the formula described by Michael and Nelson [28].

$$PIDG = (Dt-Dc)\times 100/Dt$$

-Dt: mean diameter of unconfirmed control strains
-Dc: diameter of the strains facing the antagonist

In the case of confrontations between *P. colocasiae* and bacteria, the antagonistic effect is evaluated by the percentage of radial growth inhibition (PRGI) of the colonies calculated as follows:

$$PRGI = (Re-Rr)\times 100/Re$$

-Re: radius of the strain to the furthest bacterial colony
-Rr: radius of the strain to the nearest colony

2.3.4. Bioprotection of Healthy Plants

The microorganisms retained on the basis of their inhibitory potency on *P. colocasiae* "*in vitro*" were used to inoculate healthy taro plants in the greenhouse with the *P. colocasiae* pathogen. This "*in situ*" authentication test made it possible to identify and select strains capable of ensuring the bioprotection of plants against the mildew of the taro.

(i). Revitalization and Purification of Strains

The strains of *P. colocasiae* and those from the various traps conserved at -20°C. in the LBPPO were revitalized in petri dishes containing the nutrient agar medium containing per liter of distilled water of the following composition: peptone, 5g, Glucose, 1g, yeast extract, 2.5g, agar, 15g, pH 7. The Petri dishes were poured with 15ml of medium and the streak method (for bacteria) was used to transplant the strains until pure colonies are obtained. As for the fungi, the purification was carried out in sterile kettle dishes containing the PDA medium after successive transplanting.

(ii). Plant Germination Protocol

a) *Sterilization of equipment:* The taro corms of the variety "Ibo coco" were sterilized by successive dipping in the following baths: 15% sodium hypochlorite for 2 minutes, two rinses with sterile distilled water, alcohol 90° for one minute, drying on sterile filter paper. Sand was soaked in 5% hydrochloric acid for 24 hours. It is then rinsed a dozen times with distilled water until the pH stabilizes around 6. This sand is then sterilized twice in an autoclave for one hour.

b) Preparation of inoculas of microorganisms: Pure and fruit-bearing cultures of *P. colocasiae* and those of the various fungal antagonists, all 21 days old, were each carefully brushed with a fine brush in 20ml of sterile distilled water. The sporangial suspension obtained was filtered with muslin to remove the mycelial fragments. A drop of Tween 20 was added thereto to homogenize the spore suspension which was subsequently quantified at 5 x 104 sporangia / ml using a hemacytometer and then stored at 4°C for 30 minutes in a refrigerator for stimulating the release of zoospores [29, 12]. In the case of bacteria, the sheet discs were previously soaked in the bacterial suspension for one minute and then placed in bins on a foamed plate of water. Each disk then receives 10 µl of a suspension calibrated at 3.105 zoospores/ml [30]. Only the positive control is soaked in the suspension of zoospores of the pathogen.

c) Planting and inoculation of plants: The corms thus sterilized are placed in 30×40 cm sachets filled with sand sterilized previously, at a depth of 7cm (at the rate of one corm per sachet) and watered for 10 days until the thrust of the first leaves. The plants were divided into 8 batches of 5 plants according to each treatment, comprising two control batches, one of which was inoculated only with the sporangial suspension of the pathogen *P. colocasiae* (positive control). After 1 month of culture, the leaves of each plant were washed, disinfected in a 5% bleach solution for 2 minutes, and then rinsed three times with sterilized distilled water. After drying, these leaves were circumscribed on a surface of 58.05cm^2 in order to define the similarity of the study area of each leaf.

Two leaves of each plant were lightly scraped in the center of the circumscribed area on a portion of about 4cm^2 using a knife blade. The previously injured portions were kept soaked for 15 minutes in a sporangial suspension of the antagonist (2×104 sporangium/ml antagonist mycelium), except for the controls. After 5 minutes, the pre-inoculated plants are again inoculated with a sporangial suspension of the pathogen *P. colocasiae* at the same concentration as that of the antagonists. Cotton impregnated with sterilized distilled water was placed in the inoculation zone to maintain moisture during the experiment.

The plants thus inoculated were watered every day and observed every 24 hours until the appearance of the first manifestations. The diameter of leaf rot was measured every 48 hours for up to 6 days. The disease was estimated by measuring the diameter of the infection and then calculating the percentage of foliar destruction on all the plants in each batch on a scale ranging from 1 (very tolerant, 0% foliage reached) to 9 (foliage completely destroyed) according to Ferjaoui *et al.*, [31].

2.3.5. Statistical Analyzes

The data was written into an Excel spreadsheet (Microsoft Office, USA) and analyzed with Statview software version 5.0 (SAS Institute, Inc., USA). These data were presented as mean ± standard deviation (SD) in charts and tables. ANOVA was used to make comparisons of mean diameters between batches every two days. The Newman-Keuls test (post hoc tests) was used subsequently to make comparisons two by two. Ordered variance analysis (ANOVA) in repeated measurements was used to study the variation in diameter between follow-up days for each batch. The threshold of significance was set at p-value <0.05.

3. Results

3.1. Identification of Microorganisms

The manifestation of the antagonistic effect observed after 7 days showed that of the thirty-six (36) isolates tested, only 14 showed an inhibitory effect on the growth of *P. colocasiae*. These, depending on their inhibitory power, are used as an aid to biological control. This showed that at the level of the leaves: 5 fungal strains were isolated against 2 bacterial strains and 4 bacterial strains against 3 fungal strains at the level of the soil.

The results identified 14 microorganisms including 8 fungi belonging to the genus *Pythium Sp, Trichoderma Sp, Penicillium Sp, Aspergillus Sp*, and four other unidentified isolates; 6 Gram-positive and Gram-positive bacterial isolates (bacilli and shells), with rapid growth, with production of exopolysaccharides, on the basis of morphological characteristics and the BBT test carried out, assume to belong to the genera *Bacillus,* Streptomycetes*, Rhizobium* and three Other unidentified isolates (Figure 7).

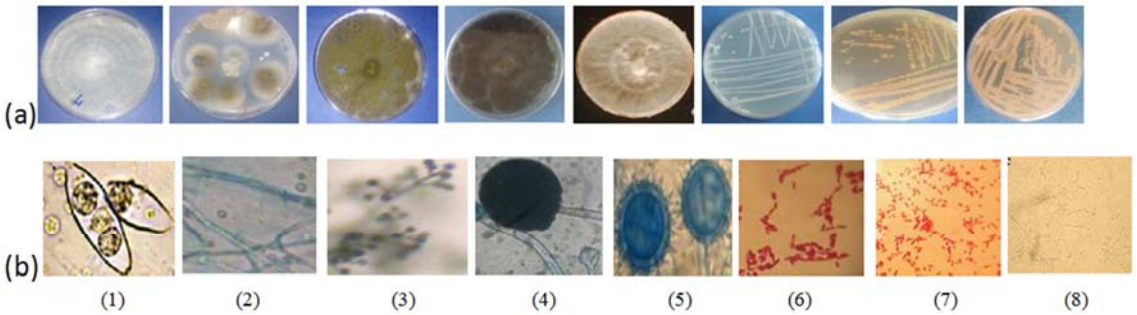

Figure 7. *Various isolated and identified antagonists of foliar and rhizospheric samples (objective 100): (a) macroscopic and (b) microscopic: (1) P. colocasiae (2) Penicillium sp. (3) Trichoderma sp. (4) Aspergillus sp. (5) Pythium sp. (6) Rhizobium (7) Bacillus (8) Streptomyces.*

3.2. Action of the Antagonists on P. colocasiae

The comparison of the antagonistic microorganisms against *P. colocasiae* (Figure 8) shows that: The smallest inhibitions were observed in the unidentified strains N.i3 (52.32%) and N.i1 (59.30) for the case of fungi, N.i5 (44.18%) and *Bacillus sp.* (65.71%) for bacteria. The greatest inhibition percentages were obtained with the fungal strains *Aspergillus sp.* (86.04%) and N.i4 (90.69%), and the bacterial strains *Streptomyces* and *Rhizobium* with respective values of 77. 61% and 91.66%.

This suggests a positive action of these latter microorganisms on their ability to exert a strong influence on the growth of the pathogen.

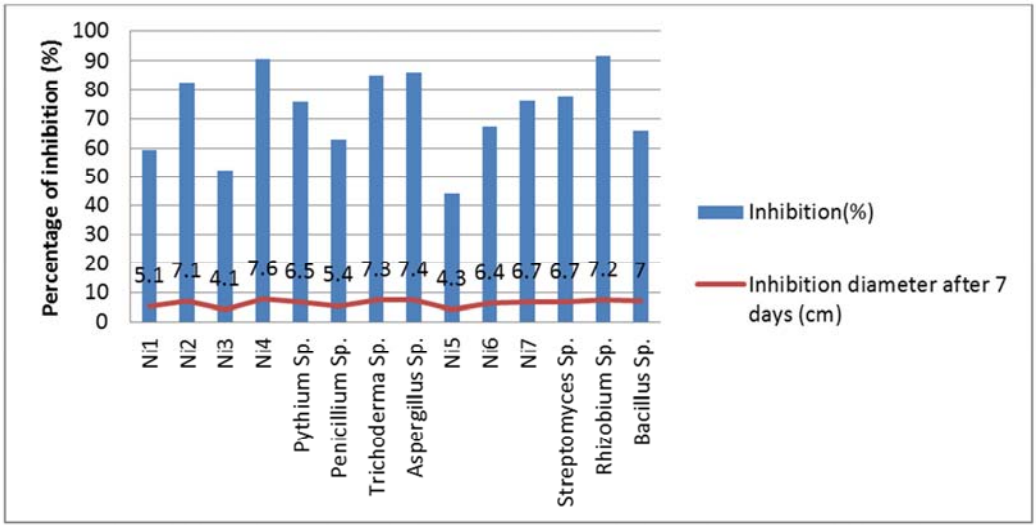

Figure 8. *Percentage of inhibition of P. colocasiae as a function of the microorganisms tested.*

3.3. Evaluation of the Antagonist Action in Planta

Of the identified isolates, six were selected for the antagonist assay. This test was carried out on the leaves of taro plant by co-inoculation of each of these antagonists with *P. colocasiae*. These isolates are: *Trichoderma sp, Aspergillus sp, Pythium sp, Bacillus sp, Streptomyces, Rhizobium* (Figure 9).

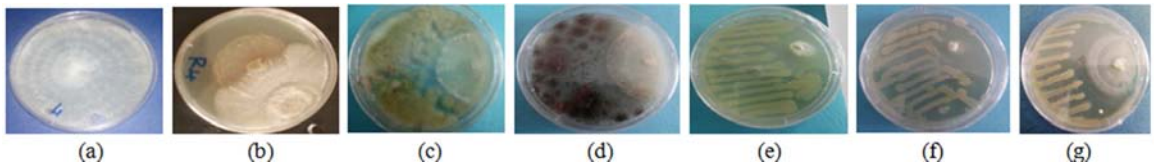

Figure 9. *Inhibitory action of antagonist microorganisms on P. colocasiae: (a) P. colocasiae/P. colocasiae (témoin), (b) Pythium Sp/P. colocasiae, (c) Trichoderma Sp./P. colocasiae, (d) Aspergillus Sp/P. colocasiae, (e) Streptomyces/P. colocasiae, (f) Rhizobium/P. colocasiae, (g) Bacillus/P. colocasiae.*

The observations made on each batch after 6 days of co-infection of each microorganism with *P. colocasiae* showed that the control plants showed severe stunting symptoms. The diameter of the lesion and the percentage of alteration were significantly higher than those of the different batches confronted with the antagonists of the experiment. Indeed, the whole control batch (T) containing only the pathogen *P. colocasiae* presented on the second day the first manifestations of the mildew on the healthy leaves. The average growth diameter measured on all the leaves was

3.45cm. This growth diameter was even more effective on the fourth day with an average of 4.73cm, followed by an almost total invasion of the standard circumference (8.2 cm) corresponding to the almost total destruction of the whole of foliage (8.6cm), with an alteration percentage of 95.34% (Figure 10).

Figure 10. *Foliar alteration, severe stunting symptoms (control group).*

The screening of the results made it possible to demonstrate the inhibition of the growth of the pathogen on two consecutive media.

On the one hand, in solid media where the smallest inhibitions obtained were manifested with the unidentified isolates N.i3 (52.32%) and N.i1 (59.30) for the case of fungi except Ni4 (90.69%), N.i5 (44.18%) and Bacillus sp (65.71%) for bacteria, while the greatest percentages of inhibition were obtained with the fungal strains *Aspergillus sp.* (86.04%) and N.i4 (90.69%), and bacterial strains *Streptomyces* and *Rhizobium* with respective values of 77.61% and 91.66%.

Other parts on healthy leaves, it was observed that the greatest percentages of alteration were obtained in Bacillus sp and *Aspergillus* sp with respective values of 28.37 and 29.76%. The smallest alterations corresponding to the greatest inhibitions occurred with the *Trichoderma* and *Rhizobium* strains with respectively% alteration 9.65 and 1.86%.

The microorganisms antagonistic in case *Trichoderma* and *Rhizobium* showed only tiny symptomatic effects with respect to plants, with very small percentages of leaf alteration compared to control (Figure 11). Their inhibitory capacity persisted in time, and remained always present even after the duration of our experiment.

However, *in vitro* inhibitory potency of *Pythium* which was lower than that of *Aspergillus* was controversial on healthy leaves. This suggests a positive action of this microorganism on their ability to exert a strong influence on the growth of the pathogen in infected plant plots.

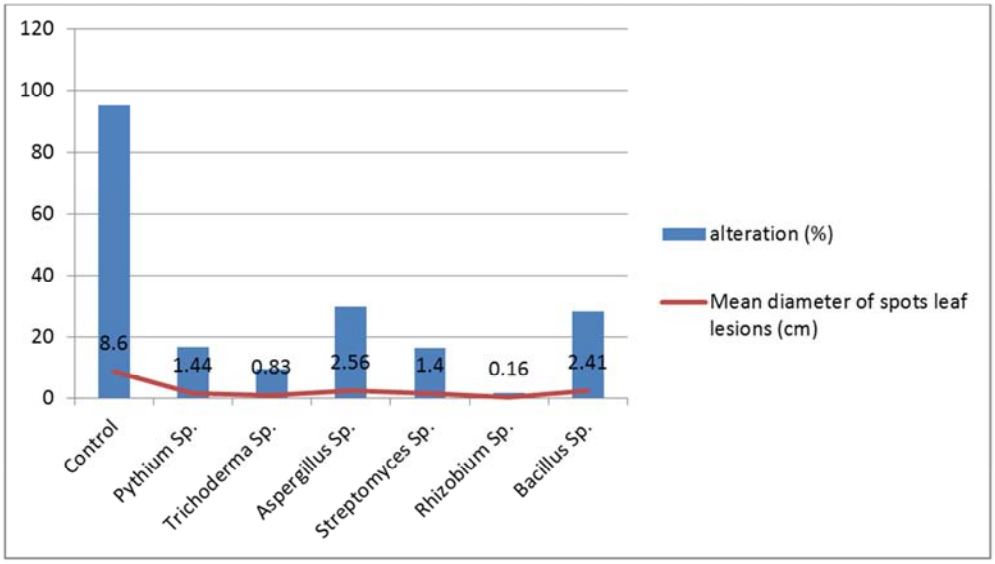

Figure 11. *Mean diameter of spots leaf lesions and percentage of alteration by antagonistic microorganisms after 6 days.*

4. Discussion

4.1. Quantitative Analysis of the Taro Microflora

Foliar ecosystem presented 7 microorganisms, corresponding to 7 species of fungi and 2 belonging to bacteria. Rhizospheric samples obtained 7 microorganisms including 3 fungal isolates and 4 bacterial isolates. These results show that the number of microorganisms varies qualitatively and quantitatively depending on the habitat chosen. Rhizospheric soil and the infected taro leaves harbor a wide range of microbial populations which, for this purpose, are sites of predilection for indigenous microorganisms, potential antagonists of *P. colocasiae*, occupying the same ecological niche. The use of the trapping technique from these two habitats significantly improves the yield of the isolates of the microorganisms, depending on whether the latter are grown in a PDA or YEMA

environment. These results are in agreement with those of Kebe *et al*. [30], which showed that the use of microorganism traps, consisting of *P. palmivora* infected cocoa pod fragments, significantly improved the yield of isolates of microorganisms at ground level, due to the affinity between *P. palmivora* and the microorganisms collected.

4.2. Action of the Antagonists on the in Vitro and in Vivo Growth of P. colocasiae

Comparison tests in the petri dish between the different antagonists and the phytopathogen, the significant differences observed between the diameter of the control leaves and that of the co-infected leaves, showed an effect ranging from inhibition to complete cessation of Mycelial growth. The reduction of growth remains a reliable characteristic to estimate microbial antagonism towards fungal organisms [32]. The results of these trials have led to the conclusion that the introduction of beneficial microorganisms in field-grown taro plots may modify the expression of the pathogenicity of *P. colocasiae* in relation to new host plants.

Observation of the antagonistic activity of the selected microorganisms on the phytopathogen caused a very high inhibition of mycelial growth. Indeed, these antagonists were very competitive from the fourth day with an exponential growth of their mycelia towards the pathogen. Spatial colonization continued with a greater invasion of the pathogen on day 7, with diameters and inhibition rates of 6.5cm/75.58%; 7.3cm/84cm; 7.4cm/86.04%; 6.7cm/77.61%; 7.2cm/91.66%; 7cm/65.71% for *Pythium sp, Trichoderma sp, Aspergillus sp, Strptomyces sp, Rhizobium sp, Bacillus sp* respectively. These results corroborate those obtained by Juliette Dedi *et al.,* [33], Mouria *et al*., [34], Rey *et al.*, [35]; Benhamou *et al.,* [36], Sempere and Santamarina [37], Lamia and Souad [38], respectively comparing the strains of *Aspergillus niger* against five fungi (*Penicillium sp., Fusarium solani, Trichoderma sp. Phoma sp.* and *Fusarium oxysporum*), *Trichoderma sp*. against the causal agent of verticillium wilt, *Pythium oligandrum* against *Fusarium oxysporum* and *Phaeomoniella chlamydospora, Penicillium Oxalicum* Currie against *Alternaria Alternata* (Fr.) Keissler, *Paenibacillus polymyxa* SGK2 against *Fusarium* graminearum*, Rhizobium* against *Fusarium oxysporum* and *Trichoderma harzianum, Streptomyces Sp* against *Fusarium oxysporum*.

These observations are consistent with the findings of Simpfendorfer *et al.,* [39] who, by analyzing the abstract activities of the *Rhizobium* biological control on the severity of plant root diseases (greenhouse study), found that *Rhizobium* isolates caused a significant reduction in the growth of *Fusarium oxysporum*. These bacterial species would have a significant potential for activities that can contribute to the establishment of beneficial interaction with certain microorganisms in the rhizosphere to improve plant growth and health. This would suggest that these bacteria would have nutritional capacities that would allow them to rapidly colonize the culture medium and subsequently deprive the fungi of nutrients and thus the impossibility of mycelial growth, which is very beneficial in the context of the biological struggle. This bacterial strain could be used as a biological control agent capable of reducing the severity of mildew.

Similarly, the experiments of Schrey and Tarkka [40] have shown that *Streptomyces* can modulate the defense of plants against phytopathogens by stimulating local or systemic defense mechanisms or on the contrary facilitate colonization of plant roots. The use of Streptomycetes would be an additional means of defense to protect plants from pathogenic organisms insofar as their diversity in the soil offers enormous potential to inhibit growth or even kill other organisms such as fungi.

5. Conclusion

In the light of laboratory and greenhouse results, it was found that of the thirty-six (36) microorganisms isolated from the foliar and rhizospheric samples fourteen (14) had a negative effect on the growth of the pathogen. The different in vitro clashes between these microorganisms showed that eleven of the fourteen isolates showed an inhibition of more than 60%. In addition to the isolates identified, unidentified isolates such as N.i2, N.i4, N.i6, N. 17 showed appreciable inhibitions with respective inhibition percentages of 82.55; 90.69; 67.18; 65.71%. The isolates identified were classified according to their inhibitory potency: *Rhizobium* (91.66%), Aspergillus Sp (86.04%), *Trichoderma Sp* (84.88%), *Pythium Sp* (75.58%), *Bacillus* (65.71%), *Penicillium Sp* (62.79%).

The growth of the disease recorded in the control in the greenhouse (95.65% foliar alteration) was clearly superior to that of the interactions. All treatments showed considerable inhibition of the disease. The foliar alteration percentages were noted as follows: Group A1 (29.76%), Group A2 (9.65%), Group A3 (16.74%), Group B1 (1.86%), Group B3 (16.74%), respectively for the genus *Aspergillus Sp, Trichoderma Sp, Pythium Sp, Bacillus, Rhizobium* and *Streptomycetes*. The greatest manifestations of biological control of the pathogen were with the *Rhizobium Sp* and *Trichoderma Sp* strains and the unidentified strains N.i2 and N.i4 which showed a high capacity for pathogenic inhibition under experimental conditions both in the (For some) in the greenhouse (for others).

Exploring antagonistic agents would be of particular benefit to farmers in the development of biological pesticides as a competitor in an environment where nutritional resources are limited. Biological control is thus a key area in integrated control, oriented with respect for the environment and therefore to provide an outlet for taro production in Cameroon.

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