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Article

Phytopathological Challenges of Corm Rot in Papa China in the Colombian Pacific

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Abstract: *Colocasia esculenta*, also known as Chinese taro or taro, is a crop of great importance for the Colombian Pacific region. However, it is threatened by corm rot, which affects the food security of Afro-descendant and indigenous communities in the area. This study aimed to identify some of the phytopathogenic agents responsible for this disease and explore their control using an aqueous extract of *Dysphania ambrosioides* (paico). Through morphological analyses and ITS gene sequencing, two fungi responsible for the rot were identified: *Fusarium solani* and *Mycoleptodiscus suttonii*, and their roles as causal agents of the rot were confirmed in greenhouse experiments. Paico proved effective in controlling the growth of these fungi, with concentrations of 12.5% and 17.5% for *M. suttonii*, and 17.5% for *F. solani*. These findings highlight the importance of organic control to ensure food security and sustainable production in vulnerable areas of Colombia, emphasizing the relevance of this study for local communities.

Keywords: paico extract; organic control of fungi; *Fusarium solani*; *Mycoleptodiscus suttonii*; phytopathogenic agents; *Colocasia esculenta*; *Dysphania ambrosioides*

1. Introduction

In the context of the Colombian Pacific, where excess moisture and clay content in the soil cause the rotting of crops such as cassava and potatoes, and low banana yields, the region's carbohydrate supply depends on Chinese yam, making this product crucial for food security and the local economy [1–5]. Among the main reported diseases are Mosaic Virus [6] and dry rot syndrome, caused by fungi and/or bacteria [7], which affect fundamental processes such as photosynthesis and carbon assimilation, leading to a reduction in corm production and postharvest losses [8]. The observed increase in the incidence of phytopathological diseases, some of which are emerging and previously undocumented, emphasizes the need to identify pathogens and formulate organic control strategies using aqueous [9] or ethanol [10] plant extracts. This research explores *Dysphania ambrosioides* (paico), which has demonstrated notable antifungal activity [7,11]. The main objective of this study was to address the problem of corm rot in *Colocasia esculenta*, mainly cultivated in the Colombian Pacific, by identifying the fungal phytopathogenic agents responsible for crop deterioration and their potential control with an aqueous extract prepared from paico.

2. Materials and Methods

Collection of Chinese Yam Samples with Corm rot and Phytopathogenic Agents Identification

Samples were collected in Buenaventura (3.81N, -77.03W, 13 meters above sea level, 91% relative humidity, 65% average annual precipitation, and 27°C average annual temperature) and Guapi

(2.57N, -77.89W, 4 meters above sea level, 87.5% relative humidity, 69% average annual precipitation, and 27°C average annual temperature) (Figure 1).



Figure 1. Location of the Chinese potato samples collected with corm rot. On the right, an Afro-descendant woman from Buenaventura harvests the Chinese potato for her daily sustenance.

The material was disinfected with potable water and cut into 5x5mm pieces. The samples were then washed in 96% ethanol for 3 seconds, 0.5% sodium hypochlorite for 2 minutes, and twice with sterile distilled water. Each sample was triplicated in Petri dishes containing Potato Dextrose Agar (PDA) culture medium. They were incubated at 28°C ±1 for 6 to 8 days. Growth was checked every 24 hours, and isolations were performed in PDA acidified with tartaric acid at 10% (approximately 14 ml/L) to inhibit bacterial growth. For the observation of reproductive structures, Water Agar at 0.015%, Potato Carrot Agar, and V8 Agar were used [12].

Morphological characteristics were obtained by microscopy (40x and 100x), and DNA extraction from pure mycelium was carried out using the NORGEN kit. The ITS1 and ITS4 regions were amplified, products were purified by ethanol precipitation, and quantified using NanoPhotometer™ A260. To determine DNA quality, A260/280 and A260/230 ratios were measured. Amplicons were sequenced at MACROGEN.

To verify corm rot caused by the isolated phytopathogenic agents, six 4-month-old plants were inoculated with each fungus. A blade cut was made and a 5mm inoculum disc was placed under greenhouse conditions. They were evaluated for 2 months until corm thickening. The negative control consisted of plants inoculated with PDA. To confirm the presence of the pathogen, a sample of the obtained rotted corm was cultured on PDA.

Preparation of the Aqueous Extract from Dysphania ambrosioides (Paico)

Dysphania ambrosioides (paico) was obtained from an organic cultivation, washed, and air-dried. 100 g of the plant were blended in 400 mL of water (w/v) and filtered through a sieve. The obtained standard concentration was 25%, from which 17.5%, 12.5%, and 7.5% concentrations were prepared. These were used to poison the solidified SABOURAUD agar medium when it was below 50°C [11]. Six replicates were inoculated with 5 mm discs of mycelial culture of each fungus and incubated at 28°C ±1. Mycelial growth was recorded at 0, 48, 96, and 144 hours using a digital caliper (±0.2mm), and the growth percentage was calculated as the radial growth percentage. Control plates with SAB medium without plant extract were inoculated separately with each isolated fungus. The percentage of mycelial growth inhibition was determined by comparing the growth diameter of the control with the different treatments using the formula proposed by Vincent 1947 [13].

$$\%ICM = \frac{Dc-D}{Dc} * 100 \quad (1)$$

where: %ICM is the percentage of mycelial growth, Dc is the diameter of the mycelium on the control Petri dish, and Dt is the mycelial growth on the Petri dish with the treatment to be evaluated.

The inhibition behavior of the two fungi isolated against the aqueous extract of paico was grouped into the following categories: Negative: Absence or percentage less than 10% in the normal colony growth, similar to the control. Low: 10-39% reduction in fungal colony growth. Medium: 40-69% reduction. High: More than 70% reduction.

To evaluate whether the extract's effect is fungicidal, discs from each fungus were taken from plates where there was no mycelial growth and transferred to Petri dishes with PDA medium without plant extract. As a control, a disc of mycelium obtained from a pure culture was inoculated on PDA. They were incubated under the same experimental conditions as the previous assay, with colony growth readings taken every 24 hours until the control presented total growth in the dish, according to NTC 6414:2020 [14].

Experimental Design

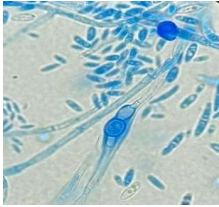
A linear mixed-effects model with repeated measures over time and 6 repetitions per treatment was employed. Assumptions of normality and homoscedasticity were evaluated. A 95% confidence level was used for all analyses, and for variance homogeneity, a residuals vs. fitted values plot was used. A model was proposed for each experimental factor (fungal growth, time, and plant extract concentrations), attributing variance heterogeneity to each of these factors. Based on this information, a factorial ANOVA was conducted. The null hypothesis (H0) posited that the control with paico extract is not effective for managing phytopathogenic fungi, regardless of the type or concentration of the extract. Quantitative data derived from the inhibition percentage estimates were used to perform descriptive statistics, homoscedasticity, and normality tests for subsequent analysis of variance and TUKEY multiple comparison test to observe how the interactions of fungi with the concentrations of paico extract vary over time. Analyses were conducted in Rstudio.

3. Results

Sample Collection During Harvest Season and Isolation of Fungal Pathogens

Samples were collected during the harvest season from corm rot-affected specimens. Two fungi were isolated: *Fusarium solani*, which showed micro and macroconidia [15] in all culture media at 5 days, and *Mycoleptodiscus Suttonii*, which required constant subculturing and lost viability after 30 days, as reported in the literature [16]. The identity was verified through ITS region sequencing. The *F. solani* amplicon presented a 627 bp band, and the sequence matched 100% with NCBI, while *M. Suttonii* amplified a 742 bp band with 97.5% identity. Each isolated fungus was corroborated by reinoculation, observing rot symptoms (Table 1).

Table 1. Macroscopic and Microscopic Characteristics of the Causal Agents of Rot in Chinese Yam. The symptoms were recorded in the corms 2 months after inoculation with the causal agents. Macroscopic characteristics were obtained on PDA medium, and for microscopic characterization, macro and microconidia of *F. solani* and aggregates of sporodochial cells in *M. Suttonii* were observed. Photographic records of microscopy were achieved under a 100X magnification objective.

Causative agent	Symptoms	Macroscopic identification	Microscopic identification. 100X
<i>Fusarium solani</i>			



When evaluating the average mycelial growth, it was observed that inhibition increased with concentrations equal to or greater than 12.5% for *M. suttonii* and 17.5% for *F. solani* (Figure 2), confirming the fungistatic activity of the extract (inhibits growth upon contact with the extract) (Table 2). Additionally, the fungicidal effect of the paico extract was confirmed, considering the percentage of inhibition, and it was found that there was no growth of any of the causal agents at those concentrations of paico.

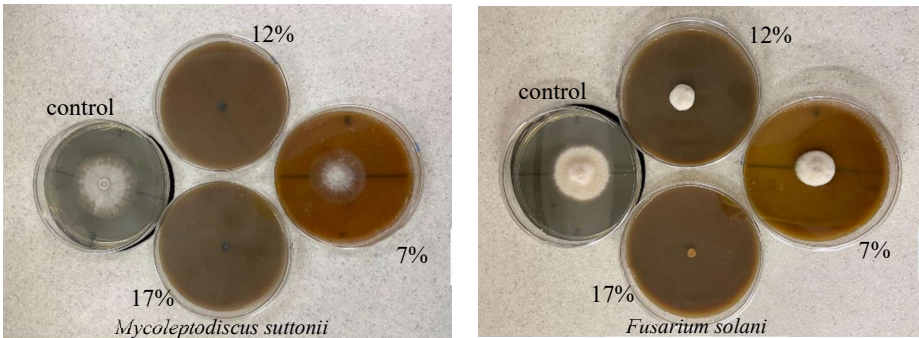


Figure 2. Mycelial growth of *M. suttonii* and *F. solani* in sabouraud medium with aqueous paico plant extract at different concentrations.

Table 2. Inhibition of growth of *Fusarium solani* and *Mycoleptodiscus suttonii* isolated from Chinese yam against aqueous plant extract of paico, evaluating the growth ranges concerning a positive control. Light green indicates a low percentage, darker green a medium percentage, and red a high percentage, as previously described.

[%Paico]	% inhibition <i>Fusarium solani</i>			% inhibition <i>Mycoleptodiscus suttonii</i>		
	48 h	96 h	144 h	48 h	96 h	144 h
7,5	12	24	33	33	33	44
12,5	49	52	48	82	93	95
17,5	78	91	94	83	93	95

The ANOVA results showed that all sources of variation (fungus, paico, time) and their interactions were significant (P value < 0.0001). The Tukey test results confirm the treatment groupings, showing differential responses starting at 48 hours, with an increase in growth except for *M. suttonii* at 12.5% and 17.5% (treatments 7 and 8) and for *F. solani* at 17.5% (treatment 4) (Figure 3).

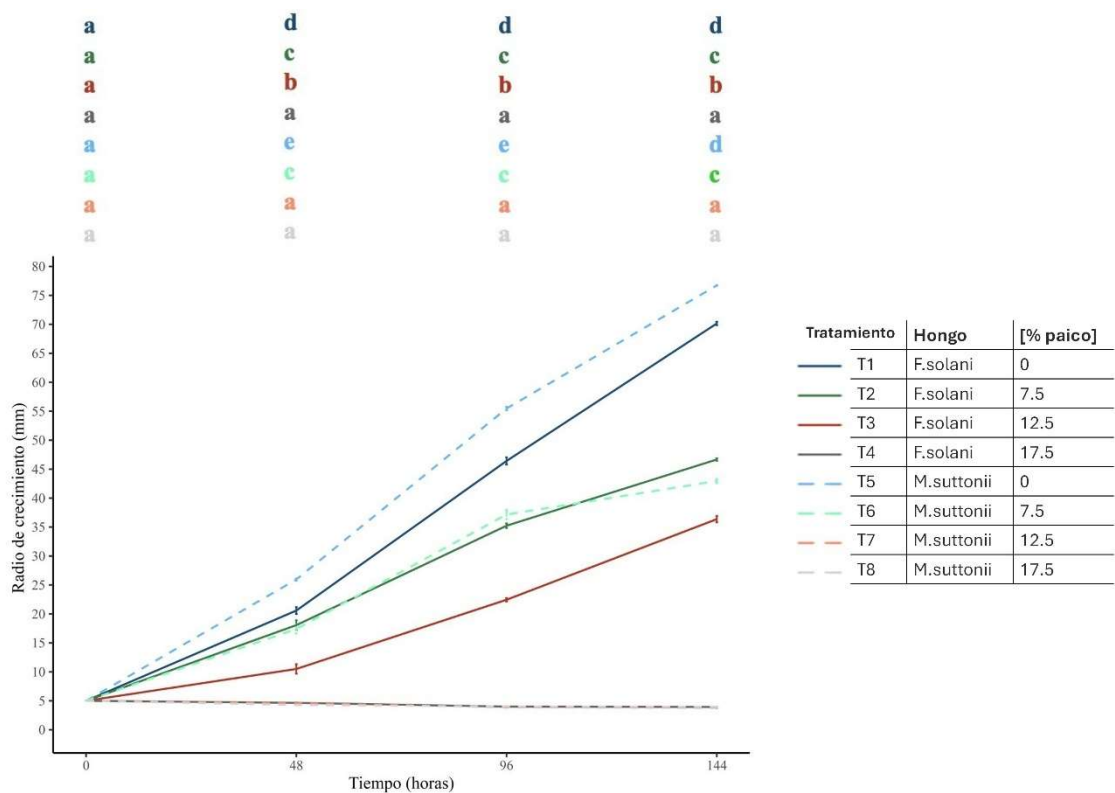


Figure 3. Growth rate (mm) of the phytopathogenic fungi *F. solani* (Treatments 1 to 4) and *M. suttonii* (Treatments 5 to 8) under three concentrations of aqueous paico extract (7.5, 12.5, and 17.5%) using PDA medium without paico extract (0%) as a control, evaluated over 144 hours with growth assessments every 48 hours. At the top, the letters represent treatment groupings based on the Tukey test, with the color of the letters corresponding to the color that identifies each treatment in the graph. An average of 6 repetitions is presented.

4. Discussion

This study reports, for the first time in the Colombian Pacific, the identification of *M. suttonii* and *F. solani* as causal agents of corm rot in Chinese yam. *F. solani* had previously been reported as causing post-harvest damage in Chinese yam [10], [17], whereas *M. suttonii* had not been reported in yam but had been found in other crops [16].

Various studies suggest that this effect is due to secondary metabolites such as alkaloids, steroids, flavonoids, terpenoids, and quinones, among others [18][19]. Specifically, in paico, a component called ascaridol (1,4-peroxide of p-menth-2-ene), the main antifungal agent [20][21][22], acts by inhibiting fungal growth by interfering with the integrity of the fungal cell membrane, producing variable responses depending on the species, race, and pathogenicity of the fungus [21]. This compound affects the synthesis of essential fatty acids in the membrane, resulting in the loss of viability and fungal growth [21]. Additionally, ascaridol and other secondary metabolites of paico work synergistically to enhance its fungitoxic effect [10].

Although the plant extract in alcohol is reported to be more effective than the aqueous extract [23][10], because alcohol could more easily solubilize phytochemicals, the aqueous extract of paico showed an evident toxic effect at low concentrations. However, it is not as fast-acting as chemicals, but this solution is less polluting to the ecosystems of the Colombian Pacific, easy to produce, low-cost, and also resolves the difficulties of transportation and procurement of inputs by using paico that grows in the region.

When evaluating the effect of paico, it was observed that only at 17.5% did *F. solani* show an effect, possibly due to having a thicker cell wall [24], or because it is a chemotroph and secretes enzymes that help it degrade organic compounds, a characteristic that confers resistance to

phytotoxins. However, it ends up being vulnerable to several of them, such as *Azadirachta indica*, *Carica papaya*, *Allium sativum* bulbs, and *Garcinia kola* seeds [10][25]. Is necessary identify the minimum effective concentration (MEC) of the paico extract that achieves a significant inhibition of the mycelial growth of *Fusarium solani* and *Mycoleptodiscus suttonii*.

5. Conclusions

The Pacific region, with its vast geographical and cultural diversity, presents unique challenges in terms of food security. Pathogens can affect crops both pre-harvest and post-harvest, putting the sustainability and economy of the region at risk.

The combination of morphological and molecular methods is essential for the precise identification of fungi, especially in complex cases such as *Mycoleptodiscus suttonii*, where sequencing of other genes (LSU, TEF, RPB2) is necessary to obtain a more accurate and reliable taxonomic resolution.

It can be concluded that the aqueous extract of paico possesses fungicidal and fungistatic properties, making it an alternative to conventional agrochemicals and promoting ecological agriculture for the production of organic food at a low cost.

New bioassays on the composition of paico are proposed, complemented with gas chromatography analysis for the separation, identification, and quantification of secondary metabolites. This will further elucidate organic control methods.

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Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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