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Research Article

ANTIFUNGAL ACTIVITY OF THREE PLANT EXTRACTS AGAINST *PHYTOPHTHORA MEGAKARYA*, THE CAUSAL AGENT OF COCOA POD BROWN ROT (*THEOBROMA CACAO* L.) IN CÔTE D'IVOIRE

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ABSTRACT. In Côte d'Ivoire, cocoa (*Theobroma cacao* L.) is cultivated for its socio-economic importance as well as its various uses, both nutritional and medicinal. Despite its value, cocoa cultivation is seriously threatened by brown pod rot, a major disease. The commonly used chemical fungicides to control this disease often have harmful effects. In this context, a study was conducted to explore alternatives to chemical control methods. The pathogenicity of *Phytophthora megakarya* was evaluated through wound inoculation on cocoa pods, confirming its pathogenic nature. The antifungal activity of aqueous extracts from three local plants (*Syzygium aromaticum*, *Carica papaya*, and *Ricinus communis*) as well as a synthetic fungicide was assessed *in vitro* against *P. megakarya*, the causal agent of brown pod rot. PDA culture medium supplemented with extract concentrations of 20%, 40%, and 60% was used for the tests. The plant extracts demonstrated antifungal activity, resulting in inhibition of mycelial growth ranging from 46.43% to 100%. Depending on the concentration, the extracts exhibited fungistatic and/or fungicidal effects. Aqueous extracts of *Syzygium aromaticum* and *Carica papaya* completely inhibited mycelial growth at the 40% concentration, similar to the effect of the synthetic fungicide commonly used in conventional agriculture. Notably, *Carica papaya* extract fully inhibited *P. megakarya* mycelial growth even at 20% concentration. These results suggest that such extracts may offer a promising avenue for the control of this pathogen under field conditions, warranting further investigation.

Keywords: *Antifungal activity, Brown pod rot, Phytophthora megakarya*

INTRODUCTION

The cacao tree (*Theobroma cacao* L.) is a plant species native to the humid tropical rainforest, originally from South America, particularly the Upper Amazon region. It is a perennial plant belonging to the family Malvaceae [1]. Cacao is primarily cultivated for its beans, which serve as raw materials for the food, cosmetic, pharmaceutical, and confectionery industries [2]. Indeed, cocoa beans have therapeutic properties that help reduce the risk of cardiovascular accidents and metabolic diseases [3]. Cocoa cultivation is also used in agroforestry systems to prevent soil erosion and thus contributes to environmental conservation [4]. Global cocoa production was estimated at 5.87 million tonnes in 2023, with a significant concentration in West Africa, particularly Côte d'Ivoire, which produced more than 2.2 million tonnes in 2023, ranking it as the world's leading cocoa producer [5]. In Côte d'Ivoire, cocoa plays a crucial role in both the social and economic lives of the population, generating about 46% of the country's export revenues [6]. However, cocoa production is facing numerous constraints that threaten its sustainability. These constraints, both abiotic and biotic, contribute to significant yield losses [7]. The biotic constraints include pest attacks and diseases. Among

these, fungal diseases, particularly black pod disease, currently represent the most destructive threat to cocoa cultivation [8].

Among the pathogens involved, species of the genus *Phytophthora* are the most widespread and damaging, causing yield losses estimated at 20% to 60% [9,10]. *Phytophthora* species infect the plant through natural openings or mechanical wounds caused by human activity or vectors [11]. Infection typically occurs through stomatal penetration, following the germination of zoospores, which release germ tubes. The pathogen secretes cell wall-degrading enzymes and produces toxins that facilitate penetration and weaken the plant's immune defenses [12]. Infected pods undergo rapid degradation, leading to internal necrosis of the beans, rendering them unsuitable for the international market [13]. To control this fungus, farmers often rely on synthetic chemical fungicides, which raise environmental concerns due to their persistence, bioaccumulation, and potential toxicity to living organisms [13]. Consequently, alternative control methods are increasingly being explored. These include the use of biopesticides or biological control agents, which are defined as plant protection products whose active ingredient is a living organism or a derivative there of. They may also consist of naturally derived substances, such as plant extracts or pheromones [14].

Plant extracts are gaining increasing attention as a potential source of bioactive natural compounds. They have demonstrated antifungal activity against a wide range of pathogenic fungi [15]. Indeed, the use of plant extracts is more affordable for farmers, environmentally friendly, and represents a viable alternative to conventional pesticides. However, no comprehensive study has yet been conducted on *Phytophthora* spp. using the three selected plant extracts.

The present study aims to contribute to this field by developing a plant-based biological control method against *Phytophthora* spp., the pathogen responsible for black pod disease in cocoa. More specifically, the objectives were to: Confirm the pathogenic potential the fungus responsible for black pod disease, and evaluate the antifungal activity of three local plant extracts against *Phytophthora megakarya.* Nonetheless, the efficacy of these aqueous extracts needs to be validated *in vivo* to confirm or refute their antifungal potential.

MATERIALS AND METHODS

Plant material

The plant material consisted of asymptomatic cocoa pods used for pathogenicity tests (Fig. 1). Fresh, healthy young leaves of papaya (*Carica papaya*) and castor (*Ricinus communis*) were collected from two farmers' fields located in the towns of Soubré and Agboville. Additionally, dried floral buds of clove (*Syzygium aromaticum*) were purchased at the Adjamé market in the district of Abidjan (Fig. 2). All collected samples (cocoa pods, fresh leaves, and clove flower buds) were transported to the Plant Health Unit Laboratory of the Plant Production Research Hub at Nangui ABROGOUA University, where the in vitro antifungal assays were conducted.

Chemical material

The synthetic chemical fungicide used in this study is officially approved for controlling brown pod rot in cocoa. The product used was CACAOFLA 72WP, a fungicide commonly employed by cocoa producers. CACAOFLA 72WP is a plant protection product based on mancozeb and cymoxanil. It is a contact and systemic fungicide with preventive, curative, and eradicative actions (Fig. 3).

It is approved for the protection of cocoa trees against *Phytophthora* spp. and has the following characteristics:

- Trade name: CACAOFLA 72WP

- Composition: 640 g/kg of mancozeb + 80 g/kg of cymoxanil
- Formulation: Wettable powder (WP)
- Approval: France, Côte d'Ivoire

Fungal material

The fungal material consisted of isolates of *Phytophthora megakarya*, with the accession number PQ157645 [16].

In vitro Experimental Design

The *in vitro* experimental setup for this study was based on two factors. Factor 1 (antifungal product) included three treatments (plant extracts) and one control (synthetic fungicide). The treatments consisted of castor leaf extract (*Ricinus communis*), papaya leaf extract (*Carica papaya*), dried clove flower buds (*Syzygium aromaticum*), and a synthetic fungicide (Mancozeb + Cymoxanil). Factor 2 (product concentration) included three levels: 20%, 40%, and 60%.

Fig. 1. *Cocoa pods collected in the cocoa plantations visited*

Fig. 2. *Plant material used for testing during the study*

A: castor leaves (*Ricinus communis*); B: flower buds (*Syzygium aromaticum*); C: papaya leaves (*Carica papaya*).

Fig. 3. *Packaging of the fungicide CACAOFLA 72WP*

Culturing of Phytophthora megakarya Strains

Preparation of Potato Dextrose Agar (PDA) Medium

Potato Dextrose Agar (PDA) medium was used to subculture fungal strains obtained from the Plant Health Unit of Nangui ABROGOUA University. To prepare one liter of the medium, 200 g of peeled and diced potatoes were boiled in 1 L of water for 30 minutes. After cooling, the broth was collected and supplemented with 20 g of D-glucose and 20 g of agar-agar, which were dissolved completely. The final volume was adjusted to 1 L with distilled water. The medium was then sterilized in an autoclave at 121 °C under 1 bar of pressure for 30 minutes. While still molten (at approximately 45 °C), the PDA medium was poured into Petri dishes under a laminar flow hood in the presence of a Bunsen burner flame [17].

Culturing of Phytophthora megakarya Strains

Phytophthora megakarya fungal strains were subcultured on Pétri dishes containing PDA medium. For this, 0.5 cm diameter mycelial disc was transferred to a fresh PDA-containing Pétri dish. This subculturing aimed to multiply the strains for subsequent pathogenicity testing and evaluation of the antifungal effects of the plant aqueous extracts.

Pathogenicity Test of Phytophthora megakarya Strains

Cacao pods were disinfected with 3% diluted sodium hypochlorite (12°) for 3 minutes and rinsed three times consecutively with sterile distilled water. The pods were then dried on blotting paper. Two superficial wounds were made on the pod epicarp using a sterile scalpel, and a 0.5 cm fungal inoculum was placed directly on each wound and covered with spathes from the pod to prevent external contamination. Control pods were inoculated with a 0.5 cm of PDA medium (Fig. 4). After inoculation, the pods were incubated in sterile plastic containers to maintain humid conditions. These containers were kept in the laboratory at ambient room temperature (25 ± 2 °C) for one week. At the end of this incubation period, symptoms developed on the pods were observed and described.

Evaluation of the Antifungal Activity of Local Plant Extracts on Phytophthora megakarya, the Fungus Responsible for Brown Rot of Cacao Pods

Selection of Plants

Local plants such as castor leaves (*Ricinus communis*), clove flower buds (*Syzygium aromaticum*), and papaya leaves (*Carica papaya*) were used for the antifungal tests. These plants were chosen based on their traditional medicinal uses, including treatment of constipation, relief of joint pain, analgesic and anti-inflammatory properties, as well as healing infections and wounds. Agronomically, they are employed as substances capable of inhibiting mycelial growth of pathogenic fungi and enhancing plant defenses. Indeed, these plants are easily cultivable and adapt well to the agroecological conditions where they grow. Previous studies by Martini et al. [18]. and Soro et al. [19]. have shown that many medicinal plants contain secondary metabolites with broad-spectrum antifungal activities against crop pathogens. However, studies specifically targeting *Phytophthora* are scarce, suggesting potential for further exploration of these plant extracts.

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Fig. 4. *Pathogenicity testing of fungal strains isolated from mature cocoa pods*

Preparation of Plant Extracts and Culture Media

Fresh, green leaves of *Ricinus communis*, *Carica papaya*, and flower buds and seeds of *Syzygium aromaticum* were used for the preparation. The collected leaves and seeds were air-dried at room temperature in the laboratory for 15 days. The dried leaves and seeds were separately ground into powder using a blender. A mass of 100 g of the resulting powder was macerated for 72 hours at room temperature in 1 L of distilled water. After maceration, the solution was filtered using filter paper lined with a layer of hydrophilic cotton placed in a funnel to remove insoluble plant particles, following the method of Guidoum and Salah [20] (Fig. 5). The medium used to test the antifungal activity of the extracts was prepared by incorporating the aqueous plant extract into molten PDA medium. Specifically, 100 ml of medium was prepared as follows: volumes of 20, 40, and 60 ml of extract were mixed with 80, 60, and 40 ml of PDA medium, respectively, to obtain final concentrations of 20, 40, and 60%. These concentrations were selected based on preliminary laboratory tests. Positive control media consisted of PDA amended with a standard fungicide mixture of Mancozeb and Cymoxanil at the recommended

concentration (200 g/ha) for brown rot control. The negative control consisted solely of PDA medium. The prepared culture media were homogenized and poured into 90 mm diameter Pétri dishes at 20 ml per dish.

Fig. 5. *Preparation process for aqueous extracts of local plants A: powdered extracts of* Syzygium aromaticum; *B:* Carica papaya *powder extracts; C:* Ricinus communis *powder extracts*

Antifungal Activity of Aqueous Plant Extracts In Vitro on the Mycelial Growth of Phytophthora megakarya

Culturing Phytophthora megakarya on Media Amended with Plant Extracts and Measurement of Mycelial Colony Growth

A volume of 100 mL of each mixture (PDA + extract) was poured under a laminar flow hood into Pétri dishes, with five replicates per concentration. A 5 mm diameter fungal inoculum, taken from a 7-day-old culture, was placed at the center of each dish to evaluate the efficacy of the aqueous extracts. Positive control media consisted of PDA amended with a standard fungicide, specifically **mancozeb** and **cymoxanil**, at the recommended concentration. The negative control consisted solely of PDA medium. The experiment was repeated three times. The Pétri dishes

were sealed with parafilm and incubated at ambient laboratory temperature for 7 days. The diameter of the *Phytophthora megakarya* mycelial growth was estimated daily by calculating the average of two perpendicular diameters measured on the bottom of the Pétri dish. Measurements were stopped once the negative control medium was fully covered by mycelium.

Determination of the Mycelial Growth Inhibition Rate of Phytophthora megakarya

The measurement of mycelial colony diameters was used to calculate the growth inhibition rate according to the following formula:

$$\mathrm{TI}(\%) = \frac{1}{5}\sum \frac{\mathrm{Dt} - \mathrm{De}}{Dt}$$

Eqn.1

- TI (%) : Inhibition rate of mycelial growth
- Dt (cm) : Diameter of mycelial growth of the control colony
- De (cm) : Diameter of mycelial growth of the treated colony
- 5 : Number of replicates per experiment

The sensitivity scale of Kumar et al. [21] was used to determine the sensitivity of the fungal strain according to the plant extracts and their concentrations (Table 1). For any given concentration of aqueous plant extract where no mycelial growth was observed, the fungal inoculum was transferred onto PDA medium without plant extract. Resumption of growth indicated a fungistatic effect, whereas absence of growth suggested a fungicidal property of the extract [22].

Table 1. *Sensitivity level of fungi as a function of inhibition rate [21]*

Échelle	**Interprétation**
I > 90 %	Highly sensitive (S+)
75 % < I < 90 %	Sensible (S)
60 % < I < 75 %	Moderately sensitive (R-)
40 % < I < 60 %	Résistant (R)
I < 40	Highly résistant (R+)

Statistical Analysis of Collected Data

The collected data were subjected to statistical analysis using RStudio software version 4.3.0. One-way analysis of variance (ANOVA 1) was performed to compare the mean inhibition rates across different concentrations. When a significant difference was observed at the 5% significance level, Fisher's Least Significant Difference (LSD) test was used to identify the optimal concentration. Subsequently, one-way ANOVA 1 was conducted to compare the mean inhibition rates according to the different products. In case of significant differences, the same LSD test was applied to determine the best-performing product.

RESULTS AND DISCUSSION

Pathogenicity of Phytophthora megakarya Strains Isolated from Cacao Pods

The pathogenicity test conducted with the *Phytophthora megakarya* strain on asymptomatic cacao pods resulted in the appearance of brown rot on the inoculated pods. No symptoms were observed on the control pods. The *Phytophthora megakarya* strain caused organ deterioration on

the inoculated pods after 5 days. This deterioration manifested as brown rot with irregular margins on the pods (Fig. 6).

Fig. 6. *Symptoms induced by Phytophthora megakarya, a: Control pod; b: Pod inoculated with Phytophthora megakarya*

Pathogenicity tests conducted on cocoa pods using the *Phytophthora megakarya* strain resulted in the development of brown rot symptoms on inoculated pods. This confirms the pathogenic capability of the tested strain and its aggressiveness as the causal agent of brown pod rot in West Africa. The absence of symptoms on uninoculated pods attests to the specificity of the observed pathogenicity and validates the experimental conditions. These results support the notion that *Phytophthora megakarya* poses a serious threat to cocoa production. Indeed, once the pathogen comes into contact with the pod, it produces spores that penetrate plant tissues through wounds. Inside the host, the pathogen establishes parasitism by secreting toxins and cell wall-degrading enzymes, thereby compromising the host plant's immune responses and leading to symptom development. These findings are consistent with those of Fofana et al. [23], who observed similar brown rot symptoms after inoculation of cocoa leaves with *P. megakarya* strains.

Antifungal Activity of Aqueous Extracts on Phytophthora megakarya

In vitro effect on radial growth of Phytophthora megakarya depending on plant aqueous extracts and chemical fungicide

The mycelial growth of *Phytophthora megakarya* varied according to the plant aqueous extracts, the chemical fungicide, and their concentrations. The different aqueous extracts from each plant and the chemical fungicide reduced the mycelial growth of the pathogen responsible for brown rot of cacao pods. Statistical analyses showed a significant difference between the mycelial growth inhibition rates of *Phytophthora megakarya* depending on the plant extracts, chemical fungicide, and applied concentrations.

No mycelial growth was observed with the aqueous extract of *Carica papaya* at any concentration tested against the pathogenic fungus. In contrast, mycelial growth was observed in the negative control (Fig. 7). Overall, the extracts of *Syzygium aromaticum*, *Ricinus communis*, and *Carica papaya* as well as the chemical fungicide at the 60% concentration completely inhibited the mycelial growth of *Phytophthora megakarya* compared to other concentrations. The sensitivity of *Phytophthora megakarya* varied depending on the aqueous plant extracts tested and their concentrations. The plant extracts demonstrated significant antifungal activity against *P.*

megakarya. This activity may be attributed to the presence of secondary metabolites such as phenols, flavonoids, tannins, saponins, terpenes, and alkaloids within the extracts. Similar to synthetic fungicides, the efficacy of plant extracts is likely related to the bioactive compound they contain. These results align with those of Saighi et al. [24], who tested the effectiveness of plant extracts against fungal pathogens of potato.

In vitro inhibition rate of Phytophthora megakarya mycelial growth according to concentrations of plant extracts and chemical fungicides

The mycelial growth inhibition caused by *Syzygium aromaticum* extract was 73.12% and 93.9% at concentrations of 20% and 40%, respectively, against *Phytophthora megakarya*. The aqueous extract of *Carica papaya* completely inhibited (100%) the mycelial growth of *P. megakarya* at a concentration of 20%. The *Ricinus communis* extract induced an inhibition rate of 99.69% at 60% concentration, while the 20% and 40% concentrations inhibited mycelial growth by 46.43% and 79.91%, respectively. Regarding the synthetic fungicide, it showed complete inhibition (100%) at 40% and 60% concentrations, and 92% inhibition at 20%. Statistical analyses revealed significant differences between the various inhibition rates according to the concentrations of plant extracts and chemical fungicide in PDA medium, forming three homogeneous groups (Fig. 8).

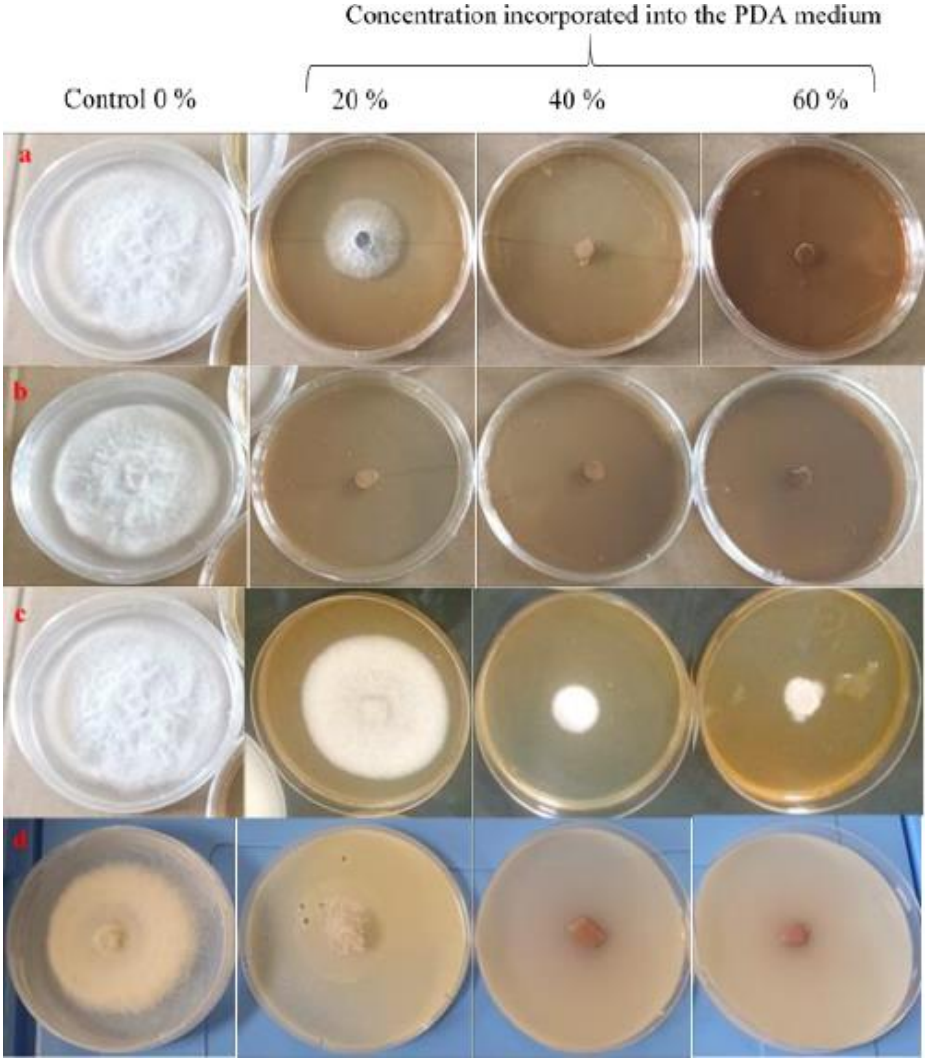

Fig. 7. *Mycelial Growth of Phytophthora megakarya on PDA Medium Amended or Not with Aqueous Plant Extracts and Standard Fungicide, a: Syzygium aromaticum; b: Carica papaya; c: Ricinus communis; d: Standard fungicide, the combination of Mancozeb and Cymoxanil*

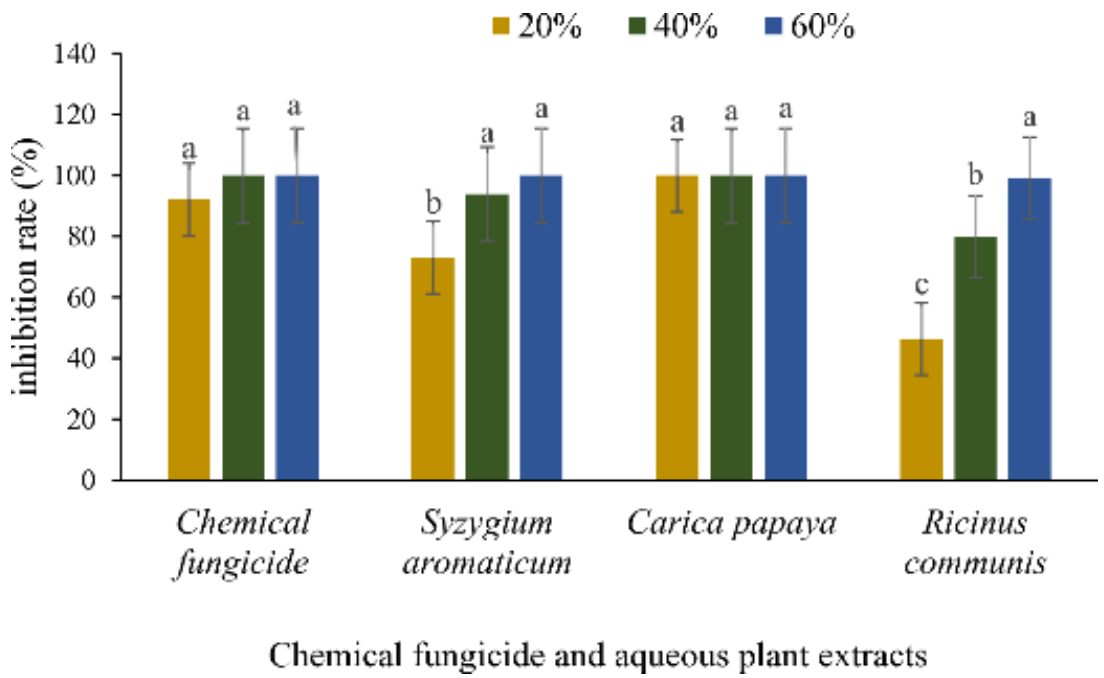

Fig. 8. *Inhibition rate of mycelial growth of Phytophthora megakarya according to concentrations of the chemical fungicide and aqueous plant extracts*

The histograms with the same letter are statistically identical at the 5% threshold according to Fisher's LSD test

The study revealed that aqueous extracts of *Syzygium aromaticum* and *Carica papaya* completely inhibited (*100% inhibition*) the mycelial growth of *P. megakarya* at a 40% concentration, matching the efficacy of synthetic fungicides commonly used in conventional agriculture. Notably, even the lowest tested concentration (20%) of *Carica papaya* extract achieved complete inhibition. This strong inhibition of *P. megakarya* mycelial growth could be explained by the presence of compounds interfering with fungal metabolism either through alteration of the cell wall, disruption of enzyme functions, interference with cellular respiration, or enzymatic inhibition. These findings are consistent with Medfouni et al. [25], who showed that extracts of *Syzygium aromaticum* and *Carica papaya* contain saponins, flavonoids, tannins, and alkaloids, molecules known for their inhibitory effects on fungal cellular structures.

Fungistatic or fungicidal effect of aqueous extracts on Phytophthora megakarya at 60% concentration

On PDA medium without added extract, no mycelial growth was observed from fungal isolates initially treated with the aqueous extracts of *Carica papaya*, *Syzygium aromaticum*, or with the synthetic fungicide at the 60% concentration. This result suggests a fungicidal effect of the *Carica papaya* and *Syzygium aromaticum* extracts against *Phytophthora* sp. In contrast, mycelial growth was observed in isolates initially treated with the *Ricinus communis* extract at the same concentration (60%), indicating a fungistatic effect of this extract on *Phytophthora* sp. (Fig. 9).

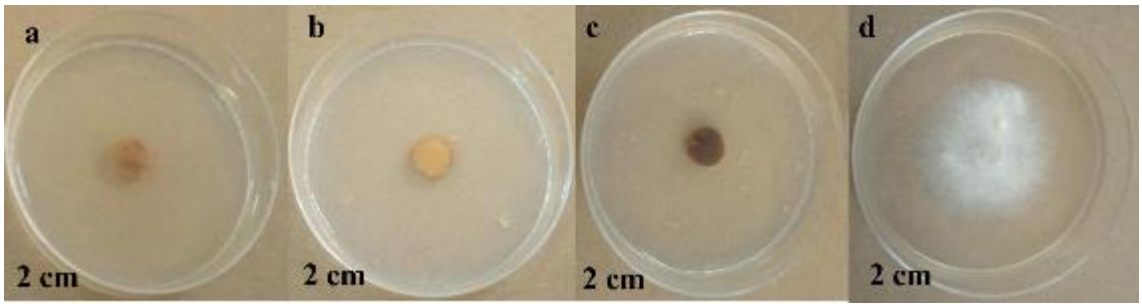

Fig. 9. *Mycelial growth of* Phytophthora megakarya *on PDA medium after culture on medium amended with aqueous extracts of local plants and synthetic fungicide at 60% concentration, a: initially treated with synthetic chemical fungicide; b: initially treated with* Syzygium aromaticum*; c: initially treated with* Carica papaya*; d: initially treated with* Ricinus communis

Overall, the results clearly demonstrate that the tested plant extracts possess antifungal properties against *Phytophthora megakarya*, exhibiting fungistatic or fungicidal effects depending on the concentration applied. Such concentration-dependent variation is characteristic of many plant-derived bioactive substances. These effects can be explained on two levels: for the fungistatic effect, *Ricinus communis* secretes metabolites that disrupt fungal metabolic processes (respiration, membrane permeability, and enzymatic synthesis), temporarily inhibiting mycelial growth without destroying fungal cells. Regarding the fungicidal effect, *Syzygium aromaticum* and *Carica papaya* may secrete substances or enzymes that cause potential destruction of fungal structures, notably hyphae and spores. These findings support those of Kossonou et al. [26], who demonstrated that the antifungal activity of extracts from plants such as *Nesogordonia papaverifera*, *Cola gigantea*, *Triplochiton scleroxylon*, *Trichilia heudelotii*, and *Celtis mildbraedii* was greater at higher concentrations against certain pathogenic fungi. These data are crucial for developing plant-based phytosanitary formulations aimed at effectively controlling *Phytophthora megakarya* while reducing reliance on synthetic chemical products.

CONCLUSION

The results revealed the pathogenicity of *Phytophthora megakarya* on cocoa pods. The aqueous extracts of *Syzygium aromaticum*, *Carica papaya*, *Ricinus communis*, as well as the synthetic fungicide, exhibited significant antifungal activity against *P. megakarya*, the fungus responsible for brown pod rot in cocoa. Indeed, the 60% concentration was effective for all three plant aqueous extracts. The aqueous extracts of *Syzygium aromaticum* and *Carica papaya* completely inhibited the mycelial growth of *Phytophthora megakarya* starting at 40% concentration, similarly to the synthetic fungicide *in vitro*. Notably, the *Carica papaya* extract fully inhibited mycelial growth even at 20% concentration. This extract could be used for field management of this pathogen to confirm its efficacy.

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