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Original Article

Evaluation of the Inhibitory Effect of Extracts of two Medicinal Plants on the Development of *Phytophthora megakarya* in *Theobroma cacao*

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Abstract *- The production of cocoa (Theobroma cacao) is threatened by the attack of pathogens, the most aggressive of which is Phytophthora megakarya. The study of the inhibitory effects of Ocimum basilicum and Psidium guajava leaf extracts on growth in vitro and in vivo conditions would be necessary to deal with this pathogen. Thus, the aqueous and ethanolic extracts of the two plants at different concentrations and slices of mycelium 5 mm in diameter were used. The in vivo sensitivity of the pathogen to the extracts was carried out by innoculating an inoculum/extract complex with a volume of 20 µL in the cavities of the pods. The means obtained were separated by Duncan's multiple tests at the 5% probability threshold. The results obtained show that the ethanolic extracts of plants under in vitro conditions completely inhibit the mycelial growth of the pathogen at a concentration of 4mg/mL and more. In contrast, the aqueous extracts of basilicum and guajava reduce their growth by 49, 53% and 32.5%, respectively, at a concentration of 8 mg/mL. The pods inoculated with the aqueous extract of basilicum at a concentration of 4mg/mL show no sign of necrosis, as well as those with ethanolic extracts of guajava and basilicum at a concentration of 6 mg/mL after six days of inoculation. These extracts have been shown to be effective and can, therefore, be used as an alternative in the control of brown rot of cocoa pods.*

Keywords *- Antifungal properties, Ocimum basilicum, Phytophthora megakarya, Plant extracts, Psidium guajava, Theobroma cacao.*

1. Introduction

The cocoa tree (*Theobroma cacao* L.), native to the lowland rainforest of the Amazon basin, is an important cash crop in West and Central Africa. This evergreen tropical plant domesticated over 1,500 years ago, is grown in more than 50 countries around the world to boost its economic power. Several producing countries seek the cultivation of this plant because of its benefits for the cosmetics and chocolate industries, not to mention the cocoa butter used in confectionery, chocolate, perfumery and pharmacy. Moreover, other secondary products are used, such as cocoa liquor, pods and shells, which are used respectively in the production of soft drinks and alcohol and in animal feed or after decomposition, constitute amendments for crops to improve their production [1,2].

Cocoa production in this African region represents around 70% of world cocoa production. Its cultivation has a high yield potential in the absence of environmental constraints and parasitic attacks. However, its production, being mostly ensured by smallholder farmers, faces unfavorable environmental conditions such as unsuitable soil-type genetic material. Likewise, other production constraints, such as the appearance of diseases and insects, lead to annual crop losses between 30 to 80% of world production, thus limiting the expression of this potential [3-6].

Cocoa production around the world is particularly threatened by three major fungal infections: brown pod rot, "witches' broom" disease and pod maniliosis [7,8]. However, brown pod rot, caused by species of *Phytophthora palmivora* and *Phytophthora megakarya*, is the most dangerous of all. In Central and West Africa and particularly in Cameroon, *Phytophthora megakarya* is the most aggressive pathogen of cocoa, frequently causing total loss of pods due to the large number of zoospores produced by the species [7,9,10]. In Cameroon, about 80% of production losses are caused by *P. megakarya* [11-13]. To remedy the attacks orchestrated by this pathogen, farmers most often use chemical fungicides. However, the latter are very expensive for small producers who use them in an illegal way, resulting in water pollution and a health risk when applying these products [14,15].

Genetic control aimed at developing disease-tolerant hybrids often proves to be a sustainable remediation strategy. However, despite the effectiveness of this technique, the establishment by hybrid growers for resistance to brown pod rot is still very low, coupled with

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the presence of aging plantations [16]. Another recourse advocating the protection of the environment is biological control, which consists of identifying microorganisms indigenous to cocoa plantations with antagonistic effects to the pathogen. With this in mind, Kebe et al. (2009) showed that *Trichoderma sp* isolates had a fungistatic and fungicidal effect in mixed culture with *Phytophthora palmivora* [17]. In the same year, Hanada et al. (2009) showed that *Trichoderma martiale* ALF 247 strains had an antagonistic effect on *Phytophthora palmivora*, and their conidia were resistant to fungicides such as copper hydroxide and fosetyl-Al [18]. Later, Tchameni et al. (2017) found that *Trichoderma asperellum* strains not only had antagonistic effects against *Phytophthora megakarya* but also promoted increased plant height and shoot weight and facilitated phosphorus uptake. However, the inoculation of these different strains in farmers' cocoa plantations is still not effective [19].

An approach by application of biopesticides based on plant extracts could be a much more ecological, economical and effective alternative based on the antifungal properties of certain medicinal plants. The antifungal properties of certain plants have been proven. Callistemon viminalis, for example, has antifungal activity against *Phytophthora infestans*. Djeugap et al. (2011) showed that the acetone extract from the leaves of this plant has strong antifungal activity under in vivo conditions against black nightshade downy mildew [20]. The extract of *Thevetia peruviana* seeds with acetone at a dose of 12.5 mg/mL inhibits the radial growth of *Phytophthora colocasiae* under in vitro conditions [21]. The utilization of the antifungal properties of plants turns out to be a promising one, respecting the principles of sustainable development, especially with the fact that it has no major impact on the environment. It is in this perspective that we propose to evaluate the aqueous and ethanolic extracts of two medicinal plants (*Psidium guajava* and *Ocimum basilicum*) on the growth of *Phytophthora megakaria*, the most aggressive causative agent responsible for brown rot of cocoa pods.

This study aims to contribute to the tolerance of the cocoa tree against the pathogenic agent *Phytophthora megakarya*, responsible for the brown rot of cocoa pods by the use of biological control from plant extracts. The results obtained will make it possible to evaluate the activity of aqueous and ethanolic extracts of *Ocimum basilicum* and *Psidium guajava* on the growth and development of *P. megacarya* in vitro and in vivo conditions.

2. Materials and Methods

2.1. Plant and Chemical Material

The plant material consists of hybrid genotypes resulting from the direct crossing of the parental genotypes ICS40 X UPA134 obtained after germination of the seeds in the nursery in the greenhouse of the Biology and Physiology of Plant Organisms laboratory of the University of Douala. During this work, cocoa pods of 4-month-old hybrid genotypes harvested from a plantation in Nkongsamba, a cocoa production area of Cameroon, were used. For plant extracts, *Ocinum basilicum* leaves were obtained from a market in Douala, while *Psidium guajava* leaves were harvested from a young three-year-old guava tree. As for the chemical pesticide, the product Ridomil Gold Plus 66 WP, whose active ingredient consists of Mefenoxame (6%) and Copper Oxide (60%), was purchased in a store selling phytosanitary products in Douala.

2.2. Isolation, Purification and Identification of Phytophthora Megakarya

The isolation of the *Phytophthora megakarya* strain was carried out according to the method of Mychiang et al. (2014), with some modifications to the concentration of sterilants [22]. Three cocoa pods showing signs of brown rot were harvested from the field and brought to the laboratory. The surface of the pods was previously washed with tap water. These pods were then subjected to a series of disinfection in 95% ethanol for 30 seconds. In 10% sodium hypochlorite for 2 minutes and finally in 70% ethanol for 2 minutes so as to eliminate the microorganisms present on the cortex. The pods were then rinsed three times with sterile distilled water to remove traces of disinfectant. The sampling area was chosen, and the superficial tissues were stripped using a sterile scalpel. Ten cubic-shaped pieces of 5 to 7 mm on a side were taken per pod in the subcortical tissues. The fragments taken were cultured on PDA medium contained in petri dishes. The growth of the mycelium in the petri dishes was then followed by a series of transplanting of this pathogen into new dishes containing the PDA medium in order to ensure its purification. Agar disks 5 mm in diameter were taken from boxes of 5 days of growth. Each disc was then placed in the center of the new dishes and incubated under the same conditions as before. This operation was carried out until a pure strain was obtained. The identification of the strain was made by visual observation of the colony in culture, by standard text-assisted microscopy and followed by a pathogenicity test [23].

2.3. Preparation of Plant Extracts

The procedure was carried out using the method of Wuthi-udomlert et al. (2010) [24]. A batch of leaves of each species was dried at room temperature (25-28°C) for 5 days, then placed in an oven at 50°C for 5 hours. One hundred grams of each sample was ground to powder in an electric mixer, and the resulting powder was transferred to reformable and labeled vials. Then 50g of each powdered sample was mixed with 200 mL of deionized distilled water for the aqueous solution and 200 mL of ethanol (70%) for the ethanolic solution in a 500 mL conical flask, then stoppered with sterile cotton and shaken at 120 rpm for 24 hours.

After shaking, the resulting solutions were filtered through four plies of muslin cloth. The residues were again extracted in the same way, and the two extracts were mixed and filtered with filter paper called Whatman n°1. All the extracts obtained were kept in an oven at 45°C until drying, and the powder was collected in a closable bottle [25].

2.3.1. Preparation of Different Concentrations of Extracts, PDA/Extract Complexes and Confrontation with Phytophthora Megakarya.

Ten grams of powder or paste of each extract were mixed with 100 mL of original solvent (ethanol or water) in a 250 mL Erlenmeyer flask, then stirred at 120 rpm for 2 hours, and the stock solutions of concentration Co= 100 mg/mL were obtained. Each stock solution was diluted with its original solvent to obtain four initial daughter solutions of concentrations 80 mg/mL, 60 mg/mL, 40 mg/mL, and 20 mg/mL for each extract. After preparation and sterilization of the culture medium (PDA), it was conducted in a horizontal laminar flow hood as well as the initial daughter solutions previously obtained for the preparation of the inhibition complexes. Five milliliters of the initial daughter solution (80 mg/mL, 60 mg/mL, 40 mg/mL, or 20 mg/mL) of each plant extract after sterilization were taken and introduced into a 250 mL Erlenmeyer flask, and the volume adjusted to 50 mL with PDA medium and then homogenized. The negative control was obtained by replacing the initial daughter solution with sterile distilled water. This is how the final daughter concentrations of C1 = 8 mg/mL, C2 = 6 mg/mL, C3 = 4 mg/mL, and C4 = 2 mg/mL were obtained respectively from the initial daughter solutions of concentrations 80 mg/mL, 60 mg/mL, 40 mg/mL, and 20 mg/mL. The daughter solutions obtained were then poured into petri dishes 90 mm in diameter at the rate of 12 mL of solution per dish with a repetition of 4 dishes per concentration, i.e. 33 treatments and 132 petri dishes. After solidification, mycelium discs 5 mm in diameter taken from five-day culture dishes was introduced into the center of each dish with the part of the mycelium facing downwards, and this at the rate of one disc per dish. These dishes were then wrapped in cling film and incubated in the dark at room temperature.

2.4. Preparation of the Positive Control and Evaluation of the Antifungal Activity of each Treatment

Referring to the dose prescribed by the manufacturer (50 g/15 L of water), 0.166 g of Ridomil Gold Plus was weighed, introduced into 50 mL of PDA medium and the whole was homogenized to obtain a solution of 3.32 mg/mL. The mixture was then poured into 4 Petri dishes, and the mycelium seeded after cooling. The antifungal activity of the leaf extracts of each treatment was evaluated from the percentage inhibition of each treatment against the pathogen. For this, a daily measurement of the diameter of the radial growth of each explant cultured was made until the mycelium had filled the control dishes. The radial growth of the inoculum was therefore evaluated by measuring two perpendicular diameters traced to the Aquarius of the petri dish. The average of these two diameters, subtracted from the diameter of the explant, represented the measurement of the radial growth of the mycelium [21].

$$Dxi = \frac{D1 + D2}{2} - D$$

Where;
Dxi = radial growth diameter of the mycelium;
D0 = explant diameter;
D1 and *D2* = diameters of the culture measured in the two perpendicular proportions of the petri dish.
The percentage inhibition (I%) of each extract was evaluated according to the formula described by Dohou et al. (2004) [26].

$$I\% = \frac{Dt0\ m - Dxi\ m}{Dt0\ m} \times 100$$

I% = inhibition;
Dt0 m = average diameter of the control batch;
Dxi m, the average diameter of the batches in the presence of the extract.

2.4.1. Harvesting and Packaging of Healthy Pods

The unripe four-month-old unripe cocoa pods and healthy were harvested were harvested first thing in the morning in an experimental field located in Nkongsamba. The pods were then packaged in a plastic bag and sprinkled with water, then taken to the laboratory.

2.4.2. Design of Notches on Pods

Once in the laboratory, the cocoa pods went through a series of disinfection. They were first carefully washed with tap water, then sterilized with alcohol at 70° for two minutes and finally rinsed with distilled water. These cocoa pods were left to rest for three hours. Then, three holes were made on each pod, respectively at the upper end, in the middle and at the lower end of the pod in an equidistant manner using a cookie cutter one centimeter in diameter and for a depth of 0.7 cm. The thickness of the removed fragments was reduced by half to serve as a lid [27].

2.4.3. Preparation of Phytophthora Megakarya Inoculum

Petri dishes containing *Phytophthora megakarya* mycelia of one week of growth were used for the extraction of the inoculum according to the method described by (Ajayi, 2019) [27]. Twenty milliliters of distilled water were added to each dish, and the mycelia scraped off using a sterile spatula. The resulting mixture was subsequently strained through a muslin cloth to remove entangled agar fragments and masses of mycelium. The filtrate obtained consists of sporangial and chlamydiospore suspensions [21].

2.4.4. Preparation of Inoculum/Extract Solutions and Infection of Pods

A volume of 10 mL of inoculum/plant extract complex was prepared for each concentration of extract from 5 mL of inoculum and 5 mL of extract. The complex obtained was homogenized and left to stand for 5 hours. For the positive and negative controls, the extracts were replaced by 5 mL of Ridomil gold solution or 5 mL of sterile distilled water, respectively. The infection of the pods was then carried out by depositing 20 μL of each complex in the wells present on the pods using a micropipette. These wells were covered by the mesocarps previously removed. Thus, the same pod was infected with the same complex solution three times in a row for each treatment. Only the pods receiving the control

complexes were represented 8 times for a total of 48 pods. The infected pods were labeled and placed in plastic basins containing paper towels soaked in sterile distilled water at the rate of 6 pods per basin, including 4 pods representing the different concentrations of the same type of extract and 2 pods representative of controls. These basins were then covered with dark plastic and incubated in the dark at room temperature.

2.4.5. Evaluation of the Inhibition of the Infection of Each Treatment

The data was collected by taking the diameters of the radial growths of the necrosis on the pods, as in the case of the *in*-vitro studies. Then, the different diameters were converted into necrotic surfaces from the following formula:

$$S = \frac{D \times d \times \pi}{4}$$

Where S represents the surface of the necrosis, D is the diameter of the necrosis in the direction of the width of the pod, and d is the diameter of the necrosis in the direction of the length of the pod.

2.5. Statistical Analysis

The data collected were transferred to Microsoft Excel software for the calculation of the percentages of inhibition of mycelial growth, and the surfaces of the necrosis observed on the various treatments were subjected to the Analysis of Variance (ANOVA) from statistical data analysis software SPSS version 22. The means obtained were separated by Duncan's multiple tests at the probability threshold of 5%.

3. Results

3.1. In vitro studies

3.1.1. Effect of Plant Extracts on the in vitro Growth of Phytophthora Megakarya

The plant extracts used variably inhibited the radial growth of *Phytophthora megakarya*. Figure 1 shows the appearance of mycelial diameters in culture at different concentrations. Thus, the percentage of mycelial inhibition increased with the increase in the concentration of the extracts to become total at higher concentrations (Tables 1 and 2).

The ethanolic extracts of the two plants completely inhibited mycelial growth at the concentration of 4 mg/mL, whereas total inhibition was not obtained with the aqueous extracts. On the other hand, mycelial growth was significantly rapid in the dishes that had received no treatment (negative control) compared to the different concentrations of the extracts tested (Figure 1).

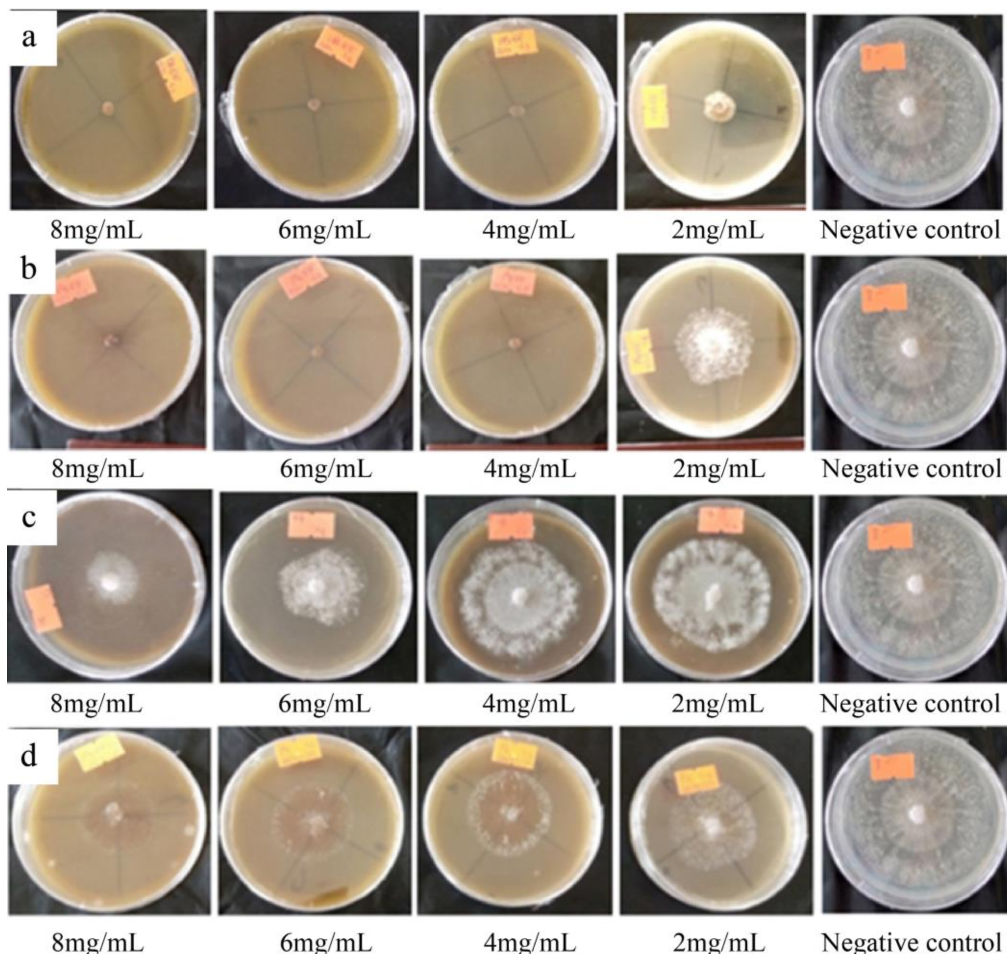

Fig. 1 Inhibitory activity of ethanolic extracts of *Ocinum basilicum* (a) and *Psidium guajava* (b), and aqueous extracts of Ocinum *basilicum* (c) and *Psidium guajava* (d)

Table 1. Percentage inhibition (%) of aqueous extracts of *Ocinum basilicum*, *Psidium guajava* and synthetic fungicide (Ridomil Gold plus) on the In vitro mycelial growth of *Phytophthora megakarya*

Concentrations / Plantes		**Inhibition of mycelial growth / Day after inoculation**		
2mg/mL		2	4	6
	O. basilicum	61,81f	23,26d	31,87d
	P. guajava	54,54g	8,76e	23,12e
4mg/mL	*O. basilicum*	65,45ef	32,32c	35,31c
	P. guajava	67, 27f	11,17e	21,87e
6mg/mL	*O. basilicum*	69,09de	51,66b	48,9b
	P. guajava	74,54cd	21,45d	22,18e
8mg/mL	*O. basilicum*	80c	56,79b	49,53b
	P. guajava	87,27b	21,45d	32,5cd
Ridomil Gold Plus		100a	100a	100a

Note: Means in the same column followed by identical letters do not show significant differences according to Duncan's test at the 5% probability threshold.

Table 2. Percentage inhibition (%) of ethanolic extracts of *Ocinum basilicum*, *Psidium guajava* and synthetic fungicide (Ridomil Gold plus) on mycelial growth of *Phytophthora megakarya*

Concentrations / Plants		**Inhibition of mycelial growth/ Day after inoculation**		
		2	4	6
2mg/mL	*O. basilicum*	100a	83,08b	90,78b
	P. guajava	100a	62,83c	66,09c
4mg/mL	*O. basilicum*	100a	100a	100a
	P. guajava	100a	100a	100a
6mg/mL	*O. basilicum*	100a	100a	100a
	P. guajava	100a	100a	100a
8mg/mL	*O. basilicum*	100a	100a	100a
	P. guajava	100a	100a	100a
Ridomil Gold Plus		100a	100a	100a

Note: Means in the same column followed by identical letters do not show significant differences according to Duncan's test at the 5% probability threshold.

3.1.2. Effect of Aqueous Plant Extracts on the in Vitro Growth of Phytophthora Megakarya

The aqueous extract of *Psidium guajava* was the least effective. The highest percentage inhibition of it one week after incubation was less than 40% (32.5%), even at the highest concentration of 8 mg/mL of the extract (Table 1). However, the aqueous extracts of the leaves of *Ocinum basilicum* performed better than those of *Psidium guajava*, with an average diameter of mycelial growth smaller (4.03 cm) and, by extension, a greater percentage of inhibition (49.53 %) after 6 days of incubation (Table 1).

3.1.3. Effect of Ethanolic Plant Extracts on the in vitro Growth of Phytophthora megakarya

The ethanolic extracts of the two plants, compared to the aqueous extracts, showed a much stronger inhibitory activity of mycelial growth. The latter was only observed in the boxes containing the treatments with extracts at a concentration of 2mg/mL for the two plants (Figure 1). Nevertheless, at this concentration, the extracts of the leaves of *Ocinum basilicum* were more inhibitory, with a percentage inhibition rate of 90.78% after one week of incubation (Table 2).

3.1.4. Verification Test of the Fungicidal or Fungistatic Properties of the Aqueous and Ethanolic Extracts of the Two Plants Studied

One week after the incubation of the various treatments, there was no growth in the dishes inoculated with the ethanolic extracts at concentrations 4:6 and 8mg/mL for the two plants studied.

Once the mycelial discs had been recovered from the various treatments and transplanted into the PDA without additives, a resumption of mycelial growth was observed in all the transplanted dishes (Figure 2).

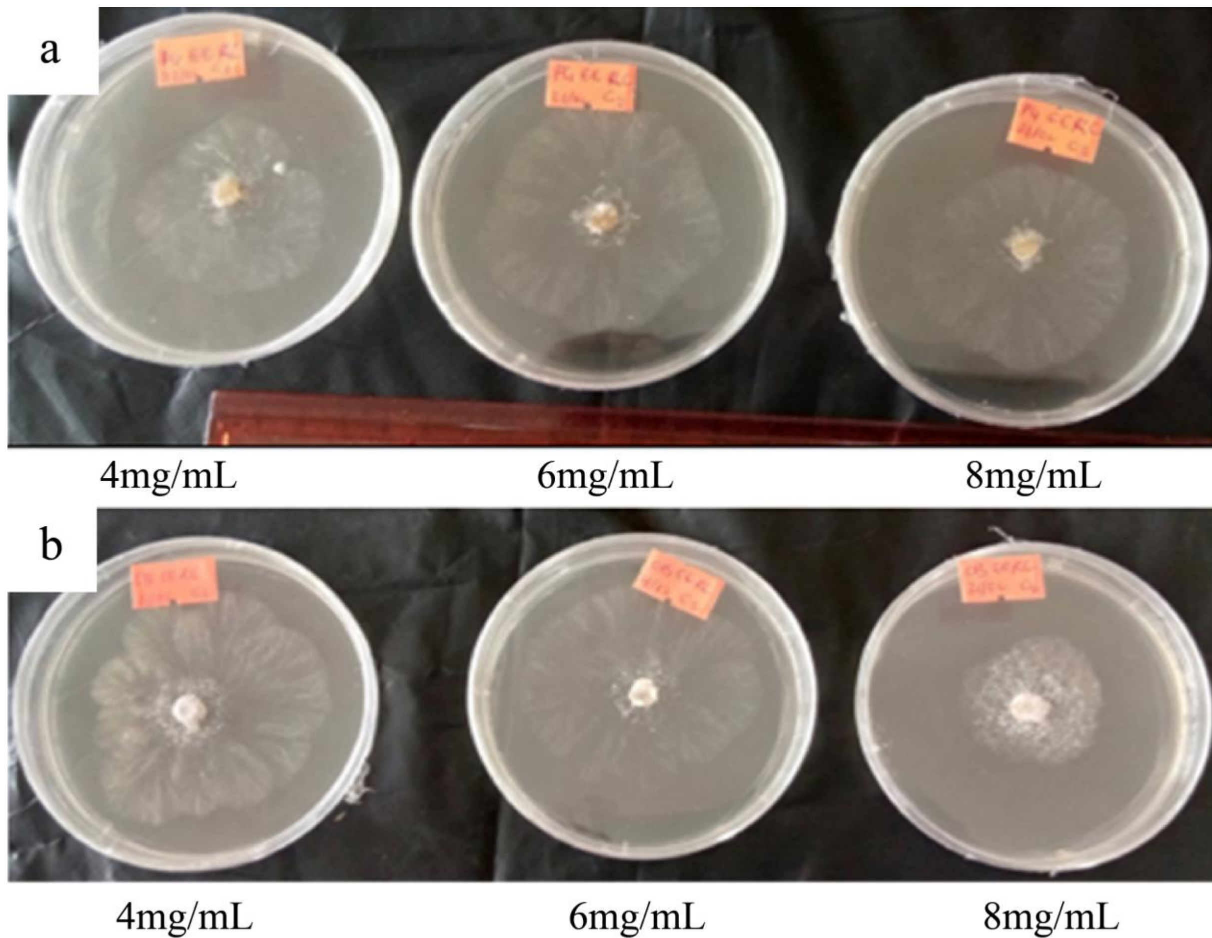

Fig. 2 Resumption of mycelial growth of explants inhibited by ethanolic extracts of leaves of *Psidium guajava* (a) and *Ocinum basilicum* (b) on PDA medium after 6 days of incubation

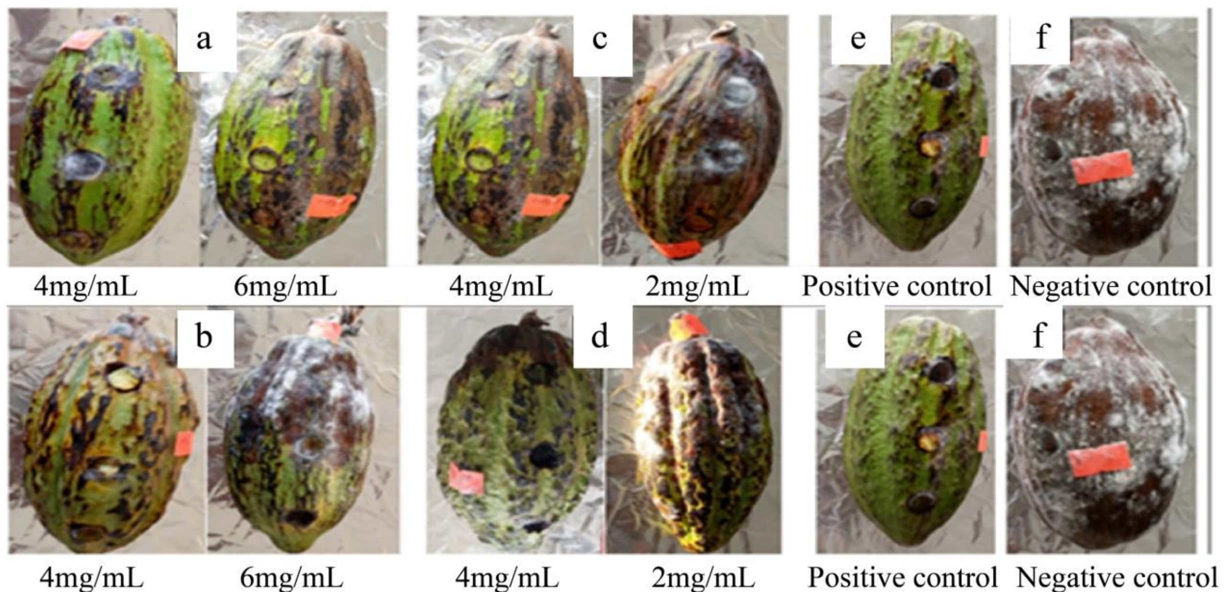

Fig. 3 Development of necrosis on pods inoculated and treated with ethanolic extracts of *Ocinum basilicum* (a) and *Psidium guajava* (b), and aqueous extracts of *Ocinum basilicum* (c) and *Psidium guajava* (d) and positive (e) and negative (f) control treatments after 6 days of incubation

3.2. In vivo Studies

The antifungal activity of the two plants was also evaluated from the development of necrosis on the detached pods.

3.2.1. Effect of Plant Extracts on the in Vivo Development of Black Pod Disease on Detached Pods

The results of the *in* vivo studies on the detached cocoa pods showed a great improvement in the percentage of inhibition of the aqueous extracts and, consequently, a

strong reduction in the necrotic surfaces after 6 days of incubation. Signs of necrosis were observed on pods treated with low concentrations of extracts (Figure 3).

The analysis of variance showed that at concentrations 2 and 4 mg/mL, the necrotic surfaces obtained were significantly lower than those obtained on the control treatment (Table 3). This suggests that at these concentrations the extracts of the two plants could slow down the rate of development of the disease without, however, completely inhibiting it. For all the treatments tested, complete inhibition was obtained from a concentration of 6mg/mL, except for the aqueous extract of *Ocinum basilicum,* which was effective at a concentration of 4 mg/mL. At high concentrations, these extracts would have antifungal properties similar to the chemical fungicide.

Table 3. Mean surfaces (cm²) of mycelial necrosis on the pods at different concentrations of aqueous and ethanolic extracts of *Ocinum basilicum*, *Psidium guajava*, synthetic fungicide (Ridomil Gold plus) and negative control

		Inhibition of mycelial growth, 6 days after inoculation	
Concentrations / Plants		**Aqueous extracts**	**Ethanolic extracts**
2mg/mL	*O. basilicum*	11,77c	2,94c
	P. guajava	25,74b	26,06b
4mg/mL	*O. basilicum*	0d	0,86c
	P. guajava	1,17d	0,60c
6mg/mL	*O. basilicum*	0d	0c
	P. guajava	0d	0c
8mg/mL	*O. basilicum*	0d	0c
	P. guajava	0d	0c
Ridomil Gold plus		0c	0d
Control		32,38a	32,38a

Note: Means in the same column followed by identical letters do not show significant differences according to Duncan's test at the 5% probability threshold.

4. Discussion

The studies on the potential inhibitory activity of aqueous and ethanolic extracts of two medicinal plants (*Ocimum basilicum* and *Psidium guajava*) under the *In* vitro and *In* vivo conditions were carried out within the framework of the improvement of the tolerance of cocoa to black pods disease caused by the pathogen *Phtophthora megakarya*.

4.1. In vitro studies

The results of *in* vitro studies on the inhibition of mycelial growth of *Phtophthora megakarya* showed not only that the aqueous and ethanolic extracts of the two plants differ in their inhibition of mycelial growth but also that the type of solvent (ethanol or water) is a determining element in the effectiveness of inhibition. These results are similar to those obtained by Mbarga et al. (2021), who showed that the screening of the antimicrobial activity of aqueous and ethanolic extracts of some medicinal plants from Cameroon and the evaluation of their synergy with common antibiotics against multi-resistant uropathogenic bacteria differ depending on the solvents [28]. They obtained a strong inhibition of the ethanolic extracts compared to the aqueous extracts, reflecting a better extraction of the antimicrobial compounds. On a general aspect, the percentage of inhibition of the mycelial growth increased with the increase of the concentration in extract, whatever the type of solvent used (water or ethanol) and decreased with the increase of the days following the inoculation. , except for the ethanolic extracts of the two plants at high concentrations. These results are also similar to those obtained by Daniel et al. (2015) when studying the *In* vitro effects of *Allium sativum* extracts on pathogenic fungi of apple, which showed complete inhibition of the pathogen *Botrytis cinerea* at high concentrations of 80% and 60% respectively of the aqueous extracts and ethanol from *Allium sativum* [29].

4.1.1. Effect of aqueous plant extracts on the in vitro growth of Phytophthora megakarya

The aqueous extract of *Psidium guajava* was the least effective. The highest percent inhibition of it at one week after incubation was less than 40% (32.5%), even at the highest concentration of 8mg/mL of the extract. These results are similar to those obtained by Djeuani et al. (2014), who worked on the evaluation of the antifungal activity of *P. guajava* leaf extracts on *Pythium myriotylum* Drechsl, a fungus endemic to Cameroonian soil causing macabo root rot (*Xanthosoma sagitttifolium*) and obtained a percentage also mediocre mycelial growth inhibition (34.18%) with the aqueous extracts under In vitro conditions after 15 days of incubation [30].

However, the aqueous extract of the leaves of *Ocimum basilicum* performed better than that of *Psidium guajava*, with an average diameter of mycelial growth smaller (4.03 cm) and, by extension, a greater percentage of inhibition (49. 53%) after 6 days of incubation. These results are in agreement with the remarks made by Nugroho et al. (2019), who obtained an effective inhibition of the mycelial growth of *Sclerotium rolfsii* (causative agent of damping-off in tomato plants) with aqueous extracts of *Ocimum basilicum* in the confrontation in petri dishes [31]. Similar observations were made on the growth of *Fusarium oxysparum*, this time with complete inhibition of mycelial growth by aqueous extracts of this same plant at a concentration of 15% [32].

4.1.2. Effect of Ethanolic Plant Extracts on the In vitro Growth of Phytophthora Megakarya

The ethanolic extracts of the two plants, compared to the aqueous extracts, presented a much more advanced inhibitory activity of the mycelial growth. The latter was only observed in the boxes containing the treatments with extracts at a concentration of 2 mg/mL for the two plants. Nevertheless, at this concentration, the extracts of the leaves of *Ocimum basilicum* were more inhibitory, with an average diameter of the mycelium of 0.73 cm and a percentage inhibition rate of 90.78% after one week of incubation. These results corroborate with the observations made by Ahmed et al. (2016), who found that the ethanolic extract of *Ocimum basilicum* caused a large zone of inhibition of Gram+ and Gram- bacteria and the fungus *Candida albicans* in a petri dish, and with a progressive increase in inhibition which was proportional to the gradual increase in the concentration of extract [33]. Studies by Yahaya et al. (2019) go in the same direction, showing the antimicrobial activity of ethanolic and aqueous extracts of the leaves and bark of *Psidium guajava* on Staphylococus aureus with a preponderant inhibition of ethanolic extracts [34].

Several studies have been conducted on the phytochemical analysis of medicinal plant extracts. The methanolic extracts of *Psidium guajava*, for example, are rich mainly in polyphenols, including phenols, flavonoids, tannins and other compounds such as alkaloids, anthraquinones, amino acids, reducing sugars and others [35,34]. In addition, phytochemical analysis of *Ocimum basilicum* leaves shows the presence of flavonoids, alkanoides, saponins, steroids, and terpenoids and the absence of tannins [36,37]. These phytochemicals would likely cause the antimicrobial properties of these plants.

4.1.3. Verification Test of the Fungicidal or Fungistatic Properties of the Aqueous and Ethanolic Extracts of the Two Plants Studied

Mycelial growth resumes once the inhibited explants are transferred to a new culture medium free of plant extracts and fungicide. This would reflect the fungistatic properties of the extracts studied instead of fungicides [38]. Plant extracts, being organic matter, are easily degradable. As they degrade, their antifungal property decreases. This can be beneficial, as the accumulation of phytochemicals in soil and produce is excluded [27].

4.2. In vivo Studies

4.2.1. Effect of Plant Extracts on the In vivo Development of Brown Rot on Detached Pods

The results of the In vivo studies on the detached cocoa pods showed a great improvement in the percentage of inhibition of the aqueous extracts and, consequently, a strong reduction in the necrotic surfaces after 6 days of incubation. Signs of necrosis were observed on pods treated with low concentrations of extracts. The analysis of variance showed that at concentrations of 2 and 4 mg/mL, the necrotic surfaces obtained were significantly lower than those obtained on the control treatment. This suggests that at these concentrations the extracts of the two plants could slow down the rate of development of the disease without, however, completely inhibiting it. Similar results were obtained by Essome et al. (2020) during studies on the evaluation of the antifungal activities of *Thevetia peruviana* seed extracts against *Phytophthora colocasiae* (Oomycetes) causative agent of taro downy mildew (*Colocasia esculenta* (L.) Schott) in Cameroon [21].

For all the treatments tested, complete inhibition was obtained from a concentration of 6 mg/ml, except for the aqueous extract of *Ocimum basilicum,* which was effective at a concentration of 4 mg/mL. At high concentrations, these extracts would have antifungal properties similar to the chemical fungicide. This is in line with the results obtained by Simo et al. (2019), who used aqueous and ethanolic extracts of pepper (*Capsicum annum*) on the development of necrosis caused by *Phytophthora megakarya* in *Theobroma cacao* and Son et al. (2018) who used aqueous extracts of *Ocimum basilicum* on the growth of *Fusarium oxysporum*, the causative agent of crown and root rot of tomatoes [38,32].

5. Conclusion

The objective of this work was to evaluate the fungal activity of aqueous and ethanolic extracts of *Ocinum basilicum* and *Psidium guajava* in *Theobroma cacao* against *Phytophthora megakarya in v*itro and *in* vivo conditions. The results obtained showed that the ethanolic extracts completely inhibit the mycelial growth of *Phytophthora megakarya* at a concentration of 4mg/mL in the same way as the synthetic fungicide with an inhibition percentage of 100%. On the other hand, the aqueous extracts do not completely inhibit the mycelial growth. However, considerably reduce the mycelial diameter to 4.03 cm and 5.4 cm with respectively the extracts of *Ocinum basilicum* and *Psidium guajava,* thus showing percentages of inhibitions respectively of 49.53% and 32.5% at the high concentration of 8mg/mL. However, in vivo studies do not show necrosis on pods treated with concentrations of 6 and 8 mg/mL for the two types of extracts in the two plants. The aqueous extract of *Ocinum basilicum* stood out with 100% inhibition from 4mg/mL of concentration on the pods. The extracts studied thus proved to be active against *Phytophthora megakarya* and can, therefore, constitute an alternative in the fight against brown rot in cocoa pods.

Authors' Contribution

SIMO Claude designed the study; BEKELE James Wheatstone collected samples, performed experiments, analyzed results and wrote the first draft of the manuscript; TENE Eric Romuald critically reviewed the first draft; ASSENG Charles Carnot critically reviewed the second draft. All authors read and made input to the final draft.

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