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Assessing the Efficacy of Lemongrass (*Cymbopogon citratus*) and Sambong (*Blumea balsamifera*) Extracts in Combating Black Pod Disease: Sustainable Solutions for Controlling *Phytophthora megakarya* in Cameroon's Cocoa Plantations

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Abstract

The use of plant extracts as antifungal agents is gaining increasing attention, particularly for the control of black pod disease in cocoa. Despite extensive research, current strategies haven't been entirely effective. This study evaluated the effectiveness of *Cymbopogon citratus* and *Blumea balsamifora* leaf extracts, both individually and in combination, against *Phytophthora megakarya*. We assessed the efficacy of the most promising combination (75% *B. balsamifera*, 25% *C. citratus*) after storage at room temperature for up to 9 days. Agar microdilution and *in vivo* bioassays were conducted to determine antifungal susceptibility and effectiveness. *Blumea* extract exhibited the highest overall inhibitory activity, with the lowest minimum inhibitory concentration (117 µl mL^{-1}) while *C. citratus* had a narrower range of MIC (146 to 233 µl mL^{-1}). The combination of *C. citratus* and *B. balsamifera* demonstrated a synergistic effect against *P. megakarya*, achieving growth inhibition on V8 media (92.72 ± 4.20% to 100%) and on artificially infected detached pod cortex (92.24 ± 4.53% to 98.75 ± 1.25%), which was not significantly

different from the positive control (Ridomil). Furthermore, this combination maintained its effectiveness for up to 9 days at room temperature. These findings suggest that combining plant extracts can enhance their antifungal properties.

Keywords

Plant Extracts, Antifungal Agents, Synergistic Effect, Black Pod Disease, Cocoa

1. Introduction

Cocoa production plays a critical role in Cameroon's economy, particularly for smallholder farmers, contributing approximately 1.2% to the GDP and 8.2% to the agricultural GDP. It generates a significant direct and indirect added value of over 200 billion CFA francs [1]. Cameroon ranks among the top cocoa producers in Africa, with an annual production exceeding 290,000 metric tons (ICCO2020). However, this vital sector faces a severe threat from black pod disease, caused by the aggressive fungus *Phytophthora megakarya*. This disease can devastate cocoa pods, leading to yield losses of over 80% under favorable conditions [2] [3]. In Cameroon, it is considered one of the primary phytosanitary problems affecting cocoa production [4]. Current control methods, such as chemical fungicides, resistant cultivars, and sanitation practices, have not been able to fully manage the disease [5]. Although chemical fungicides remain the primary approach, their use can potentially lead to the emergence of fungicide-resistant strains of *P. megakarya* [6] [7], raising concerns about their long-term effectiveness. Moreover, the environmental impact, human health risks, and increasing costs associated with chemical fungicides necessitate the exploration of alternative and sustainable solutions [8]. This urgency is further amplified by the specific threat posed by *P. megakarya* in Cameroon. Unlike the more widespread but less destructive *P. palmivora* [9], *P. megakarya* causes significant yield losses [4] [9] [10]. This highlights the need for innovative and eco-friendly management strategies [11]. Previous research has demonstrated the effectiveness of plant extracts in controlling fungal diseases, including studies showing the inhibitory activity of extracts from *Parthenium hysterophorus*, *Nerium oleander*, *Oscimum basilicum*, and *Thevetia peruviana* against various *Phytophthora* species, including *P. megakarya* and *P. capsici* [12]-[17].

This study aims to investigate the potential of ethanolic extracts from two aromatic plants, *Cymbopogon citratus* L. (lemongrass) and *Blumea balsamifera* L. (Sambong), known for their antimicrobial properties [18]. The objective is to evaluate the efficacy of these extracts in inhibiting the growth of the fungal pathogen in a controlled environment and explore their potential as a sustainable management strategy for black pod disease in Cameroon. Lemongrass extracts contain various bioactive compounds with antioxidant, antimicrobial, and

anti-inflammatory properties [19]. Sambong extracts also possess a range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects, suggesting their potential for plant-based disease control [20]-[24]. By exploring plant-based alternatives, this study aims to contribute to the development of ecological solutions to combat black pod disease, promote sustainable cocoa production practices, and improve the livelihoods of cocoa farmers in Cameroon.

2. Materials and Methods

2.1. Experimental Site and Source of Materials

The plant material, including *Cymbopogon citratus* and *Blumea balsamifera* leaves, was collected in Bokito, a small village located approximately 132 km from Bafia. The extracts were prepared in the chemistry laboratory of the University of Yaounde I (Ngoa Ekele). Diseased cocoa pods were obtained from a plot without recent fungicide application in Nkoemvone, one of Cameroon's high-incidence cocoa pod rot disease basins [4] [9] [25] [26], located in the southern region of Cameroon. Laboratory work including the isolation and identification of fungal pathogens, Koch's postulates, and evaluation of extracts *in vitro* and *in vivo*, were carried out at the Innovative Plant Health Laboratory of the FASA Annex in Bafia.

2.2. Isolation and Purification of *Phytophthora megakarya*

Isolates of *Phytophthora megakarya* were obtained from cocoa pods exhibiting well-developed brown rot symptoms. The pods were collected from one of the plots at Nkoemvone, Southern Cameroon, belonging to the Institute of Agricultural Research for Development (IRAD). Prior to collection, it was ensured that the plot had not been recently treated with any fungicide. To isolate the fungus, the surfaces of the pods were initially cleaned with tap water, disinfected using 95% ethanol, and then cut into fragments from the marginal zones of the lesions (between healthy and diseased tissue) using a flamed scalpel [27]. The fragments were dried on blotting paper and placed on V8 agar medium [26] [27]. Five pieces of cubic fragments were placed on each V8 plate and then incubated at room temperature in darkness. After 4 - 5 days, a single mycelial fragment with distinct morphology was transferred to a fresh V8 medium. This process was repeated until a pure culture of *P. megakarya* was obtained [16] [26] [27]. To confirm the pathogenicity of the isolate, the Koch postulate test was conducted.

The Koch postulate test involved artificially infecting healthy cocoa pods disinfected with a 1% sodium hypochlorite solution and 95% ethanol. The cortex of the pods was then divided into four pieces and disinfected once again. Using an 11 mm diameter punch, holes were created on each disinfected cortex. A mycelial explant from the pure culture of *P. megakarya* was placed in each hole, covered with sterile cotton wool soaked in distilled water, and incubated in darkness at room temperature (24 ± 2˚C) for seven days. The development of disease

symptoms on the cocoa pod cortex confirmed that *P. megakarya* was the causal agent of brown rot [16] [26] [27].

2.3. Sources of Plant Materials and Preparation of Extracts

Fresh leaves of *Cymbopogon citratus* and *Blumea balsamifera* were collected from the Bokito locality and transported to the Innovative Plant Health Laboratory of the FASA Annex in Bafia. The leaves were shade-dried for two weeks and then crushed using a mortar and pestle. A total of 500 grams of powdered *C. citratus* and *B. balsamifera* were mixed with 1 Lof 95% ethanol (solvent) and macerated for 48 hours [28]. The mixture was filtered using Whatman No. 1 filter paper, and the ethanol was removed from the extract using a rotary evaporator (Buchi-R-200) at 60˚C, resulting in the crude extract [26] [28]. The extracts of *C. citratus* and *B. balsamifera* were stored at 4˚C for future use.

2.4. Effect of Plant Extracts on *Phytophthora megakarya* Growth Inhibition *in Vitro*

Antifungal tests were conducted in Petri dishes using V8 medium amended with separate concentrations of crude extracts of *C. citratus* and *B. balsamifera*: 25 µl/ml, 50 µl/ml, 100 µl/ml, 250 µl/ml, and 500 µl/ml. Pure colonies of *P. megakarya* (with a diameter of approximately 8 mm) were placed in the center of the amended V8 medium. The positive control consisted of V8 medium supplemented with the fungicide Ridomil (5000 µg/ml, as recommended by the manufacturer), while the negative control contained V8 medium without any additions. Each treatment was replicated four times. All the V8 plates were incubated in darkness at room temperature (24 ± 2˚C) for seven days [26]. The colony diameter was measured daily using two perpendicular lines drawn on the V8 plates. The percentage inhibition (PI) of mycelial growth was calculated using the formula: $PI = [(A - B)/A] \times 100$, where PI represents percentage inhibition, A is the colony diameter in the negative control, and B is the colony diameter in the treatment.

2.5. Evaluation of the Antifungal Potential of Combined Extracts *in Vitro*

The most effective concentration of the crude extract tested was prepared from individual plants of *C. citratus* and *B. balsamifera*, following the method described above. The extract was then added to V8 medium alone or combined at different ratios (1:1, 1:3, and 3:1). The positive control and negative control consisted of V8 medium supplemented with Ridomil (fungicide) and V8 medium without any additions, respectively. The V8 plates were incubated at room temperature (24 ± 2˚C) in darkness, and the colony diameter was measured daily for seven days [26]. Additionally, mycelial explants from V8 plates where no colony growth occurred at the end of the assay were transferred to a freshly prepared V8 medium without extract or fungicide and incubated for an additional 7 days

at room temperature. The treatment that allowed growth resumption was considered fungistatic, while the treatment that prevented mycelial growth resumption was assumed to be fungicidal [29].

2.6. Evaluation of the Antifungal Potential of Combined Extracts *in Vivo*

The evaluation of the antifungal potential of the combined extracts was performed *in vivo* using healthy detached cocoa pods. The same treatments and proportions as the *in vitro* tests (section 2.6) were applied. Before conducting the tests, the cocoa pods were disinfected according to the details mentioned in section 2.3. Each pod was cut into four pieces, approximately 4 cm × 4 cm in size. Using a paintbrush, 500 µl of each treatment was applied to the cortex of the pod pieces and left to dry for a few minutes. Once the pods were dry, a cylindrical hole with a diameter of 8 mm and a depth of approximately 0.5 cm was created at the center of each pod using a flamed cookie cutter. An 8 mm diameter mycelial fragment of *P. megakarya* was inserted into each pod and covered with a moist cotton pad soaked in water to maintain humidity [16] [26]. The treated pods were incubated at room temperature in a completely randomized design. The progression of necrotic lesions was monitored daily for seven days. The necrotic surface area was calculated using the formula:

$$S = D \times d \times \Pi$$

where: S represents the necrosis surface area in cm^2, D is the major diameter of the lesion in cm, d is the minor diameter of the lesion in cm, and Π is the value of pi (approximately 3.14).

2.7. Effect of Storage Time on the Antifungal Potency of the Best Combined Extract *in Vivo*

The combined plant extract of *B. balsamifera* and *C. citratus*, which demonstrated the highest antifungal potential (i.e., complete inhibition), was prepared and stored at room temperature. After 3, 6, and 9 days of storage, the extract was retrieved and used to treat detached cocoa pod cortex following the protocol described in section 2.7. Mycelial growth was measured daily for seven days to assess the impact of storage time on the extract's antifungal activity.

2.8. Statistical Analysis

Data analysis for the experiments was conducted using R software version 4.3.0. A multivariate analysis of variance (ANOVA) was performed to compare the colony growth and inhibition rate obtained for the isolates in cultures with different treatments. The main treatment effects considered were treatment, isolates, and concentration. The mean colony growth and inhibition were compared using Tukey's test with a significance level of 5%. The minimal inhibitory concentration of 50% was determined with 95% confidence limits (CL) using probit analysis with the "ecotox" package [30].

3. Results

3.1. Concentration Response of *Cymbopogon citratus* and *Blumea balsamifera* Extracts in Inhibiting the Growth of *Phytophthora megakarya*

The analysis of variance (ANOVA) results for the day 7 data revealed significant variations in colony growth among different treatments, concentrations, isolates, and their interactions (Table 1). Moreover, the analysis indicated that as the concentration decreased, there was an increase in colony growth.

Table 1. Analysis of variance (ANOVA) of the effect of concentration on the growth of four *P. megakarya* isolates.

Effect	Df	SS	MS	F	P
Treatments	3	15463.20	5154.40	3303.40	<0.0001
Isolates	3	675.50	225.20	144.31	<0.0001
Concentration	4	12644.80	3161.20	2025.98	<0.0001
Treatments × Isolates	9	292.80	32.50	20.84	<0.0001
Treatments × Concentration	4	30.30	7.60	4.86	0.001
Isolates × Concentration	12	235.50	19.60	12.58	<0.0001
Treatments × Isolates × Concentration	12	173.60	14.50	9.27	<0.0001
Residuals	144	224.70	1.60	-	-

Colony Growth and Inhibition Percentage

Table 2 presents the efficacy of *Blumea balsamifera* and *Cymbopogon citratus* extracts in inhibiting the growth of *P. megakarya*. Both *B. balsamifera* and *C. citratus* extracts significantly reduced colony growth at all concentrations (25, 50, 100, 250, and 500 µl/ml) for all four isolates (M2-C, M2-O, M3-N, and M3-O) compared to the negative control (no extract). These findings indicate that these plant extracts possess antifungal properties against *P. megakarya*. In general, there was a trend of increasing inhibition with higher concentrations of both extracts for all isolates, indicating that higher extract concentrations hindered fungal growth.

For three out of the four isolates (M2-C, M2-O, and M3-N), there were no significant differences in the effectiveness of *B. balsamifera* and *C. citratus* at the highest concentration (500 µg/ml), suggesting similar inhibition of growth for these isolates at this concentration. However, at the highest concentration, Tukey's test revealed a significantly greater reduction in colony growth for isolate M3-O treated with *C. citratus* (80.65 ± 1.49%) compared to *B. balsamifera* (59.28 ± 3.82%). This indicates that isolate M3-O is more sensitive to *C. citratus*. The positive control (ridomil) completely inhibited growth for all isolates, except for M3-N, which had a colony growth of 2.50 ± 0.00 mm and an inhibition percentage of 94.76 ± 0.06%, demonstrating its resistance to the treatment.

Table 2. Colony growth and inhibition percentage of the four *P. megakarya* isolates seven days post-inoculation.

Isolates	Treatments	Concentration (µl/ml)	Colony growth (mm)	Inhibition (%)
M2-C	*B. balsamifera*	25	35.75 ± 0.82bc	17.80 ± 0.98fg
		50	28.87 ± 0.55d	33.50 ± 2.17e
		100	19.62 ± 0.37ef	54.84 ± 0.98cd
		250	18.37 ± 0.31f	57.71 ± 0.95c
		500	8.75 ± 0.62g	79.88 ± 1.38b
	C. citratus	25	38.06 ± 0.48b	12.43 ± 1.29g
		50	33.12 ± 0.37c	23.70 ± 2.39f
		100	21.62 ± 0.37e	50.26 ± 0.49d
		250	18.12 ± 0.89f	53.54 ± 2.15cd
		500	11.12 ± 0.23g	74.40 ± 0.62b
	Negative control	0	43.50 ± 0.93a	0.00 ± 0.0h
	Positive control	5000	0.00 ± 0.00h	100.00 ± 0.00a
M2-O	*Blumea balsamifera*	25	36.37 ± 0.62bc	16.31 ± 1.57fg
		50	28.87 ± 0.55d	33.50 ± 2.17e
		100	19.62 ± 0.37ef	54.84 ± 0.98cd
		250	18.37 ± 0.31f	57.71 ± 0.95c
		500	8.75 ± 0.62g	79.88 ± 1.38b
	C. citratus	25	39.00 ± 0.35b	10.26 ± 1.27g
		50	34.12 ± 0.51c	21.40 ± 2.48f
		100	21.87 ± 0.23e	49.67 ± 0.68d
		250	18.12 ± 1.46f	58.45 ± 2.64c
		500	11.00 ± 0.54g	74.71 ± 1.06b
	Negative control	0	43.50 ± 0.93a	0.00 ± 0.00h
	Positive control	5000	0.00 ± 0.00h	100.00 ± 0.00a
M3-N	*B. balsamifera*	25	36.81 ± 0.40b	13.12 ± 0.25f
		50	34.25 ± 0.25c	19.14 ± 0.90e
		100	22.50 ± 0.61e	46.93 ± 0.80c
		250	23.50 ± 0.35e	44.49 ± 1.43c
		500	14.56 ± 0.52f	65.58 ± 1.59b
	C. citratus	25	35.00 ± 0.35bc	17.39 ± 0.60ef
		50	30.00 ± 0.35d	29.16 ± 1.39d
		100	24.00 ± 0.61e	43.38 ± 0.85c
		250	23.25 ± 0.62e	45.15 ± 1.01c
		500	15.75 ± 0.43f	62.83 ± 0.84b
	Negative control	0	42.37 ± 0.51a	0.00 ± 0.00g
	Positive control	5000	0.00 ± 0.0g	100.00 ± 0.00a

Continued

M3-O	*B. balsamifera*	25	41.62 ± 0.51b	12.82 ± 9.44g
		50	34.62 ± 0.68c	27.48 ± 1.13f
		100	29.00 ± 1.17d	39.28 ± 2.19e
		250	25.87 ± 0.77de	45.75 ± 2.05de
		500	19.37 ± 1.63f	59.28 ± 3.82c
	C. citratus	25	40.18 ± 0.23b	15.80 ± 0.86g
		50	33.12 ± 0.71c	30.64 ± 0.81f
		100	26.75 ± 0.25de	43.95 ± 0.81e
		250	23.00 ± 0.54ef	51.82 ± 1.02cd
		500	9.25 ± 0.77g	80.65 ± 1.49b
	Negative control	0	47.75 ± 0.59a	0.00 ± 0.00h
	Positive control	5000	2.50 ± 0.00h	94.76 ± 0.06a

Means followed by the same letters by isolate in the same column are not significantly different with the Tukey multiple tests at threshold of 5%.

Minimal Inhibitory Concentration (MIC)

Minimal inhibitory concentration (MIC) obtained with ethanolic extracts from each of the plant species showed MIC values ranging from 117 to 269 µl mL^{-1} and 146 to 233 µl mL^{-1} for *B. balsamifera* and *C. citratus*, respectively. The V8 amended with *B. balsamifera* showed the lowest MIC (117.0 µl mL^{-1}) against isolate M2-C as shown in Table 3. However, *C. citratus* had a narrower range of MIC able to inhibit all fungal isolates, suggesting a more consistent inhibitory effect against different fungal isolates.

Table 3. Minimal Inhibitory Concentration (MIC) at 50% of extracts from *Blumea balsamifera* and *Cybompogon citratus* plant species against *Phytophtora megakarya*.

Fungal Isolates	Treatments	LC_{50} (µl mL^{-1}) (95% FL)	Slopes	Chi-square
M2-C	*B. balsamifera*	117 (100 - 137)	1.9	30.4
	C. citratus	160 (136 - 190)	2.02	36.4
M2-O	*B. balsamifera*	119 (101 - 140)	1.9	34.2
	C. citratus	156 (135 - 182)	1.95	35.1
M3-N	*B. balsamifera*	227 (183 - 296)	2.39	43.1
	C. citratus	233 (197 - 283)	1	16.5
M3-O	*B. balsamifera*	269 (219 - 346)	1.38	24.8
	C. citratus	146 (126 - 170)	1.6	28.8

FT = Fluicidal limits; LC50 = lethal concentration inhibiting fungal growth at 50%.

3.2. Combined Effect of *Blumea balsamifera* and *Cybompogon citrates* against *P. mgakarya in Vitro*

Results of the experiment on evaluation of the combination of the two plant ex-

tracts showed significant differences between isolates (df = 3; F = 7.5; P < 0.0001), treatments (df = 6; F = 615.31; P < 0.0001) as well as interaction isolate-treatment (df = 18; F = 19.15; P< 0.0001). The colony growth ranged from 1.50 ± 1.06 to 31.62 ± 0.59 mm, 0.81 ± 0.81 to 40.37 ± 1.85 mm, 00 ± 00 to 42.37 ± 0.51 mm, 2.50 ± 0.00 to 47.75 ± 0.59 mm for M2-C. M2-O, M3-N, and M3-O, respectively. For the isolate M2-C, the low value of lesion diameter was obtained on the V8 medium amended with *B. balsamifera* and *C. citrates* (TC25B75) at ratio of 3:1, which was not significantly different from the positive control (T+). A similar trend was observed for the three other isolates (Table 4). This treatment (TC25B75) drastically inhibited the colony growth of M3-N (100%), with high inhibition percentages of 92.72 ± 4.20, 93.61 ± 4.72, and 98.04 ± 1.95% for M3-O, M2-C, and M2-O respectively. This indicates that the combination of extracts can be highly effective in inhibiting fungal growth for some isolates but less effective for others. Compared to single extract treatments (TB100 and TC100), the combination treatment (TC25B75) generally exhibited superior antifungal activity. This suggests a synergistic effect on inhibiting the growth of *Phytophtora megakarya*, where the combination is more effective than either extract alone. However, the 1:1 combination of both extracts (TB50C50) showed limited antifungal activity for some isolates, suggesting that a specific ratio (3:1) might be crucial for optimal effect. The fact that fungal growth resumed after sub-culturing the mycelial explant from TC25B75 treatment suggests a fungistatic effect rather than fungicidal.

Table 4. Combined effect of *B. balsamifera* and *C. citratus* on the growth and inhibition of *P. megakarya* colonies *in vitro*.

Isolates	Treatments	Colony growth (mm)	Inhibition (%)
M2-C	T-	26.25 ± 1.36b	0.00 ± 0.00e
	T+	5.00 ± 0.00ef	80.78 ± 1.06ab
	TB100	8.75 ± 0.62de	66.34 ± 2.99bc
	TB50C50	31.62 ± 0.59a	−21.61 ± 7.47f
	TC100	11.87 ± 0.65d	54.08 ± 4.68c
	TC25B75	1.50 ± 1.06f	93.61 ± 4.72a
	TC75B25	18.37 ± 0.82c	29.10 ± 6.30d
M2-O	T-	40.37 ± 1.85a	0.00 ± 0.00d
	T+	3.00 ± 0.00d	92.51 ± 0.37a
	TB100	13.75 ± 2.74c	66.33 ± 6.12b
	TB50C50	28.56 ± 1.36b	28.49 ± 6.01c
	TC100	11.00 ± 0.54c	72.59 ± 1.74b
	TC25B75	0.81 ± 0.81d	98.04 ± 1.95a
	TC75B25	9.50 ± 0.35c	76.39 ± 0.86b

Continued

M3-N	T-	42.37 ± 0.51a	0.00 ± 0.00e
	T+	0.00 ± 0.00e	100.00 ± 0.00a
	TB100	13.81 ± 1.16c	67.30 ± 3.06c
	TB50C50	25.62 ± 0.55b	39.47 ± 1.80d
	TC100	15.75 ± 0.43c	62.83 ± 0.84c
	TC25B75	0.00 ± 0.00e	100.00 ± 0.00a
	TC75B25	9.75 ± 1.16d	77.07 ± 2.48b
M3-O	T-	47.75 ± 0.59a	0.00 ± 0.00e
	T+	2.50 ± 0.00e	94.76 ± 0.06a
	TB100	19.37 ± 1.63bc	59.28 ± 3.82cd
	TB50C50	24.56 ± 1.12b	48.58 ± 2.08d
	TC100	9.25 ± 0.77d	80.65 ± 1.49b
	TC25B75	3.43 ± 1.98e	92.72 ± 4.20a
	TC75B25	14.37 ± 1.43cd	69.96 ± 2.75bc

Means followed by the same letter in the same column for each isolate are significantly different using the Tukey multiple tests at threshold of 5%. T-: no amended PDA. T+: PDA amended with Ridomil. TB100: PDA amended with *B. balsamifera* extract only. TB50C50: PDA amended with *B. balsamifera* extract and *C. citratus at* 50% each. TC100: PDA amended with *C. citratus* extract only 500 *µl*. TC25B75: PDA amended with 75% *B. balsamifera* and 25% *C. citratus* TC75B25: PDA amended with 25% *B. balsamifera* and 75% *C. citratus*.

Comparison of Antifungal Activity of Combined Extracts *in vivo*

The results obtained following the application of the treatments *in vivo* demonstrated a significant difference in lesion diameter between the isolates (df = 3; F = 58.92; P < 0.0001) and between the treatments (df = 6; F = 593.35; P < 0.0001) (Table 5). Furthermore, a significant difference was observed in the treatment-isolate interaction (df = 18; F = 32.39; P < 0.0001). Lesion diameter of plant extracts was notably lower than that of the negative control (T-) for all isolates (Figure 1). This confirmed an antifungal effect of the combined extracts and suggested that the effectiveness of the extracts varied depending on the fungal strain.

The percentage inhibition followed a similar pattern to that observed for lesion diameter, with a significant difference between isolates (df = 3; F = 7.45; P < 0.0001), and between treatments (P < 0.05). However, no significant difference was observed in the treatment-isolate interaction (df = 18; F = 0.91; P = 0.56). A significant difference was observed in the necrotic area between isolates (df = 3; F = 38.03; P < 0.0001), between treatments (df = 6; F = 219.17; P < 0.0001), and in the treatment-isolate interaction (df = 18; F = 26.31; P < 0.0001).

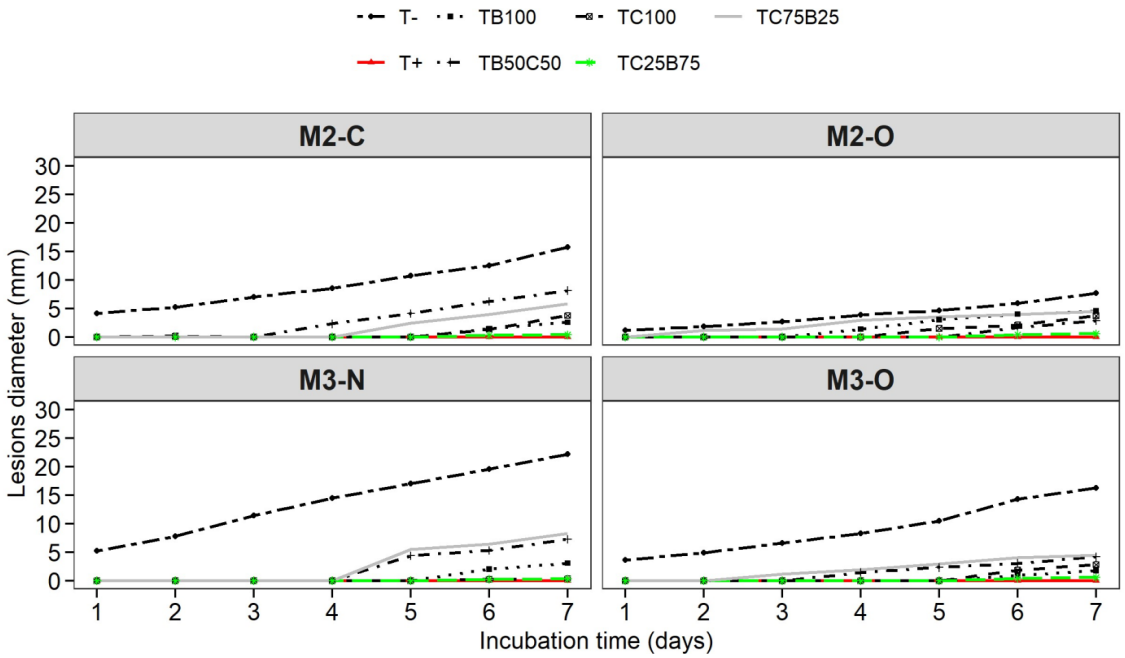

Figure 1. Progression of lesion due to *P. megakarya* during incubation time after application of treatments *in vivo*.

Table 5. Percentage of inhibition and surface lesions caused by *P. megakarya* on the cortex of cocoa pod seven days after application of both *Blumea balsamifera* and *Cybompogon citratus*.

Isolates	Treatments	Inhibition (%)	Surface lesions (mm^2)
M2-C	T-	0 ± 0f	196.93 ± 23.96a
	T+	100 ± 0a	0 ± 0c
	TB100	83.34 ± 3.17bc	5.45 ± 1.50c
	TB50C50	47.82 ± 3.80e	52.07 ± 4.29b
	TC100	75.74 ± 3.21cd	11.28 ± 1.90bc
	TC25B75	97.65 ± 1.46ab	0.24 ± 0.18c
	TC75B25	62.21 ± 5.95de	27.34 ± 5.80bc
M2-O	T-	0.00 ± 0.00d	46.59 ± 3.64a
	T+	100 ± 0a	0 ± 0d
	TB100	40.33 ± 3.27c	16.39 ± 1.14b
	TB50C50	62.39 ± 3.51b	6.62 ± 1.11cd
	TC100	51.29 ± 6.88bc	11.1 ± 2.30bc
	TC25B75	92.24 ± 4.53a	0.50 ± 0.30d
	TC75B25	41.03 ± 4.15c	15.96 ± 1.28b
M3-N	T-	0 ± 0d	388.52 ± 45.59a
	T+	100 ± 00a	0 ± 0b
	TB100	85.97 ± 0.90c	5.54 ± 0.94b
	TB50C50	65.02 ± 2.30b	41.89 ± 5.93b
	TC100	83.76 ± 0.52b	9.91 ± 1.80b
	TC25B75	98.75 ± 1.25a	0.30 ± 0.3b
	TC75B25	62.13 ± 1.91c	54.42 ± 3.64b

Continued

	T-	0 ± 0e	207.73 ± 11.27a
	T+	100 ± 0a	0 ± 0b
	TB100	89.22 ± 1.07b	2.47 ± 0.45b
M3-O	TB50C50	74.25 ± 1.16d	13.89 ± 1.48b
	TC100	82.53 ± 2.56c	6.53 ± 1.70b
	TC25B75	96.60 ± 1.53a	0.40 ± 0.21b
	TC75B25	72.64 ± 1.17d	15.51± 1.07b

Means followed by the same letter in the same column for each isolate are significantly different using the Tukey multiple tests at threshold of 5%. T-: distilled water. T+: positif control with Ridomil. TB100: *Blumea balsamifera* extract only. TB50C50: *B. balsamifera* extract and *C. citratus* at 50% each. TC100: *C. citratus* extract only. TC25B75: 75% *B. balsamifera* and 25% *C. citratus* TC75B25: 25% *B. balsamifera* and 75% *C. citratus*.

4. Effect of Storage on the Fungicidal Potential of the Best Combination of the Two Extracts

During storage (0 to 9 days), inhibition percentages ranged between 91.38 ± 2.25% and 97.65 ± 1.46%, 71.55 ± 24.06% and 100 ± 00%, 92.70 ± 2.65% and 98.75 ± 1.25%, and 92.63 ± 0.9% and 96.60 ± 1.53% as shown in Figure 2. Importantly, statistical analysis revealed no significant differences in inhibition between isolates (df = 3; F = 0.473; P = 0.70), storage time (df = 3; F = 1.21; P = 0.31), or the interaction between isolates and storage time (df = 9; F = 1.165; P = 0.338). Notably, inhibition percentages remained above 75% for the entire storage period, suggesting good stability of the inhibitory effect.

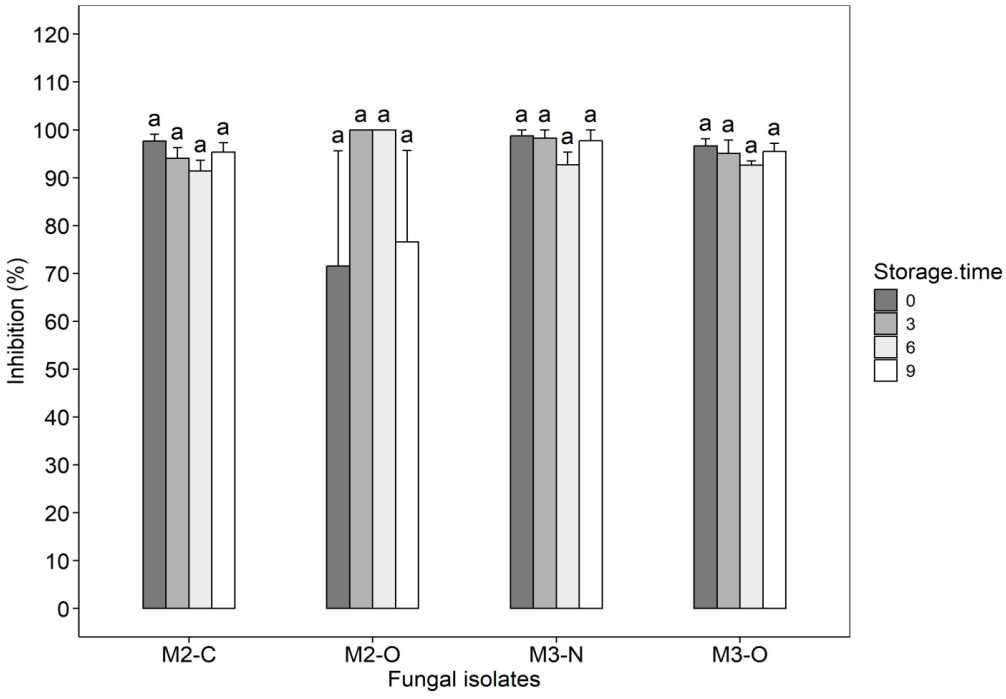

Figure 2. Inhibition of fungi growth using treatment *TC25B75* with *Blumea balsamifera and Cybompogon citrates* at ratio of 3:1, stored for 9 days at ambient temperature.

5. Discussion

Many phytoextracts from medicinal plants [31] or not including *Rosmarinus officinalis*, *Thevethia puriviana*, *Azachitiracha indica*, *Allium sativum*, *Zingiber officinale*, *Thymus algeriensis*, *Chromoleana odorata*, and *Ageratum conyzoides*, are already known for their antifungal properties against *Phytophtora* spp., causal agent of black pod [12] [13] [27] [32]. However, most studies have focused on assessing the efficacy of a single extract, whereas combining extracts can improve the efficacy of plant extracts. In the context of agroecology, there is a need to discover more fungicidal plants and their combined effects.

The present study examined the antifungal potential of *Blumea basalmifera* and *Cymbopogon citratus* against *Phytophthora megakarya*. The two plant extracts were assessed separately and in combination. Colony growth and inhibition percentages are the most widely quantified biological traits used to understand the interaction between plant extract and pathogen activity [27] [32].

The results obtained in this study suggest that extracts from both *Blumea balsamifera* and *Cymbopogon citratus* have the potential to inhibit the growth of *P. megakarya* fungi. The effectiveness of *B. basalmifera* and *C. citratus* may be due to bioactive components in the plant materials, such as secondary metabolites, which are known as important sources of microbicides, pesticides, and many pharmaceutical drugs. Ragasa *et al*. [33] reported that *B. basalmifera* contains icthyothereol acetate and cryptomeridiol, which have antifungal activities. *Cymbopogon citrates* also can contain 2 to 12 components depending on the geographic origin of the plant [34] [35]. Sawadogo *et al*. [34] reported a high content of citral (99.99%) and geranial (55%), which also have antimicrobial properties. The ethanolic extract of *C. citrates* may contain higher concentrations of bioactive components such as caffeic acid (1.432 mg/g), synapic acid (6.743 mg/g), and benzoic acid (7.431 mg/g), although the quantity and quality vary between geographic locations within different or even the same country [35]. Additionally, *C. citrus* has been widely used worldwide against various microbial diseases due to its antibacterial, antifungal, and analgesic properties.

Our study revealed that the antifungal activity of both plant extracts increased with increasing extract concentration. Different extract concentrations are typically obtained through dilution. However, this process does not increase the quantity of active components; it simply concentrates them in a smaller volume. This explains the observed trend of higher effectiveness at higher concentrations. Ambang *et al*. [32] also reported a similar increase in antifungal potential with increasing concentration of crude *Thevetia peruviana* seed extract against *P. megakarya*. Our findings are further supported by the reports of Djeugap *et al*. [36] and Adeyemi *et al*. [37], who observed a concentration-dependent increase in antifungal activity of ethanolic extracts of *Thymus algeriensis* and *Chromoleana odorata*, respectively, against *P. megakarya*.

Blumea balsamifera extract exhibited higher antifungal activity against three isolates, with the lowest Minimum Inhibitory Concentration (MIC) compared to

C. citratus, which was highly effective against only one isolate. The greater effectiveness of *B. balsamifera* compared to *C. citratus* could be attributed to the qualitative and quantitative differences in their active components. These are generally affected by environmental conditions, especially the geographical location where the plants are grown [38]. The susceptibility of *P. megakarya* isolates to *B. balsamifera* and *C. citratus* varied. One of the four fungal isolates studied was less susceptible to both extracts. This difference in response among the fungal isolates could be related to their genetic variability, virulence, conidial production, and fungal growth speed [16]. Our findings highlight that isolates within the same fungal species can exhibit varying susceptibility. This suggests that validating plant extracts as an effective management strategy for cocoa black pod disease may require screening against various pathotypes from different agroecological zones. Our results align with those of Ambang *et al*. [32] who reported that one of the four *P. megakarya* isolates from the Centre region of Cameroon displayed resistance to *Thevetia peruviana* seed extract *in vitro*. Similarly, Mboussi *et al*. [27] showed that the inhibitory potential of *T. peruviana* and *Azadirachta indica* depended on the specific *P. megakarya* strain studied.

When combining plant extracts, synergism is often the most desirable effect because it optimizes efficacy, can affect resistant pathogens, and broadens the spectrum of activity compared to a single extract [39]. Previous research on using combinations of plant extracts against cocoa pathogens has shown promising results. For example, *African panaxia* extract and combinations like *Cymbopogon citratus* and *C. schoenanthus* essential oil or *C. citratus* combined with *Azadirachta indica* extract all demonstrated improved antimicrobial potential [36] [40]. In our study, a synergistic effect was observed when *B. balsamifera* and *C. citratus* were applied in a 3:1 ratio in V8 media (resulting in 92.72 ± 4.20% to 100% growth inhibition) and on detached pod cortex artificially infected (achieving 92.24 ± 4.53% to 98.75 ± 1.25% inhibition) against *P. megakarya*. This suggests that *in vitro* assays could be reliable for screening plant extracts against pathogens. Synergism may have been induced by several mechanisms, including the inhibition of a common biochemical pathway and protective enzymes, increased concentration of antifungal compounds at the inoculation sites, and the use of cell wall active agents that enhance the uptake of other antimicrobials [39]. One of the extracts may have facilitated entry into the pathogen by serially blocking metabolic pathways or inactivating microbial enzyme [39].

Our study observed high stability in the combined extract during the 9-day storage period, with antifungal activity against *P. megakarya* remaining above 75%. The content of bioactive components in plant extracts can vary depending on the plant species and storage duration. Some plants exhibit higher phenolic and flavonoid content in fresh material compared to stored material, while the opposite is true for others. Additionally, some species maintain similar levels of bioactive components in both fresh and stored material [41]. The consistent antifungal activity observed in our study, before and after storage, could be attri-

buted to the presence of stable bioactive components in both the fresh and stored extracts.

Generally, microbial proliferation within plant extracts can accelerate decomposition or fermentation processes [42]. The observed stability of our extract could be due to the potential presence of natural biopreservatives within the plant material itself [43]. Further studies are warranted to optimize extraction formulations for long-term preservation.

6. Conclusion

The current study revealed that extracts of both *C. citratus* and *B. basalmifera*, alone or in combination, exhibited antifungal potential against *P. megakarya*. However, the antifungal effect was optimized when the two extracts were combined synergistically. This synergistic effect was concentration-dependent and varied among fungal isolates. Fungal growth inhibition increased with increasing extract concentration. Notably, the combination of 25% *C. citratus* and 75% *B. basalmifera* exhibited the strongest synergistic effect. The observed consistency between *in vitro* and *in vivo* results suggests that *in vitro* assays can be a reliable tool for screening plant extracts with high potential for controlling various phytopathogens. Further research is warranted to explore the underlying mechanisms of this synergy between *C. citrates* and *B. basalmifera*. Additionally, investigating the potential for optimizing the extract ratio for different *P. megakarya* isolates could improve the overall efficacy of this approach.

Authors' Contributions

Conceptualization: G. Membang, J.M. Tchotet Tchoumi; Methodology: G. Membang, J.M. Tchotet Tchoumi; Investigation and Data curation: G. Membang, J.M. Tchotet Tchoumi, E.Y. Mba Ela, S.L. Lontsi Dida, L. Tchuenkam Tsango, P.L. Ekango Mbonjo; Formal analysis: G. Membang, J.M. Tchotet Tchoumi, E.E.Y. Mba; Resources: F.O. Tabi, G. Ntsomboh Ntsefong, G. Membang, J.M. Tchotet Tchoumi, E.Y. Mba Ela, S.L. Lontsi Dida,; Supervision: F.O. Tabi, G. Ntsomboh Ntsefong; Writing original draft: J.M. Tchotet Tchoumi, G. Membang; Manuscript revision: J.M. Tchotet Tchoumi, G. Membang, F.O. Tabi, G. Ntsomboh Ntsefong. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declared no competing interests.

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