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Evaluation of the effectiveness of some plant extracts against cowpea charcoal rot

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ABSTRACT

The charcoal rot of cowpea nowadays represents one of the major constraints of the crop, with 100% losses. The present study aims to evaluate the effectiveness of essential oils (EOs) from *Cymbopogon schoenanthus* (L.) Spreng., *Lippia multiflora* (Moldenke), and *Ocimum americanum* L., as well as aqueous extracts from *Eclipta alba* (L.) Hassk. *and Balanites aegyptiaca* (L.) Delile against the disease as an alternative to the use of chemical fungicides. Thus, the aqueous extracts and essential oils were inoculated into PDA at a concentration of 1%, and the fungus was then cultivated to evaluate its radial growth *in vitro* in the presence of a reference treatment, Calthio C, inoculated into PDA at a rate of 2.5 g per liter, and, as a positive control, simple PDA. *In vivo*, the essential oils and Calthio C at the same doses were used to treat the seeds of Komcallé and Tiligré cowpeas in the presence of an untreated control. The results showed that all the tested treatments exhibited antifungal activity *in vitro* on the fungus, with a complete (100%) inhibition of mycelial growth by the essential oils, and Calthio C. *E. alba* and *B. aegyptiaca* exerted partial inhibition with radial growths of 3.97 and 3.92 mm, respectively. *In vivo*, in the greenhouse, the EO of *L. multiflora* exerted a considerable antifungal effect compared to Calthio C, whereas in the field, the EOs showed low efficacy. Essential oils exhibit interesting antifungal effects.

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INTRODUCTION

The primary approach to controlling fungal diseases such as charcoal rot caused by *Macrophomina phaseolina* continues to be the application of synthetic chemical pesticides. The misuse of these products can be harmful to both humans and animals, as well as contribute to environmental degradation (Zongo *et al.*, 2015).

Environmental toxicity associated with chemical products and the developments of resistance in plant pathogens have prompted researchers to explore alternative approaches for controlling plant pests and diseases (Ogunnupebi *et al.*, 2020). In the context of agro-ecological transition, it is important to identify sustainable and ecological alternatives to reduce dependence on conventional fungicides. In this regard, several studies in the world have reported the antifungal activity of plant-derived compounds (Banaras *et al.*, 2021). Studies have shown that all parts of *Acacia nilotica* (L.) Delile exhibit antifungal activity against *M. phaseolina*, with the floral extract being the most effective (Rafiq *et al.*, 2024). Similarly, the combination of *Trichoderma harzianum* with the leaf biomass of *Azadirachta indica* A. Juss. proved highly effective in controlling *M. phaseolina* causing cowpea disease (Shoaib *et al.*, 2018).

In Burkina Faso, Bonzi *et al.* (2013) and Tiendrébéogo *et al.* (2017) reported that plant extracts often contain bioactive compounds that have antifungal properties and can be used to control the development of pathogenic fungi. Toé *et al.* (2022) emphasised the antifungal efficacy of three biofungicides derived from essential plant oils (*Lippia multiflora*, *Cymbopogon schoenanthus*, and *Ocimum americanum*) against predominant fungi, including *M. phaseolina*, linked to cowpea seeds. The present study seeks to evaluate botanical extracts derived from *Cymbopogon schoenanthus* (L.) Spreng., *Lippia multiflora* (Moldenke), *Ocimum americanum* L., *Eclipta alba* (L.) Hassk., and *Balanites aegyptiaca* (L.) Delile, in order to identify those capable of effectively combating the charcoal rot of cowpea an emerging disease in Burkina Faso. Previous studies have shown a high prevalence of the disease, with more than 90% of cowpea seed samples infected, and it is present in all cowpea-growing areas.

MATERIALS AND METHODS

Seed treatment products

Plant species

The plant extracts used are composed of three essential oils derived from three aromatic plants which are *C. schoenanthus*, *L. multiflora*, and *O. americanum*, and two aqueous extracts, prepared from *E. alba* and *B. aegyptiaca*. These oils were chosen based on their antifungal properties revealed in the literature.

Reference check

Chemical fungicide, Calthio C, which is made up of 25% Chlorpyrifos-ethyl and 25% Thiram, is a fungicide approved for seed treatments. In this study, it was used as a reference control.

Acquisition of treatment products

The essential oils were sourced from the Laboratory of Environmental Technologies and Natural Products at the Institute of Research in Applied Sciences and Technologies. These oils were extracted from the leaves of *C. schoenanthus*, *L. multiflora*, and *O. americanum* by hydrodistillation (Fig. 1).

Fig. 1. Essential oils of *Cymbopogon schoenanthus*, *Lippia multiflora*, and *Ocimum americanum*

The aqueous extracts were obtained from the barks of *B. aegyptiaca* and the whole plants of *E. alba*. The plant organs were harvested, dried in the shade, and ground into powder. Next, aqueous extracts were prepared by mixing 10 g of the powder from each plant with 100 ml of distilled water. The mixtures were left to macerate under laboratory conditions for 20 hours before being filtered through a thin fabric, then through Millipore filters (Ø = 0.45 µm).

Evaluation of the effect of the products on the radial growth of *M. phaseolina*

The test consisted of preparing PDA nutrient media to which different essential oils and aqueous extracts were added at a concentration of 1% (Fig. 2) in accordance with the dose applied by Ugilino *et al.* (2018). The fungus was then cultured on these media according to the methodology used by Zida *et al.* (2008). The control treatment consisted of the PDA medium untreated by the products, and the reference treatment, the PDA medium to which Calthio C was added at a rate of 2.5 g of product per liter of PDA. To evaluate the effect of the products, mycelial explants of 4 mm in diameter were taken from a 7-day-old pure culture and then placed at the center of a Petri dish containing each treatment to be tested. The Petri dishes were incubated at the ambient temperature (25-30°C) of the laboratory. The experimental setup used is a Fischer block consisting of seven (7) treatments, which are the pure medium (untreated), the medium treated with Calthio C., the medium treated with aqueous extracts of *E. alba* and *B. aegyptiaca*, the essential oils of *L. multiflora*, *O. americanum*, and *C. schoenanthus*. Each treatment was repeated 4 times, with one box used per treatment and per repetition.

Fig. 2. Process of incorporating aqueous plant extracts into the PDA medium. (a) Aqueous extract before filtration; (b) filtration of extracts using Millipore filters.

The diameter of the colonies was measured in each box 24 hours after the incubation of the explants, and then each day until the 3rd day (on the 3rd day, the fungus had reached its maximum growth on the untreated PDA). The result, expressed in millimeters, was the average of two diameters measured perpendicularly to each other, minus 4 mm (explant diameter). Mycelial growth is calculated using the formula:

$$V = [(D1 - 4) + (D2 - 4)] / N \times 2$$

Where V= growth rate of the fungus in mm/day; D1 and D2 are the two perpendicular diameters of the colony; N is the number of days after the explant was replanted; and 4 is the diameter of the explant.

The most effective products, that is, those that completely inhibited the radial growth of the fungus, were selected to be evaluated in seed treatment in a real-environment setting.

Evaluation of the effectiveness of products in treating seeds naturally infected by *M. phaseolina*

Seed treatment with essential oils

At the end of the in vitro trial, the treatments based on essential oils (*Lippia multiflora*, *Ocimum americanum*, and *Cymbopogon schoenanthus*) that resulted in the strongest inhibition of mycelial growth were selected for the *in vivo* trial. To this end, two improved varieties of cowpea, Komcallé and Tiligré, developed by research in Burkina Faso and appreciated by producers, were used. The seeds of these varieties were collected in 2023 from the seed bank of the Kamboinsé Research Center CREAF/K. They were initially infected by *Macrophomina* with infection rates of 20.11% for Komcallé and 27.49% for Tiligré. The seeds of the two varieties mentioned above were naturally contaminated by *M. phaseolina*, then mixed with a little distilled water in a glass vial (100 µl of water per gram of seeds) to soften the seed coats and allow the oils to be absorbed by the seeds. After adding 1µl of essential oil (Fig. 3), the seeds were vigorously mixed and kept in the vial, protected from light, for about 20 hours, before being air-dried for at least three hours. The seeds treated only with distilled water (100µl of water per gram of seeds), those treated with Calthio C (2.5g/kg of seeds), and those that received no treatment served as negative control, reference control, and absolute control, respectively.

Fig. 3. Cowpea seeds packaged for treatment with essential oils

Evaluation of the effectiveness of essential oils in a greenhouse

Inside the greenhouse, seeds of the Tiligré variety were sown in seedling trays filled with previously sterilized sand, at a rate of 32 seeds per treatment and per repetition, and then incubated at 27°C under alternating lighting of 12 hours of daylight and 12 hours of darkness for 15 days. The experimental design used was a Fisher block with 4 repetitions. At the end of the incubation, the seedlings were carefully uprooted. In this study, the observations consisted of counting the normal plants (plants with complete essential structures (roots, leaves, stem) and without infection from the mother seed); the abnormal plants (plants exhibiting abnormalities in the roots, stems, leaves, plants affected by disease due to infection from the breeder seed); the ungerminated seeds, and express the results as a percentage of the number of seeds sown, and the plants (normal plants and abnormal plants) which were then gathered and weighed, with the weight expressed in grams.

On-farm evaluation of the effectiveness of the essential oils

Description of the study site

The study was conducted on the experimental field of the Center for Environmental, Agricultural, and Training Research (CREAF) in Kamboinsé. Established in 1985, CREAF is one of the Research Centers of the Institute of Environment and Agricultural Research (INERA) located about twelve kilometers North of Ouagadougou on the Ouagadougou-Kongoussi axis. The station receives an average annual rainfall of 700 mm. The soils are of the remoulded tropical ferruginous type and are, due to their topographical situation, very sensitive to water erosion.

Soil preparation and setup of the experimental trial

In the field, soil preparation consisted of plowing the field, delineating the elementary plots, and marking the sowing lines. The experimental design was a split plot with 4 replications, with the cowpea varieties assigned to the main plots and the treatments to the subplots. Each elementary plot consisted of 4 rows, each 4 meters long. The sowing was done with 3 seeds per sowing hill, with a spacing of 80 cm between the rows, 40 cm between the sowing hills in the row, and 2 m between the repetitions. A thinning was carried out two weeks after sowing, leaving 2 plants per clump.

Maintenance of the trial

The maintenance of the trial consisted of the application of the fertilizer NPK, applied three (3) weeks after sowing at a dose of 100 kg/ha. Two weedings were realized, the first was one month after sowing and the second before the maturity of the pods. Two insecticide treatments (Pacha and K. optimal) at the recommended dose (2 ml per 1 liter of water) were applied at the stages of 50% floral bud formation and 50% pod formation.

Data collection and statistical analyses

The observations were made on the two central rows of each elementary plot and focused on:

1. The number of seedlings emerged, 15 days after sowing (DAS) and expressed as a percentage;
2. The total damping-off (pre-emergence damping-off and post-emergence damping-off) at 30 DAS; and
3. The severity of the disease at maturity, expressed as a percentage.
4. The seed yield, expressed in kg/ha.

The severity was rated using the brown spot disease rating scale used by Sérémé (1999), which has been adapted to charcoal rot. This scale consists of 5 classes ranging from 1 to 5:

1: No symptoms;

2: Small lesions (circular or elongated) brown-black or black on the stem;

3: Coalescent lesions on the stem and branches;

4: Coalescent lesions on the stems and branches with the presence of sclerotia but the plant survive;

5: Senescence and wilting of the plant, infected stems covered with black sclerotia, dead plants, ungerminated seeds.

The disease severity index was calculated using the formula proposed by Allen *et al.* (1981):

$$I(\%) = \frac{\Sigma(Xi-1)ni*100}{[E(Xi-1)]N}$$

With Xi = disease score for each plant, ni = number of individuals in category Xi, N = total number of observed plants, E(Xi) = range of the scale.

At the end of the evaluations and based on the severity indices, the accessions were classified according to their level of resistance or sensitivity to the disease as follows:

Severity (S) = 0%: Immune Plant (I);

0% < S ≤ 5%: Highly Resistant Plants (HR);

5% < S ≤ 10%: Resistant Plants (R);

10% < S ≤ 20%: Moderately Resistant (MR) or Moderately Susceptible (MS) Plants;

20% < S ≤ 50%: Susceptible Plants (S);

S > 50%: Highly Susceptible Plants (HS).

The data were subjected to an analysis of variance followed by a separation of means in case of significant differences between treatments, as previously described.

RESULTS

In vitro effect of plant extracts on the mycelial growth of *M. phaseolina*

Table 1 presents the mycelial growth diameters of *M. phaseolina* measured 1, 2, and 3 days after incubating the mycelial explants in the different PDA media. The growth diameters varied from 0 to 3.77 mm, from 0 to 7.03 mm, and from 0 to 7.90 mm, respectively, on the 1st, 2nd, and 3rd day after Transplanting (DAT) the explants.

Very significant differences (p <0.0001) were observed in the mycelial growth of the fungus depending on the treatments applied, at all observation periods. The results revealed that Calthio C and the essential oils of *L. multiflora*, *O. americanum*, and *C. schoenanthus* completely inhibited growth by completely blocking the development of the fungus (zero growth) regardless of the observation period (Fig. 4). During the evaluation period, the diameters obtained with the PDA media treated with aqueous extracts of E. alba (1.08-3.97 mm) and *B. aegyptiaca* (0.97-3.92 mm) were significantly lower than those obtained with untreated PDA (3.77-7.90 mm).

The aqueous extracts of *E. alba* and *B. aegyptiaca* had statistically similar effects on the growth of *M. phaseolina*, reducing the development of the fungus by 71.35%, 65.14%, and 49.74% for the *E. alba* extract, and 74.27%, 65.43%, and 50.37% for the *B. aegyptiaca* extract, respectively at the 1st, 2nd, and 3rd DAT of the fungal mycelial explants (Fig. 5). At the end of this test, the essential oils and Calthio C were retained for on-farm evaluation.

Effects of essential oils on germination parameters, normal and abnormal plants, ungerminated seeds, and the fresh weight of cowpea seedlings at 15 DAS

In the greenhouse, the results concerning the percentages of normal plants, abnormal plants, ungerminated seeds, and the fresh weight of plants following seed treatment with essential oils are presented in Table 2.

These results revealed significant differences between the treatments for all parameters except for the rate of normal plants. Among the treatments used, the essential oil of *L. multiflora* recorded a higher plant emergence rate and fresh plant weight (85.15% and 47.16 g, respectively) than the reference fungicide, which had a 72.65% emergence rate and a plant weight of 31.99 g. This treatment also has the lowest rate of abnormal plants (34.38%) and ungerminated seeds (14.84%) compared to the other treatments (38.28-42.19% of abnormal plants and 19.53-29.68% of ungerminated seeds).

Table 1. Effects of treatments on the radial growth rate of *M. phaseolina*

Treatments	Growth rate 1 DAT mm/Day	Growth rate 2 DAT mm/Day	Growth rate 3 DAT mm/Day
Untreated	3.77 a	7.03 a	7.90 a
Eclipta alba	1.08 b	2.45 b	3.97 b
Balanites aegyptiaca	0,97 b	2.43 b	3.92 b
Calthio C	0 c	0 c	0 c
C. schoenanthus	0 c	0 c	0 c
L. multifora	0 c	0 c	0 c
O. americanum	0 c	0 c	0 c
Probability	<0.0001	<0.0001	<0.0001

The differences are considered significant for $p < 0.05$.

Table 2. Emergence rates and proportions of normal plants, abnormal plants, ungerminated seeds, and fresh weight of plants, following seed treatment with essential oils in the greenhouse after 15DAS.

Treatments	Emergence rate (%)	Normal plants rate (%)	Abnorrmal plants rate (%)	Ungerminated seeds rate (%)	Fresh weight of plants rate (g)
Untreated	80.15 ab	39.84	40.62 a	19.53 ab	34.04 b
Calthio C	72.65 b	32.81	39.34 a	27.34 a	31.99 b
L. multiflora	85.15 a	50.78	34,38 b	14.84 b	47.16 a
O. americanum	70.33 b	32.03	38.28 a	29.68 a	31.19 b
C. schoenanthus	80.46 ab	38.28	42.19 a	19.53 ab	34.09 b
Probability	0.0045	0.1571	0.0373	0.0045	0.0258

The differences are considered significant for $p < 0.05$.

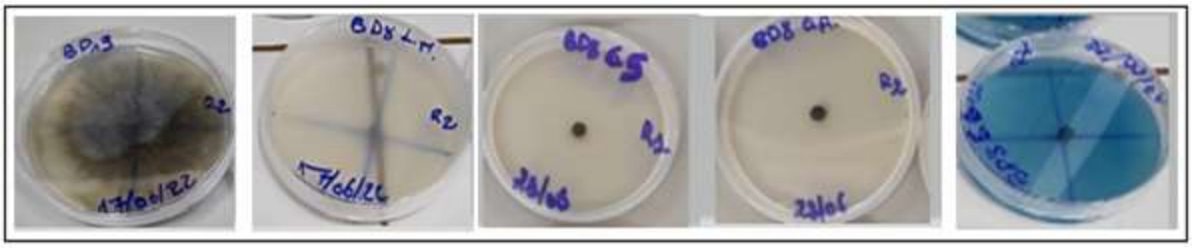

Fig. 4. Growth of the fungus on the medium treated with essential oils and Calthio C. BD19: simple medium; LM: *Lippia multilora*; CS: *Cymbopogon schoenanthus*; OA: *Ocimum americanum*; cc: Calthio C.

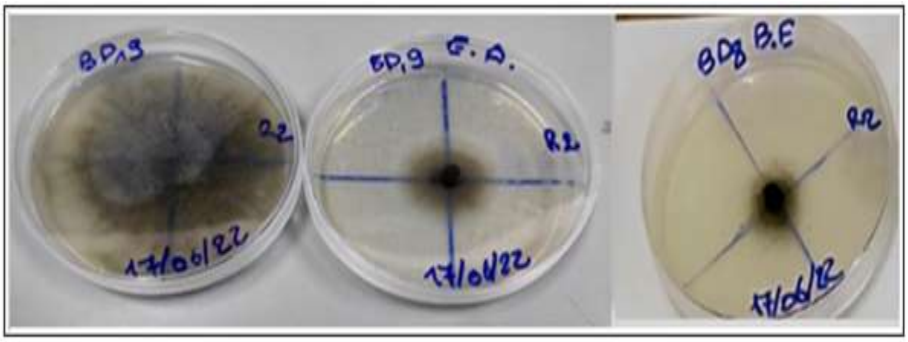

Fig. 5. Growth of the fungus on the medium treated with aqueous extracts. BD19: simple medium; EA: *Eclipta alba*; BE: *Balanites aegyptiaca*.

Table 3. Emergence rate of plants, seedling damping-off, severity of charcoal rot, and seed yield of cowpea, following seed treatment with essential oils under real conditions

Treatments	Emergence rate (%) 15 DAS	Seedling damping-off rate (%) 30DAS	Severity of charcoal rot rate (%)	Seed yield (kg/ha)
Untreated	57.57	33.14a	40.72	2094
Calthio C	67.42	14.01b	29.69	2235
Eau distillée stérile	52.65	32.95a	26.86	2214
L. multiflora	53.22	19.69ab	33.97	1988
O. americanum	44.50	30.87a	27.37	2102
C. schoenanthus	45.45	28.59a	27.53	1887
Probability	0.0559	0.0260	0.2104	0.9773

The differences are considered significant for $p < 0.05$.

Table 4. Plant emergence rates, seedling mortality, severity of charcoal rot, and seed yield of two varieties of cowpea, following seed treatment with essential oils under real conditions

Treatments	Emergence rate (%)	Seedling damping-off rate (%)	Severity of charcoal rot rate (%)	Seed yield (kg/ha)
Komcallé	53.03	48.04 a	29.94	2327
Tiligré	53.91	5.05 b	32.11	1847
Probability	0.8450	<0.0001	0.5512	0,0914

The differences are considered significant for $p < 0.05$.

Fig. 6. Symptoms of charcoal rot on cowpea in a natural infestation. (a) collar rot; (b) stem rot; (c) seedling wilting; (c) pod rot

Effects of plant essential oils on emergence, seedling damping-off, severity of charcoal rot, and seed yield of cowpea in a real-environment setting

In a real-environment setting, symptoms of charcoal rot were observed on plants from naturally infected seeds, whether treated with essential oils or not (Fig. 6). These included significant lesions on the stems and branches with or without the presence of microsclerotia, stem rot, pod rot, wilting, etc. Table 3, presenting the results of the effectiveness of the 6 treatments applied to cowpea seeds naturally infected by *M. phaseolina*, revealed a significant difference between the treatments regarding emergence (slight difference with p=0.0593) and seedling damping-off at 30 DAS (p=0.0260), but also a highly significant difference between the two varieties concerning seedling damping-off (p<0.0001). No significant interaction was noted between the treatments and the varieties for all the parameters studied. Regarding plant emergence, the essential oil of *Lippia*, with a 53.22% emergence rate, did not have any particular influence on plant sprouting compared to the various controls used (52.65-67.42%). On the other hand, the EO of *Ocimum* and the EO of *Cymbopogon*, with emergence rates of 44.50% and 45.45%, respectively, significantly reduced plant emergence compared to the reference control (67.42%). *L. multiflora* also exhibited low levels of seedling mortality (19.69%), similar to the reference control (14.01%). Regarding the severity of the disease and the seed yield, no difference was noted between the treatments. In general, the results of the study showed that seedling losses were more significant in the Komcallé variety (an average of 48.04% losses) than in the Tiligré variety (5.05%) (Table 4). For the other variables, the two varieties exhibited statistically similar behaviours.

DISCUSSION

The incorporation of essential oils from *L. multiflora*, *O. americanum*, and *C. schoenanthus*, along with the chemical fungicide Calthio C into the PDA culture medium at a dose of 1%, resulted in strong antifungal activity with a complete (100%) inhibition of the mycelial growth of *M. phaseolina*. Previous studies have shown the inhibitory effect of several essential oils on the growth of various pathogens.

Indeed, Nikiéma (2017) showed that a concentration of 0.1% completely inhibited the growth of *Bipolaris oryzae*. Also, Tiendrébéogo *et al.* (2017) indicated that at different doses, the essential oil of *L. multiflora* led to a complete inhibition of the mycelial growth of *Fusarium moniliforme*. These results reinforce the perspective of using essential oils from this plant in the protection of crops against fungal diseases. Studies conducted by Zida *et al.* (2008) on sorghum and millet have also highlighted the effectiveness of essential oils from *C. schoenanthus*, *C. giganteus*, and *C. nardus* against several fungi. The essential oils of plants in the Poaceae family are a significant source of fungitoxic chemicals, according to reports from other authors (Shukla, 2016).

The essential oils of *O. americanum* showed antifungal activity against toxinogenic strains of *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, and *Fusarium oxysporum* (Adjou and Aoumanou, 2013).

Similarly, *Ocimum basilicum*, another species of the same genus, inhibited fungi at different concentrations, affecting the germination of maize and cowpea (Koffi *et al.*, 2006).

The use of aqueous extracts of *E. alba* and *B. aegyptiaca* significantly inhibited the development of *M. phaseolina*, but at a much lower level than essential oils. This indicates a low antifungal activity of the aqueous extracts compared to the essential oils, possibly due to a better concentration of active principles in the essential oils than in the aqueous extracts. The antifungal effect of aqueous extracts of *B. aegyptiaca* was previously reported by Sérémé (1999) and Soalla (2011) on the mycelial growth of *Colletotrichum capsici*, the agent responsible for the brown spot disease of cowpea. This activity of *B. aegyptiaca* was also reported for C. *graminicola*, responsible for sorghum anthracnose (Zida, 2009).

In semi-controlled conditions (greenhouse), the application of essential oil from *L. multiflora* as a seed treatment significantly improved the seed emergence rate compared to the control treated with Calthio C. and the other evaluated treatments. This oil also recorded the lowest rates of abnormal plants and ungerminated seeds. These results suggest an antifungal effect of *L. multiflora* essential oil in the treatment of cowpea seeds. They corroborate the observations of Dossa *et al.* (2021), who reported similar effects on fungi associated with sorghum seeds. Moreover, the studies by Cissé *et al.* (2020) also demonstrated its effectiveness against *Rhizopus sp.* in maize.

Essential oils were found to be ineffective against cowpea charcoal rot when used in seed treatment under natural conditions. The antifungal activity of essential oils seen in vitro and in the greenhouse was not confirmed by the results. This could be explained by the fact that essential oils are volatile.

CONCLUSION

This study sought to assess the antifungal efficacy of three essential oils (*L. multiflora*, *O. americanum*, and *C. schoenanthus*) and two aqueous extracts (*B. aegyptiaca* and *E. alba*), both in vitro and in vivo, as well as the sensitivity of cowpea, Komcallé, and Tiligré, with regard to the substantial losses caused by cowpea charcoal rot, primarily caused by *M. phaseolina*.

The initial hypothesis was that certain plant extracts could significantly reduce the impact of this pathogen, thus offering sustainable alternatives to synthetic phytosanitary products. The results showed that all the tested treatments exhibit antifungal activity in vitro on *M. phaseolina*, with a complete (100%) inhibition of mycelial growth by the three essential oils. The aqueous extracts showed partial inhibition with radial growths of 3.97 and 3.92, respectively, for *E. alba* and *B. aegyptiaca*, indicating moderate efficacy. In comparison to the reference control, the essential oil of *L. multiflora* demonstrated a significant antifungal effect during the in vivo experiments in the greenhouse. However, for the cowpea varieties, Tiligré proved to be less susceptible to charcoal rot than Komcallé.

These results confirm the existence of plant extracts, particularly *L. multiflora* oil, which can be used as a potential antifungal agent to reduce the effects of *M. phaseolina* on cowpea. The combination of this essential oil and the Tiligré variety constitutes a promising option for the sustainable and healthy management of cowpea charcoal rot.

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