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ANTIFUNGAL ACTIVITY OF *COCHLOSPERMUM PANCHONII* HOOK RHIZOMES ESSENTIAL OIL ON EIGHT PHYTOPATHOGENIC FUNGI

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ABSTRACT: Pesticides are the chemicals used to protect crops against bio-aggressors. However, they can pose risks to human health and environment. In order to propose less toxic and more biodegradable substitution products, the effect of *C. planchonii* rhizomes essential oil was tested on the mycelial growth of eight phytopathogenic fungi frequently encountered on major food crops in Burkina Faso. This essential oil has also been tested on sorghum and maize seedlings seed germination and growth. The essential oil was extracted by hydrodistillation and evaluated for its antifungal activity in vitro on *Alternaria alternata*, *Colletotrichum dematum*, *Colletotrichum graminicola*, *Curvularia lunata*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium verticiloïdes* and *Macrophomina phaseolina*. Antifungal tests were carried out on Potato Dextrose Agar (PDA) medium in Petri dishes. Calthio C was used as a reference fungicidal control. The toxicity of this essential oil was evaluated according to the ISTA method. Considering the different concentrations of essential oil (0.10%, 0.25% and 0.50%) there are significant differences between the treatments performed. The highest inhibition of mycelial growth was obtained on *Colletotrichum graminicola* at 0.50% of essential oil. At this concentration, there was a significant reduction in the mycelial growth of *Colletotrichum graminicola* (81.70%) followed by *Curvularia lunata* (54.58%) and *M.phasoelina* (53.89%) seven days after incubation. In terms of phytotoxicity of this oil on maize seedlings germination and the growth, there was no significant difference with the controls samples. These results suggest the use of this antifungal agent oil especially in maize seed treatment.

KEYWORDS: *C. planchonii*, essential oil, antifungal, phytotoxicity.

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1.INTRODUCTION

Agriculture is the main source of income and employment in Burkina Faso. Unfortunately it is slowed by enormous parasitic challenges among which we have phytopathogenic fungi. These fungi are one of the major causes of plant diseases [1], [2], [3] leading to heavy losses before and after harvest. Notable fungal diseases include anthracnose caused by *Colletotrichum graminicola* (Ces.) [4] which is a major constraint to sorghum production in West Africa [4]. We can also mention the melting of seedlings caused by *Fusarium moniliforme* (Sheld.), *Phoma sorghina* (Sacc) and *Aspergillus niger* (Micheli) [5]. The use of synthetic chemicals by farmers to increase production yields [6] has shown its inadequacies by many environmental challenges. In addition, several studies have shown that some plant natural substances can have fungicidal properties, preserve the environment and improve food safety [7],[8]. Some essential oils are part of these natural substances that can have antifungal properties. The essential oils of *Cymbopogon schoenanthus*, *C.giganteus*, *Lippia multiflora*, *Ocimum basilicum* and *Securidaca longepedonculata* have antifungal properties [9]. Some authors have shown that the essential oil of *Cymbopogon citratus* is effective on *Phoma sorghina* and *Fusarium moniliforme* in seed treatment [10]. In this study we have been focused on the evaluation of the in vitro antifungal activity of the essential oil of *C. planchonii* whose chemical composition had already studied [11]. Indeed, *C. planchonii* is a perennial semi-woody plant generally growing in African savannas [12]. It belongs to the dicotyledonous class, to the Order of the Violales, to the Family *Cochlospermaceae*, to the genus *Cochlospermum*. The present study consisted in extracting the essential oil of *C. planchonii* and assessment of its potential effects on some major phytopathogenic fungi and sorghum and maize growth parameter.

2. MATERIALS AND METHODS

Plant material

Our work focused on rhizomes of *C. planchonii* (Photo1).

Whole plant Flower Rhizomes

Photo 1: *Cochlospermum planchonii* Hook

Seeds used

Certified seeds of *Sorghum bicolor* of the "Kapelga" variety and those of *Zea mays* of the "Barka" variety were used for the phytotoxicity test.

Phytopathogenic fungi

The fungi used (Table 1) in this study were provided by the Phytopathology Laboratory of the Nazi BONI University.

Table 1: Phytopathogenic fungi

Phytopathogenic fungi	Origin
Alternaria alternata (Fr) Keissler	Tomato (*Solanum lycopersicum* L)
Colletotricum dematium (Pers. Ex Fr) Grove	Cowpea (*Vigna unguiculata* (L.) Walp)
Colletotrichum graminicola (Ces) Wilson	Sorghum (*Sorghum bicolor* (L) Moench)
Curvularia lunata (Wakker) Boedijn	Maize (*Zea mays* L.)
Fusarium moniliform Sheldom	Sorghum (*Sorghum bicolor* (L) Moench)
Fusarium oxysporum F.sp. Radicis-Lycopersici (Sheld)	Tomato (*Solanum lycopersicum* L)
Fusarium verticillioides (Sacc) Nirenberg	Maize (*Zea mays* L.)
Macrophomina phaseolina (Tassi) Goid	Cowpea (*Vigna unguiculata* (L.) Walp)

Rhizomes harvesting and essential oil extraction

The rhizomes have been harvested in the Dindéresso classified forest, located about 15 kilometers in the west of Bobo-Dioulasso city. Extraction of the essential oil was carried out by hydrodistillation

with a Clevenger type extractor. The rhizomes were stripped of their impurities, cut into small slices and placed in a 500 ml (29/32) flask containing distilled water and heated. Essential oil has been condensed in separator funnel and separated from water by density difference. The essential oil is collected in a glass vial and dried with anhydrous sodium sulphate and stored at 4ºC. The average oil yield of three successive extractions was calculated according to the following formula:

$$R = \left(\frac{m}{M}\right) * 100$$

R: essential oil extracting yield; m: essential oil mass; M: rhizome mass.

Assessment of antifungal activity

Mycelial growth

To evaluate mycelial growth, three types of tests were carried out: the water control, the treatment with the essential oil and the fungicidal control. The water control is obtained by adding 4.2 g of Potato Dextrose Agar PDA (Liofilchem®, Italy) in 100 ml of distilled water. The mixture is autoclaved (PbI) at 121 ° C for 30 minutes. After cooling to 60ºC, it is distributed in 90 mm diameter petri dishes (Aptaca Italy) under a laminar flow hood (Napflow 12STD GV2EFR). For the preparation of the medium containing the essential oil, 4.2 g of PDA were introduced into 100 ml of distilled water. The mixture is sterilized as in the previous conditions. After cooling the culture medium to 60 ºC, the essential oil was emulsified with agar (0.01%) to obtain a final concentration of 0.10%. Two other media rich in essential oils at concentrations of 0.25 and 0.50% were prepared to test the fungi most sensitive to the essential oil at 0.10%. The Calthio C medium has been achieved by adding 4.2 g of PDA to 100 ml of distilled water and sterilized under the same conditions as above. After cooling to 60 ° C, 0.4 g of Calthio C has been added. The mixture was distributed in Petri dishes under aseptic conditions.

Inoculation of the media and incubation

From a five-day-old mycelial colony, 5-mm diameter mycelial fragments were removed from the frontal portion of the active growth zone of each fungus. The explants were placed in the center of the Petri dishes containing culture media. The Petri dishes thus inoculated were sealed with parafilm paper (Prafilm®, Neemah, Wi54956) and incubated at 25 ° C under 12 hours of alternating UV light (PhilipsTLD 36W / 08) with 12 hours of darkness for seven days [4].

Mycelial growth measuring

It was done four and seven days after incubation. For each evaluation of mycelial growth, an average of the different measurements made on the two perpendicular axes is considered. The inhibition percentage of mycelial growth is calculated according to the following formula:

$$I = \left[\frac{Dt - Df}{Dt}\right] * 100$$

I: inhibition percentage; Dt: average diameter of water control; Df: average diameter of the treatment

with the essential oil or the fungicide.

Phytotoxicity assessment

To achieve phytotoxicity tests, four types of treatment were carried out due to 100 seeds per treatment according to the International Seed Testing Association (ISTA) method. The different treatments are: The absolute control, the simple clay control, the fungicidal control (20 g Calthio C / 5 kg seed) and the essential oil at 0.50% which corresponds to the maximum concentration of essential oil having given maximal inhibition of mycelial growth. The mass of the clay used is proportional to the mass of the 100 seeds used according to the following formula [4].

$$Mc = \frac{A * 800}{5000}$$

Mc: mass of the clay; A: mass of 100 seeds.

The treated seeds were incubated at room temperature in the laboratory for 24 hours and sown in pots containing sterile fine sand in four repetitions due to 25 seeds per repetition. After sowing, the pots were placed under a tunnel at room temperature. Root length, height and weight of seedlings were evaluated seven day after sowing for maize and ten day for sorghum. The measurement of these biometric growth parameters was performed on 10 seedlings per speculation randomly chosen by treatment and by repetition.

Data analysis

The variance analysis was performed with the XLSTAT 2007 Software. The comparison of the averages was performed at the 5% threshold using the Student Newman Keuls test.

3. RESULTS AND DISCUSSION

Extracting yield

After three successive extractions by hydrodistillation, an average yield of 0.08 ± 0.01% was obtained.

Mycelial growth

The results of the variance analysis performed on the mycelial growth measurements after four day (Table 2) and seven day (Table 3) showed very highly significant differences between the controls and the treatment with essential oil at the concentration of 0.10%. The analysis of the results of the fourth day showed a more significant decrease in the mycelial growth of *Macrophomina phaseolina*, *Curvularia lunata, Colletotrichum graminicola* compared to the other fungi. Analysis of these seventh-day results showed that the most sensitive fungi remain the same as at day four. The three fungi most sensitive to the 0.10% essential oil were retained for further work at 0.25 and 0.50% essential oil.

Table 2: Mycelial growth four day after incubation

Treatment	Mycelial growth (cm)							
	A.a	C.d	C. g	C. l	F. m	F.ox	F. v	M. ph
WC	1.417[a]	3.467[a]	3.250[a]	5.600[a]	3.933[a]	3.383[a]	3.917[a]	8.417[a]
EO 0.1%	1.600[a]	3.600[a]	2.417[b]	4.083[b]	3.950[a]	3.333[a]	3.467[b]	4.017[b]
FO	0.500[b]	0.500[b]	0.500[c]	0.500[c]	0.500[b]	0.500[b]	0.500[c]	0.500[c]
p- value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

In the same column, the averages with the same letter "a" are not significantly different, unlike "b" and "c" at the 5% threshold; WC: water control; EO: treatment with essential oil; TF: fungicidal control. A.a : *Alternaria alternata ;* C.d : *Colletotrichum dematum ;* C.g : *Colletotrichum graminicola ;* C.l : *Cuvularia lunata;* F.ox : *Fusarium oxysporum ;* F.v :*Fusarium verticilloïdes;* M.ph: *Macrophomina phaseolina*

Table 3: Mycelial growth seven day after incubation

Treatment	Mycelial growth (cm)							
	A.a	C.d	C.g	C.l	F,m	F.ox	F.v	M.ph
Wc	2.717[a]	5.550[a]	4.883[a]	8.500[a]	6.783[a]	5.883[a]	6.750[a]	9.000[a]
EO 0.1%	2.783[a]	5.483[a]	3.950[b]	6.467[b]	6.400[a]	5.850[a]	5.567[a]	6.833[b]
FO	0.500[b]	0.500[b]	0.500[c]	0.500[c]	0.500[b]	0.500[b]	0.500[b]	0.500[c]
p-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

In the same column, the averages with the same letter "a" are not significantly different, unlike "b" and "c" at the 5% threshold; WC: water control; EO: treatment with essential oil; TF: fungicidal control; A.a : *Alternaria alternate;* C.d : *Colletotrichum dematum ;* C.g: *Colletotrichum graminicola* ; C.l : *Cuvularia lunata;* F.ox : *Fusarium oxysporum;* F.v :*Fusarium verticilloïdes;* M. ph: *Macrophomina phaseolina*

Comparison of mycelial growth at different concentrations of essential oil

The comparison of mycelial growth shows that the higher the essential oil concentration, the lower the mycelial growth of the three tested fungi. Considering the different concentrations of essential oil (0.10%, 0.25% and 0.50%) there are significant differences between the treatments performed. The highest inhibition of mycelial growth was obtained on *Colletotrichum graminicola* at 0.50% of essential oil (Table 4 and Figure 1, Photo 2).

Table 4: Mycelial growth at different concentrations of essential oil four and seven days after incubation

Treatment	Mycelial growth (cm)					
	C. graminicola		*C. lunata*		*M. phaseolina*	
	4 DAI	7 DAI	4 DAI	7 DAI	4 DAI	7 DAI
Wc	3.700[a]	7.817[a]	4.783[a]	7.817[a]	6.750[a]	9.000[a]
EO (0.1%)	2.417[b]	3.950[b]	4.083[b]	6.467[b]	4.017[b]	6.833[b]
EO (0.25%)	1.133[c]	2.217[c]	3.233[c]	5.517[c]	2.983[c]	6.033[b]
EO (0.50%)	1.050[c]	1.317[d]	2.417[d]	3.550[d]	1.967[d]	4.150[c]
FO	0.500[d]	0.500[e]	0.500[e]	0.500[e]	0.500[e]	0.500[d]
p-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

In the same column, the averages with the same letter "a" are not significantly different, unlike "b"; "c" and "d" at the 5% threshold; WC: water control; EO: treatment with essential oil; FO: fungicidal control.

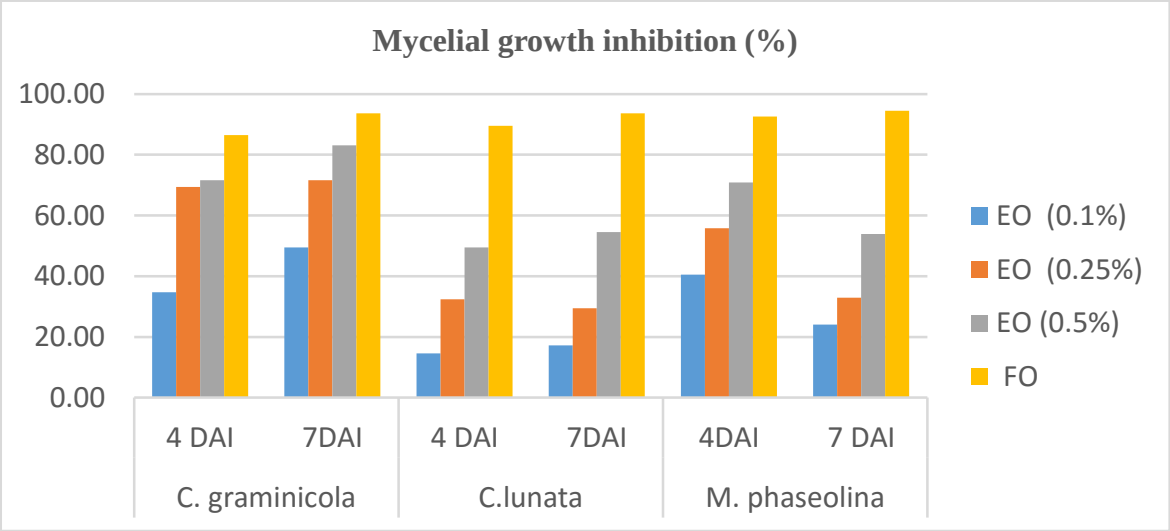

Figure 1: Mycelial growth inhibition percentage

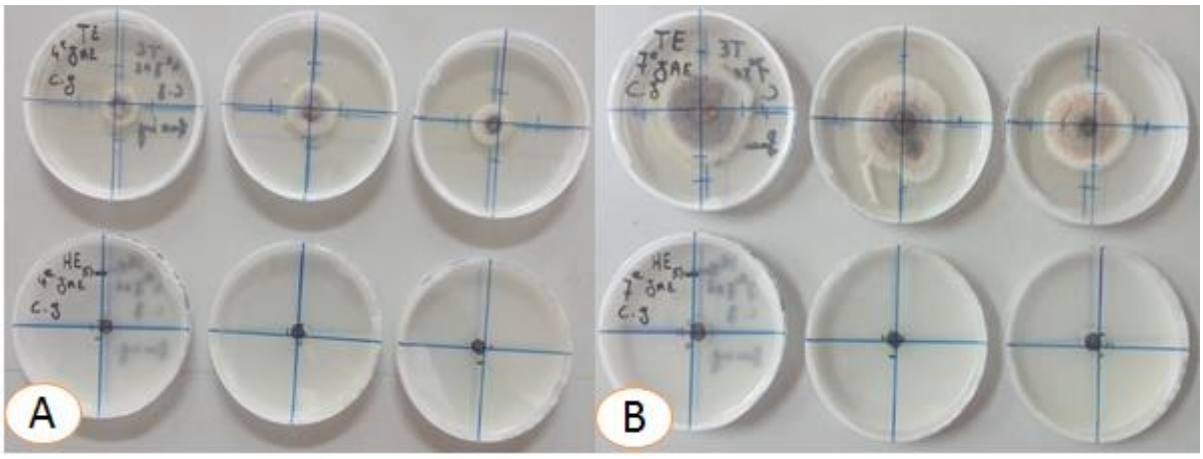

Photo 2: Mycelial growth of *C. graminicola* at 0.50% essential oil

Phytotoxicity: effect of essential oil on sorghum and maize seedlings growth

Table 5 show the results obtained on the vigor of seedlings of sorghum and maize. On the sorghum the essential oil has slightly inhibited the seedlings height. In fact, we have an average height of 7.65 cm with the essential oil against 8.47 cm for the water control. However, the variance analysis does not show any significant differences between the treatments on the roots length and the seedlings weight. The results obtained on maize seedlings, an average height of 11.6 cm was noted in the treatments that received the essential oil against an average height of 12.60 cm in the water controls. Regarding the weight of the seedlings, there was no significant difference in the different treatments. These results show that the essential oil compared to water has no significant effect on seedling growth parameters of maize and sorghum.

Table 5: Effect of essential oil on sorghum and maize seedlings growth

Treatment	Seedlings growth					
	Maize			Sorghum		
	Roots (cm)	Stem (cm)	Weight (g)	Roots (cm)	Stem (cm)	Weight (g)
Water control	15.600[a]	12.600[a]	1.80 [a]	23.550[a]	8.470[a]	0.284[a]
Clay control	20.830[b]	11.990[a]	1.746[a]	27.270[b]	8.230[a]	0.267[a]
Essential oil (0.5%)	15.380[a]	11.600[a]	1.459[a]	21.970[a]	7.650[b]	0.218[a]
Fungicid control	21.100[b]	12.630[a]	1.626[a]	23.680[a]	8.660[a]	0.263[a]
p-value	0.001	0.826	0.72	0.082	0.042	0.091

In the same column, the affected averages of the same letter are not significantly different at the 5% threshold according to Newman Student Keuls test.

DISCUSSION

The average yield of essential oil after extraction is 0.080 ± 0.01%, which is slightly lower than that obtained (0.12%) by Ouattara and collaborators [11].This difference could be explained by the difference in the soil and climate characteristics of the places where the samples were taken [13].

Measurement of the essential oil effect on mycelial growth showed variable activities on the tested fungi. At 0.1% essential oil, there was no significant difference between the mycelial growth of the water control and the essential oil treatment for the following fungi: *A. alternata*, *F. moniliforme*, *F. oxysporum and F. verticiloids*. In contrast at the same concentration the fungi having shown a notable sensitivity are *C. lunata*, *M. phaseolina* and *C. graminicola*. The second serie of tests showed a significant reduction in mycelial growth of *C. lunata*, *M. phaseolina* and *C. graminicola* at 0.25% and 0.5% compared to the water control. The maximum activity of this oil for the tested concentrations was obtained on *C. graminicola* with a reduction of 83.15% mycelial growth at the seventh day after incubation. This antifungal activity is probably due to the chemical compounds it

contains. The essential oil of *Cochlospermum planchonii* rhizomes has a particular chemical composition. It contains a high proportion of oxygenate compounds with a predominance of ketone and esters compounds (86.4%). Its main constituents are tetradecan-3-one (30.6%), tetradecene-3-one (15.3%), tetradecylacetate (15.0%) and dodecylacetate (12.4%) low proportion of sesquiterpenes and monoterpene [9]. Several studies have shown that essential oils rich in terpenoids may have antifungal properties [14], [15] on *C. graminicola* [16], [17], *C. lanata* [18], [19] and *M phaseolina* [20]. Monoterpenes could act on permeability but also other functions in cell membranes, thereby disrupting membranar exchanges [13], [21]. *C. planchonii* rhizomes essential oil used at 0.50%, showed a slight phytotoxicity on sorghum seedlings height. Phytoxicity of essential oils has been mentioned in the literature by many authors [22], [23], [24], [25], [26]. Regarding the study of the phytotoxicity of this oil on germination and the growth of maize seedlings, there was no significant difference with the controls samples. These results suggest the use of this antifungal agent oil especially in maize seed treatment.

4. CONCLUSION

The present study showed that the essential oil of *C. planchonii* has antifungal properties. At 0.1%, this essential oil did not significantly affect the mycelial growth of *A. alternata*, *C. dematium*, *F. moniliforme*, *F. oxysporum* and *F. verticillioides*. In contrast, at 0.5%, it significantly inhibited mycelial growth of *C. graminicola, C. lunata* and *M. phaseolina* with an inhibition rate more than 50% seven days after incubation.

In the next step of this work, we will undertake tests to appreciate the effect of higher concentrations of this oil on fungi species that showed resistance to low concentrations. This study could also be extended to other fungi and other plant pests. The results confirmed the antifungal activity of *C. planchonii* rhizome essential oil. This oil can be used in seed protection following further studies.

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CONFLICT OF INTEREST

The authors of this work state that there are no conflict of interest.

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