

Antifungal Activity and Phytotoxicity of *Vitex simplicifolia* Oliv. Leaves Essential Oil

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Abstract

Agriculture faces many difficulties related to phytopathogenic fungi leading to quantitative and qualitative losses of crops. The use of essential oils against these fungi is one of the alternatives. This study focused on the antifungal activity and phytotoxicity of *Vitex simplicifolia* Oliv. leaves essential oil. Firstly, we extracted the essential oil from the leaves by hydrodistillation. Subsequently, seven fungal isolates were evaluated for their sensitivity to the essential oil on Potato Dextrose Agar (PDA) media at 0.1; 0.25 and 0.50%. Evaluation of the phytotoxicity of the essential oil was performed on sorghum seeds on a sterilized sand substrate. At the seventh day after incubation, the most sensitive fungi to the essential oil are *Fusarium moniliforme*, *Colletotrichum graminicola* and *Curvularia lunata*. The most sensitive fungus to the essential oil at 0.50% is *Colletotrichum graminicola* with 77.21% inhibition rate at the seventh day after incubation. Phytotoxicity evaluation showed that for all biomass parameters measured there is no significant difference between treatment with the essential oil and treatment with the fungicide control. At the end of this study, it appears that *Vitex simplicifolia* essential oil has a high antifungal activity. This essential oil has not shown toxicity on sorghum growth parameters. Therefore, this oil can be used in organic farming.

Keywords: essential oil, *Vitex simplicifolia* Oliv, antifungal, phytotoxicity

1. Introduction

In Burkina Faso, cereals production is focused on sorghum, millet, maize and rice. In 2016, total cereal production was estimated at 4736000 tons (FAOSTAT, 2016). This cereal production faces many difficulties related to the parasites such as phytopathogenic fungi (Somda, Sanou, & Sanon, 2008) leading to quantitative and qualitative losses of crops. The most common control method of parasites is based on the use of chemicals (Hmiri et al., 2011). This method is limited by chemicals cost, environment pollution and human intoxication linked to anarchic use. The use of natural plant extracts against phytopathogenic fungi is one of the alternatives. Some extracts from plants have already been studied and have demonstrated their effectiveness in controlling phytopathogenic fungi (Nébié, 2006; Somda, Sanon, Michaud, & Sanou, 2003; Ouoba et al., 2018). In this context we aimed to assess the antifungal activity of *Vitex simplicifolia* leaves essential oil on seven phytopathogenic fungi. *Vitex simplicifolia* is a shrub 2-6 m tall, short bole, open top. The leaves are opposite, whorled or sometimes alternate, simple or trifoliate, more or less pubescent on both sides. The flowers color are pink to purple. The fruit is a globose glabrous and blackish drupe at maturity (Arbonnier, 2002).

Preliminary studies carried out on this oil had shown that it was rich in terpene compounds (Ouoba, Koudou, Somé, & Guissou, 2009). These terpene compounds are known to have antifungal properties (Nazzaro, Fratianni, Coppola, & De Feo, 2017), which justifies the relevance of this study.

2. Materials and Method

2.1 Materials

2.1.1 Plant Material

The plant material consists of *Vitex simplicifolia* leaves harvested during the months of May and June 2017 in the Dindéresso classified forest (Figure 1) and sorghum seeds of the local variety “kapelga” supplied by a company specialized in the production of certified seeds in Burkina Faso.



Figure 1. *Vitex simplicifolia*

2.1.2 Fungal Material

Fungi were provided by the Plant Clinic Laboratory of the Nazi Boni University (Table 1).

Table 1. Phytopathogenic fungi

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

2.2 Method

2.2.1 Leaves Harvesting and Essential Oil Extraction

The harvest of the leaves was achieved in the Dindéresso classified forest, located at 15 kilometers in the western part of Bobo-Dioulasso city and transported to the shelter of light at the laboratory of Biochemistry of the University Nazi Boni.

Extraction of the essential oil was carried out by hydrodistillation (Ouattara et al., 2007) with a Clevenger type extractor. The principle is as follows: The leaves were stripped of their impurities, cut into small slices and introduced into a 500 ml (29/32) flask containing distilled water and heated to gentle boiling. Water vapor and oil are entrained and condensed at the separator funnel. The different phases (oil/water) are separated by density difference. The essential oil is collected in a glass vial and dried with anhydrous sodium sulphate and stored at a

temperature of 4 °C. The average oil yield of three successive extractions was calculated according to the following formula:

$$Y = \left(\frac{m}{M} \right) \times 100 \quad (1)$$

Where, Y: essential oil yield; m: essential oil mass; M: plant material mass.

2.2.2 Evaluation of Antifungal Activity *in vitro*: Mycelial Radial Growth

Preparation of Culture Media: To evaluate mycelial growth, three treatments 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 at 121 °C for 30 minutes. After cooling to 60 °C, it is distributed in Petri dishes (Aptaca Italy) in aseptic conditions 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 °C, we prepared media rich in essential oil at the concentration of 0.10% for all fungi tested. Two other media rich in essential oil at concentrations of 0.25 and 0.50% were prepared to test the fungi most sensitive to the essential oil at 0.10%.

For the medium containing the fungicide Calthio C, 4.2 g of PDA were added 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 is added thereto.

Media Inoculation and Incubation: From a five-day-old colony, 5 mm diameter mycelial fragments were collected from the frontal portion in the active growth zone of each fungus. The explants were placed in the center of each Petri dish containing one of the culture media. The petri dishes thus inoculated were sealed with parafilm paper (Prafilm®, Neemah, Wi54956) and incubated in an incubation chamber under 12 hours of near-UV light (PhilipsTLD 36W/08) and 12 hours in darkness for seven days (Mathur & Kongsdal, 2003).

Measurement of Mycelial Growth: it was done four and seven days after incubation. For each evaluation of mycelial growth, an average of the different measurements is considered. The mycelial growth inhibition percentage is calculated according to the following formula:

$$I = \left[\frac{D_w - D_o}{D_w} \right] \times 100 \quad (2)$$

Where, I: inhibition percentage; D_w: average diameter of the water control; D_o: average diameter of treatment with essential oil or fungicide.

2.2.3 Phytotoxicity Assessment of the Essential Oil

Phytotoxicity was assessed on sorghum (Mathur, Njala & Kongsdal, 2003). Three treatments were performed due to 100 seeds per treatment. The different treatments are: The water control, the clay control, the fungicide control (20 g of Calthio C for 5 kg of sorghum seeds) and the essential oil at 0.5%. The mass of the clay used is proportional to the mass of 100 seeds used. This mass is calculated according to the following formula (Zida, Lund, & Néya, 2012; Ouoba et al., 2018):

$$Mc = A \times \left(\frac{800}{5000} \right) \quad (3)$$

Where, Mc: mass of clay; A: mass of 100 seeds

The treated seeds were incubated at room temperature 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 on the fourth and tenth day after sowing. The measurement of these biometric growth parameters was performed on 10 seedlings randomly selected by repetition. The number of emerged plant at the different assessment dates was used to calculate the percentage of seedling emergence by the following formula:

$$E = \left(\frac{n}{N} \right) \times 100 \quad (4)$$

Where, E: percentage of emergence; n: the number of emerged seedlings and N: the number of seeds sown.

2.4 Data Analysis

Radial growth averages of each fungus and averages of emergence rate, root length, shoot height, and seedling mass for each treatment were compared. The variance analysis was performed using the Student Newman Keuls multiple classification test at the 5% threshold. The XLSTAT version 2007 software was used.

3. Results and Discussion

3.1 Results

3.1.1 Extracting Yield

After three extractions we obtained an average yield of essential oil of $0.35 \pm 0.01\%$.

3.1.2 Mycelial Growth

The essential oil, at a concentration of 0.1%, reduced the mycelial growth of four fungi i.e. *C. lunata*, *C. dematium*, *F. moniliforme*, and *F. oxysporum* fungi on the fourth day after incubation (Table 2). On the seventh day, the three most sensitive fungi to the essential oil are *F. moniliforme*, *C. graminicola* and *C. lunata* (Table 3).

Table 2. Effect of essential oil on mycelial growth of fungi tested after four days incubation

Treatment	Mycelial growth (cm)						
	<i>A. alter</i>	<i>C. luna</i>	<i>C. dema</i>	<i>C. grami</i>	<i>F. moni</i>	<i>F. oxys</i>	<i>F. vert</i>
Water control	2.33b	4.48c	2.33c	2.21b	2.88c	3.15c	3.78b
Fungicide control	0.50a	0.50a	0.50a	0.50a	0.50a	0.50a	0.50a
Essential oil (0.10%)	2.40b	3.03b	2.18b	1.80b	2.05b	2.60b	3.53b
<i>p-value</i>	0.0001	0.0001	0.0001	0.00023	0.0001	0.0001	0.0001

Note. In the same column, the averages with the same alphabetical letter are not significantly different at the 5% threshold according to the Student Newman and Keuls test. *A. alter*: *A. alternata*; *C. luna*: *C. lunata*; *C. dema*: *C. dematium*; *C. grami*: *C. graminicola*; *F. moni*: *F. moniliforme*; *F. oxys*: *F. oxysporum*; *F. vert*: *F. vertillioides*.

Table 3. Effect of essential oil on mycelial growth of fungi tested after seven days incubation

Treatment	Mycelial growth (cm)						
	<i>A. alter</i>	<i>C. luna</i>	<i>C. dema</i>	<i>C. grami</i>	<i>F. moni</i>	<i>F. oxys</i>	<i>F. vert</i>
Water control	3.15b	9c	3.833b	5.650c	6.617c	5.067c	6.03c
Fungicide control	0.50a	0.50a	0.50a	0.50a	0.50a	0.50a	0.50a
Essential oil (0.10%)	3.13b	6.70b	3.583b	4.067b	4.367b	4.30b	5.16b
<i>p-value</i>	0.0001	0.0001	0.0001	0.0013	0.0001	0.0001	0.0001

Note. In the same column, the averages with the same alphabetical letter are not significantly different at the 5% threshold according to the Student Newman and Keuls test. *A. alter*: *A. alternata*; *C. luna*: *C. lunata*; *C. dema*: *C. dematium*; *C. grami*: *C. graminicola*; *F. moni*: *F. moniliforme*; *F. oxys*: *F. oxysporum*; *F. vert*: *F. vertillioides*.

Table 4 gives the values of the mycelial growth respectively at 0.25 and 0.5% essential oil. The essential oil significantly reduced the growth of the three fungi. The most sensitive fungus is *C. graminicola*. For this fungus the treatment with the essential oil did not show any significant difference compared to the treatment with the fungicide control. Figures 2 and 3 illustrate the high sensitivity of this fungus to the essential oil. The data in Table 4 on mycelial growth made it possible to calculate the percentage inhibition of mycelial growth (Figure 4). At 0.5% in essential oil, the inhibition percentage varies from 45.58% to 77.21%. At this concentration the maximum inhibition percentage for all these fungi is reached at the fourth day after incubation

Table 4. Effect of essential oil on mycelial growth four and seven days after incubation (DAI)

Treatments	Mycelial growth (cm)					
	<i>C. lunata</i>		<i>C. graminicola</i>		<i>F. moniliforme</i>	
	4 DAI	7DAI	4 DAI	7DAI	4 DAI	7DAI
Water control	6.23d	7.35d	3.70c	7.81c	4.41d	7.73d
Fungicide control	0.50a	0.50a	0.50a	0.50a	0.50a	0.50a
Essential oil (0.25%)	2.33c	2.90c	1.33b	1.95b	2.96c	5.05c
Essential oil (0.50%)	1.96b	2.43b	0.63a	1.78b	2.40b	4.18c
<i>p-value</i>	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Note. In the same column, the averages with the same alphabetical letter are not significantly different at the 5% threshold according to the Student Newman and Keuls test.

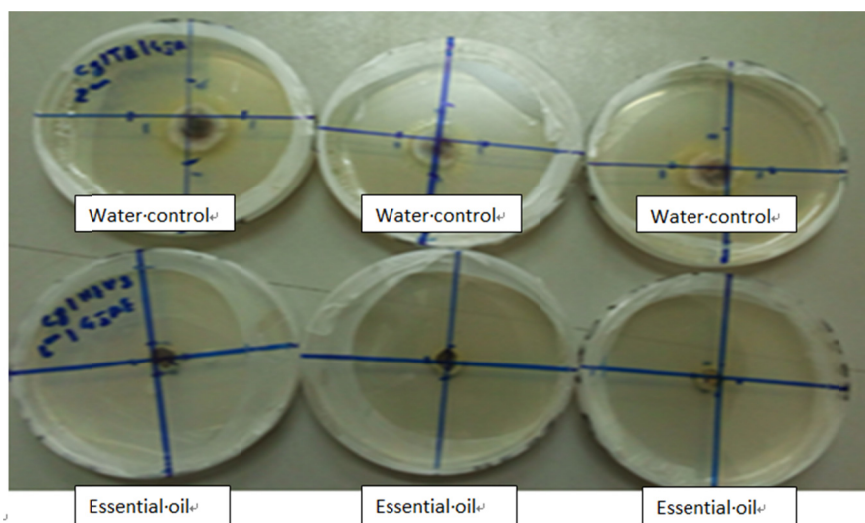


Figure 2. Mycelial growth of *C.graminicola* seven days after incubation in presence of essential oil at 0.25%

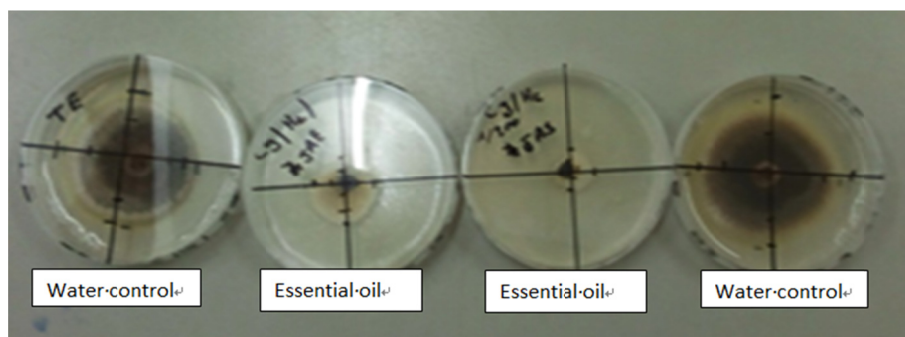


Figure 3. Mycelial growth of *C.graminicola* seven days after incubation in presence of essential oil at 0.50%

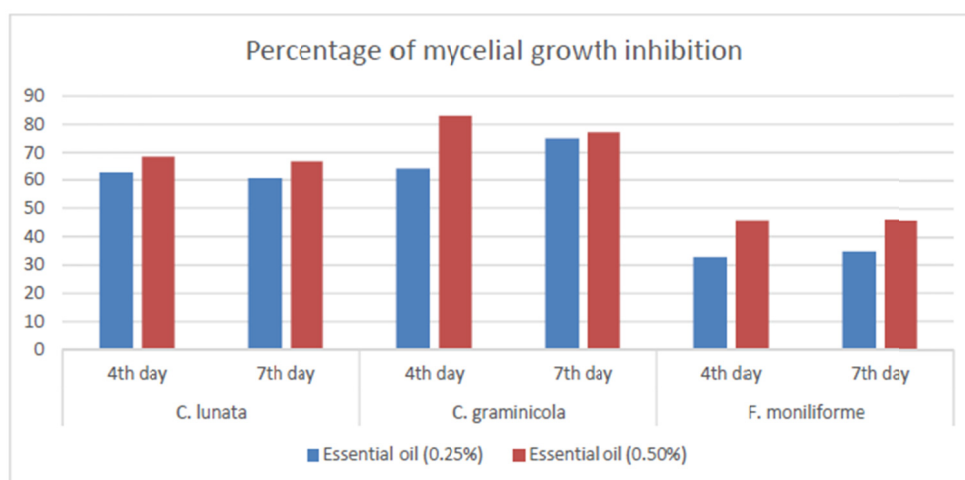


Figure 4. Inhibition percentage of the three most sensitive fungi to the essential oil four and seven days after incubation

3.1.3 Phytotoxicity of the Essential Oil on Sorghum

Table 5 gives the emergence rate, the averages of roots and stems lengths and that of the biomass. These results show that there is no significant difference for all these parameters between the treatment with the essential oil and the treatment with the fungicide control. The essential oil does not affect the growth of sorghum seedlings.

Table 5. Effects of treatments on the emergence, length and biomass of Sorghum seedlings

Treatments	Emergence rate (%)	Roots length (cm)	Stems length (cm)	Mass (g)
Water control	90 a	33.75b	11.90 a	0.27 a
Fungicide control	87 a	30.07a	13.24 a	0.26 a
Clay control	91 a	28.91a	12.78 a	0.24 a
Essential oil (0.50%).	89 a	29.66a	11.99 a	0.28 a
<i>p-value</i>	<i>0.81</i>	<i>0.03</i>	<i>0.16</i>	<i>0.23</i>

Note. In the same column, the averages with the same alphabetical letter are not significantly different at the 5% threshold according to the Student Newman and Keuls test.

3.2 Discussion

The extraction of the essential oil from the leaves of *Vitex simplicifolia* gave a yield of 0.35%. This value is in the yield range obtained by Nebié (2006) on the same plant which was 0.31 to 0.55%. It is relatively weaker than that obtained by Ouoba, Koudou, Somé, and Guissou (2009) which was 0.8%. Several factors such as harvest period, age of plant, stage of growth, pedoclimatic conditions, distilled part and extracting method may explain these differences (Satrani, 2001).

Taking into account the results on mycelial growth, there was a variable sensitivity to the essential oil of the seven fungi. At a concentration of 0.1% essential oil there was a reduction in mycelial growth of four fungi *C. lunata*, *C. dematium*, *F. moniliforme*, *F. oxysporum* at the fourth day after incubation. On the seventh day, the three most sensitive fungi are *F. moniliforme*, *C. graminicola* and *C. lunata*. These three fungi having been tested at 0.25 and 0.50% essential oil showed inhibition percentages ranging from 32.88% to 82.97% at the fourth day after incubation. This essential oil at 0.5% significantly reduced mycelial growth of *C. lunata*, *C. graminicola* and *F. moniliforme* with a higher activity on *C. graminicola* (77.21%) on the seventh day after incubation. These results show that this essential oil is endowed with antifungal activity. This could be explained by the nature of its chemical composition. Indeed, the work of Ouoba, Koudou, Somé, and Guissou (2009) on the chemical composition of the leaves of *Vitex simplicifolia* has shown that it contains mainly 71.02% of monoterpenoids, 64.70% of monoterpene hydrocarbons and 22.03% of sesquiterpene hydrocarbons with myrcene as major compound (53.50%). Several authors have shown that essential oils rich in monoterpenoids may have inhibitory activities on fungi (Hmiri et al., 2001) such as *C. graminicola*, *F. moniliforme* (Ouoba et al., 2018) and *C. lunata* (Ouoba et al., 2018; Gbogbo et al., 2005). These monoterpenes act on the mitochondria membrane of fungal cells and block their functioning (Nazzaro, Fratianni, Coppola, & De Feo, 2017; Yoshimura, Sawai, Tamotsu, & Sakai, 2011). For the three fungi tested at 0.25 and 0.50% essential oil, only *F. moniliforme* has an inhibition percentage less than 50% at the fourth (45.58%) and at the seventh day (45.92%) after incubation. This resistance to plant essential oils is also noted for several species of the genus *Fusarium* (Ouoba et al., 2018; Park et al., 2017). In an earlier study of the antifungal activity of the essential oil of *Zanthoxylum Zanthoxyloides* several fungi of the genus *Fusarium* (*F. verticillioides*, *F. moniliforme*) showed a resumption of growth four days after total inhibition of mycelial growth (Ouoba et al., 2018).

The phytotoxicity test of the essential oil used at a concentration of 0.50% has no phytotoxic effect on the emergence of sorghum seedlings. The biomass parameters of seedlings did not indicate a significant difference with the control fungicide. These results are similar to other studies that have shown that some plant essential oils have no toxicity on seeds and seedlings (Sorro et al., 2011). On the other hand, some authors have shown that the essential oils of certain plants can be toxic on seedlings (Vidal et al., 2018; El idrissi, EL Hurri, Amechrouq, & Boughdad, 2014). The essential oil of *Vitex simplicifolia* did not show a toxicity in this study deserves particular attention as for its valuation in organic farming.

4. Conclusion and Perspectives

The essential oil of *Vitex simplicifolia* showed a variable antifungal activity on the seven phytopathogenic fungi studied. An increase in antifungal activity was noted with increasing concentration of essential oil. The increase in

the amount of essential oil on the three most sensitive fungi (*F. moniliforme*, *C. graminicola* and *C. lunata*) accentuated its inhibitory effect on mycelial growth. For the three fungi tested at 0.25 and 0.50% essential oil, only *F. moniliforme* has an inhibition percentage less than 50% at the fourth (45.58%) and at the seventh day (45.92%) after incubation. The fungus which showed a high sensitivity to the essential oil at 0.5% is *C. graminicola* with an inhibition percentage of 77.21%. In addition, the phytotoxicity test conducted on Sorghum seeds indicates a non-toxicity of this essential oil.

Based on these results, the essential oil of *Vitex simplicifolia* leaves could be used as a natural fungicide for crop protection against phytopathogenic fungi. Achieving these objectives involves maintaining the specie in the plant heritage of our forests. In fact, the irrational use of the plant by farmers and traditional healers gradually leads to its disappearance in our forests. Therefore, extensive studies of its antifungal activity should be conducted on other crops and per fungus, to determine optimal and non-phytotoxic concentrations.

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