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RESEARCH PAPER

Evaluation of antifungal activity and phytotoxicity of the essential oil of *Zanthoxylum zanthoxyloides* fruits

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Abstract

The aim of this study is to find ways of biological control against the fungi responsible for the seed rot of three main food crops in West Africa: maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and cowpea (*Vigna unguiculata* (L.)Walp). Pathogenic fungi such as *Colletotrichum graminicola* (Ces) Wilson, *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliform* Sheldom, *Fusarium verticillioides* (Sacc) Nirenberg and *Macrophomina phaseolina* (Tassi) Goid. can reduce yields and limit pre- or post-harvest conservation. Antifungal activity and phytotoxicity tests of the essential oil of *Zanthoxylum zanthoxyloides* obtained by hydrodistillation were carried out respectively on the five pathogenic fungi and on the seeds of the three food crops. The antifungal activity of this essential oil was studied on the five pathogenic fungi by measuring the inhibition of mycelial growth on PDA medium containing the essential oil. Phytotoxicity was assessed by seedling emergence tests. The essential oil of *Z. zanthoxyloides* at 0.1% was very active against the five pathogenic fungi with a very strong inhibitory effect against *Colletotrichum graminicola* and *Curvularia lunata* where there is a total inhibition (100%). The essential oil did not show an inhibitory effect either on seed germination or seedling growth of the three food crops. The different properties of this essential oil suggest possible applications in the field of the fight against seedlings damping-off and seeds conservation.

Key words: *Zanthoxylum zanthoxyloides*, essential oil, Antifungal activity, phytotoxicity

Introduction

Food crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and cowpea (*Vigna unguiculata* (L.) Walp) occupy a central place in the diet of the peoples in West Africa. In Burkina Faso, in 2010, production of maize and sorghum seeds was estimated at 1,133,450 tons and 1,505,543 tons, respectively (Josserand, 2013). These statistical data show how

important these plants are in strategic and economic issues

Unfortunately, among the parasite pressures that have a significant impact on the production of these seeds, there are fungi that can also reduce yields and limit pre- or post-harvest conservation (Somdaet al., 2008). Among these pathogenic fungi there are *Colletotrichum graminicola* (Ces) Wilson, *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliform* Sheldom, *Fusarium verticillioides* (Sacc) Nirenberg (Somdaet al., 2008). Nowadays, the method commonly used to control pathogenic fungi remains the use of synthetic molecules (Hmiriet al., 2011) that are beyond the reach of the average grower and that pose risks to the environment and human health. One of the alternatives to chemicals that could help control these plant pathogenic fungi is the use of natural substances from local plant species. It is in this dynamic that we are interested in the essential oil of the fruits of *Zanthoxylum zanthoxyloides*. Several authors have shown the effectiveness of essential oils of species of the genus *Zantoxymum* in the fight against phytopatogenic fungi (Prieto, et al. 2011 ; Manandhar and Tiwari, 2005). Thus, essential oil of *Zanthoxylum zanthoxyloides* could be used as an alternative to the expensive synthetic fungicides for the Burkinabé farmers if its antifungal efficacy was proven. The fruits of this specie are rich in essential oil with an extraction yield up to 3.88% (Negi et al., 2011).

Indeed, *Zanthoxylum zanthoxyloides* belongs to Rutaceae family. It is a shrub or small tree, thorny up to 12 m high. The bark is gray to beige, rough, with fine vertical fissures and often with woody protuberances bearing prickles. The leaves are alternate, glabrous, imparipinnately compound, with 5 to 11 opposite or alternate leaflets and with numerous glandular points. The inflorescence is a terminal or axillary panicle 5-25 cm long, the fruits are ovoid dehiscent follicles, 5-6 mm in diameter, red and with black and shiny seeds (Berhaut, 1971). This plant is found in the tropical zone in West and Central Africa (Arbonnier, 2002). In Burkina Faso this specie is very common in some woodlands and savannas in the south-west of the country (Ouoba, 2006).

This plant is well known for its essential oil. Studies on a fruit sample from Benin have shown that the essential oil of this plant contained 85.5% of monoterpenoids among which we can cite linalool, geranial, beta-ocimen (Menutet al., 2011) . The essential oil of this plant has been studied by several authors for its biological properties (Ouattara, 2001; Adesina, 2005; Ouattara et al., 2009) but few of them has been interested in its use in phytopathology in the fight against the fungi of the cultures. The objective of this study is to evaluate the antifungal potential of this essential oil against five strains of plant fungi commonly grown in Burkina Faso. This work is a part of Burkina Faso's plant resources valorization.

Materials and method

Materials

Plant material

The fruits of *Zanthoxylum zanthoxyloides* were harvested from Dinderosso (Itm:30p: X = 0347000; Y =1241500) and Kua (itm:30p: X = 367237; Y = 1233166) Natural Reserves around the town of Bobo-Dioulasso. In this region, the flowering of *Zanthoxylum zanthoxyloides* begins in July. The fruits (photo 1) are available from October to March.

Photo 1: Ripe fruit of *Zanthoxylum zanthoxyloides*

Fungal material

Antifungal activities were evaluated on five fungal strains isolated from maize, cowpea and sorghum seeds provided by the Phytopathology Laboratory of Nazi BONI University (Table 1).

Table 1: Origin of the fungal strains used for the evaluation of the antifungal activity of the essential oil of *Z. zanthoxyloides*

Fungal strains	Origin
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 verticillioides (Sacc) Nirenberg	Maize (*Zea mays* L.)
Macrophomina phaseolina (Tassi) Goid.	Cowpea (*Vigna unguiculata* (L.) Walp)

Methods

Fruits harvesting

The fruit harvest took place in November and December 2016 in Bobo-Dioulasso. From 100 to 500 g of fruit were collected per individual depending on the availability of fruit. In the laboratory, the fruits were kept in a refrigerator (4 ° C) before the extraction of the essential oil by hydrodistillation.

Extraction of essential oil

Extraction of the essential oil was carried out by hydrodistillation with a Clevenger type apparatus (Clevenger, 1928). The extraction protocol can be described as follows: One hundred grams (100g) of crushed fresh fruit were introduced into a glass flask surmounted by a cooling column connected to a cryostat (temperature of the water circulating between 2 and 4 ° C). The whole is deposited on a furnace and heat up to boiling. The vapors of essential oil are precipitated at the level of the refrigerant and separated by decantation. After 4 hours of distillation, the essential oil, containing the residual water molecules, was dried with anhydrous sodium sulphate (Na 2 SO 4), collected in a flask and stored at 4 ° C.

Assessment of antifungal activity

Preparation of the culture media: water control, medium containing the essential oil

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 120 ° C for 30 minutes. After cooling to 60 ºC, 25 ml of medium are introduced in each Petri dishes (Aptaca Italy) under aseptic conditions using a laminar flow hood (Napflow12STDGV2EFR).

For the preparation of the medium containing the essential oil, 4.2 g of PDA is also put into 100 ml of distilled water. The mixture is sterilized as in the previous conditions. After cooling to 60 ° C, 100 microliters of the essential oil were introduced into 99.90 ml of PDA. The whole is then well homogenized to be distributed in Petri dishes under aseptic conditions.

Seeding of the media and incubation

The fungi to be tested are grown on PDA medium. From five (05) days mycelial colony, fragments of young mycelia of 5 mm size are delineated using a punch. These fragments are taken from the front of the petri dish. The explants were then placed in the center of the petri dishes containing the culture medium without essential oil and the culture medium containing the essential oil. The inoculated dishes were well sealed with parafilm (Parafilm®, Neemah, Wi54956) and placed in an incubation room under near UV light (PhilipsTLD 36W / 08) for 12 hours, alternating with 12 hours darkness for 7 days (ISTA 1999, Mathur and Kongsdal, 2003). We performed the triplicate test for each fungus.

Measurement of mycelial growth of fungi

Once the fungi are incubated, the measurement of their mycelial growth is made on the fourth and seventh day after incubation (DAI). For this, two perpendicular lines are drawn on the lid of the Petri dishes. The point of intersection of these lines must correspond to the center of the explant deposited on the culture medium. For each measurement of diameter, an average of the different measurements made on the two axes is considered. The mycelial growth inhibition percentage is calculated according to the following formula:**I (%) = (Dc –Dt /Dc)* 100.**

I (%): inhibition percentage; Dc: average diameter of the control colonies; Dt: average diameter of the treated colonies.

Phytotoxicity evaluation

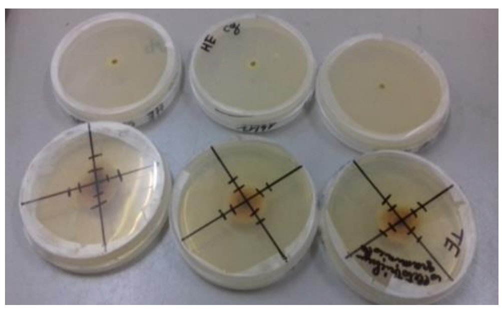
Phytotoxicity was assessed on three crops: maize, cowpea and sorghum.200 seeds per treatment at a rate of 20 seeds per repetition are sown in pots containing sterilized fine sand. The pots are placed in a tunnel between 20 and 35°C for 14 days. The number of emerged seedlings is evaluated four and seven days for maize, four and ten days for sorghum and five and eight days for cowpea after sowing. The measurement of biomass, ie the length of the roots, the length of the stems and the weight of the seedlings, is carried out 14 days after sowing. For this measurement, 10 seedlings are taken randomly by treatment. The different treatments are: (1) Absolute control (untreated seeds); (2) Fungicidal control (seeds treated with calthio C (25% chloroform-ethyl + 25% Thirame, WS)) (Zida, et al. 2012); the doses tested are 20 g of products for 5 kg of seed and (3) essential oil of *Z. zanthozyloides* at 100 microliters per treatment. The essential oil is used by trapping in fine powdered clay. The mass of clay that is used varies according to the crop because it takes into account the weight of the seeds of each species; it is determined by the following formula: Weight of 200 seeds x 800/5000.

Results and discussion

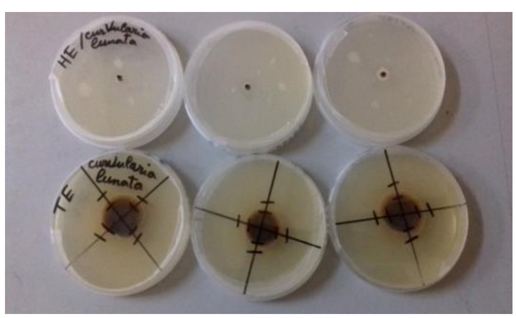
Results

Antifungal activity of the essential oil

Photo 2 shows the inhibitory effect of the essential oil at 0.1% on the mycelial growth of the five fungi studied in comparison with the absolute controls after seven days incubation. The summary of mycelial growth data is shown in Table 2. These data allowed us to calculate percentages of inhibitions of mycelial growth (Table 3). Total mycelial growth inhibition (100%) of *Colletetricum graminicola* and *Curvularia lunata* was observed with essential oil at 0.1% after seven days incubation. For other fungi tested, there is a relatively low resistance to the essential oil when compared to the absolute control. Nevertheless both species of *Fusarium* are more sensitive to the essential oil than *Macrophomina phasoelina*. The essential oil inhibits at 70.02, 65,74 and 44.41% the mycelial growth of *Fusarium moniliforme Fusarium verticilloide*s and *Macrophomina phasoelina* respectively after seven days incubation.



A



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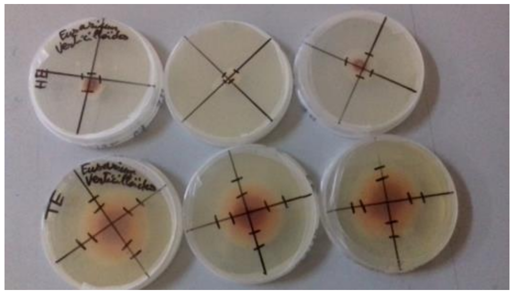

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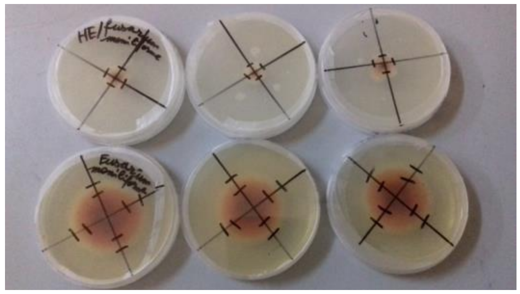

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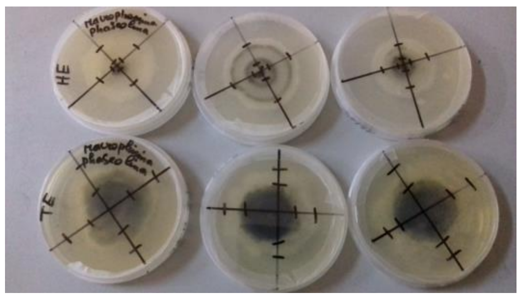

E

Photo 2: Mycelial growth on the seventh day

Table 2: Mycelial growth (in cm) of the five phytopathogenic fungi

Treatments ▼	*Colletotrichum graminicola*		*Curvularia lunata*		*Fusarium verticillioides*		*Fusarium moniliforme*		*Macrophomina phaseolina*	
	4th day	7th day	4th day	7th day	4th day	7thday	4th day	7thday	4th day	7thday
Essential oil	0,00 ± 0,00	0,00 ± 0,00	0,00± 0,00	0,00± 0,00	0,00 ± 0,00	1,48 ± 0,64	0,00 ± 0,00	1,37 ± 0,15	1,1 ± 0,38	3,52±0,65
control	1,92 ± 0,06	3,30 ± 0,13	2,27 ± 0,42	3,58 ± 0,33	2,92 ± 0,21	4,32 ± 0,03	2,95 ± 0,09	4,57 ± 0,06	3,45±0.45	6,22 ± 0,63

Table 3: Percentage of mycelial growth inhibition

Days after incubation ▼	*Colletotrichumgraminicola*	*Curvularialunata*	*Fusariumverticillioides*	*Fusariummoniliforme*	*Macrophominaphaseolina*
4	100	100	100	100	68.12
7	100	100	65.74	70.02	44.41

Phytotoxicity of the essential oil on seeds and seedlings

The seedling emergence test

The essential oil does not show any toxicity on the seeds of the three plants (maize, cowpea and sorghum). In fact, for the twenty seeds sown by repetition, at least 18 out of20 germinated (Table 4). These average germination rates observed with the seeds treated with the essential oil did not differ significantly (at a threshold of 5%) from the germination rates observed with the seeds of the absolute controls and the fungicidal controls. In the case of maize seeds, the emergence rate on the fourth day is greater ($p <0.05$) than the seed germination rates of the absolute control and the control fungicide. This suggests that the essential oil has a stimulating effect on the germination speed of maize seeds.

Table 4: Average germination rate of maize, cowpea and sorghum seeds; the values in the table indicate the number of seeds germinated on the twenty seeds sown by repetition.

Treatments ▼	Germination rate					
	Maize		Cowpea		Sorghum	
	4th day	7th day	5th day	8th day	4th day	10th day
Absolute control	15,6[b]	19,7[a]	11,1[a]	19,3[a]	16,1[a]	17,7[a]
fongicide Control	13,1[b]	18,9[a]	7,8[a]	19,3[a]	16,4[a]	18,5[a]
Essential oil	16,3[a]	19,5[a]	10,4[a]	18,1[a]	16,6[a]	18,3[a]
Probability	0,046	0,108	0,098	0,114	0,498	0,336

Within the same column, the mean values followed by the same letter are not significantly different (at 5% threshold) according to the Newman-Keuls test

Effect of essential oil on seedling growth

The essential oil does not show toxicity on seedling of maize, cowpea and sorghum. In fact, for sorghum, seedling size (length of roots, stems and weight of seedling) from seeds treated with the essential oil is not different from the seedlings of the absolute control and the control fungicide (Table 5). This essential oil shows, for maize and cowpea seedlings, growth stimulation of the roots, stems or weight of seedlings (Table 5). Thus, for cowpea, the stems of the seedlings treated with the essential oil are significantly longer than those of the absolute control and the fungicide control (Table 5). For maize, the stems and roots of the seedlings obtained from seeds treated with the essential oil are significantly longer and the weight of the seedlings wider than the seedlings of the absolute control and the fungicide control.

Table 5: Mean values of the length of the root and stem and mean values of seedling weight

Treatments ▼	rootLength (cm)			stem Length (cm)			seedling weight (g)		
	Maize	Cowpea	Sorghum	Maize	Cowpeaw	Sorghum	Maize	Cowpea	Sorghum
Absolu control	16,8[b]	10,2[a]	10,9[a]	15,6[b]	17,8[b]	12,2[a]	54,8[b]	181,1[a]	22,8[a]
Fongicide control	19,6[b]	12[a]	11,2[a]	18[a]	16,8[b]	12,1[a]	82,6[b]	140,3[a]	22[a]
Essential oil	39,8[a]	9,2[a]	12,6[a]	18,2[a]	20,6[a]	11,5[a]	183[a]	159,6[a]	24,1[a]
Probability	0,0001	0,05	0,33	0,031	0,024	0,506	0,0001	0,869	0,456

Within the same column, the mean values followed by the same letter are not significantly different (at 5% threshold) according to the Newman-Keuls test

Discussion

The results show that the essential oil of *Z. zanthoxyloides* harvested in western Burkina Faso has an inhibitory action on the five phytopathogenic fungi (*C. graminicola*, *C. lunata*, *F. moniliforme*, *F. verticillioides* and *M. phaseolina*) with complete inhibition of mycelial growth on *Curvilaria lunata* and *C. graminicola*. Several works have highlighted the antifungal activities of plants belonging to the genus *Zanthoxylum*. Similar work conducted with the essential oils of species of the genus *Zanthoxylum* against phytopathogenic fungi have shown that their essential oils have an inhibitory effect on several species of pathogenic fungi of the genus *Colletotrichum* and *Fusarium*. For example, the essential oil extracted from the fruits of *Zanthoxylum fagara* has shown strong activity against *Colletotrichum acutatum* and that of *Zanthoxylum. monophyllum* is very active against Fusarium oxysporum (Prieto, et al. 2011). Similarly, the essential oil of *Zanthoxyluma rmatum* showed a strong inhibition on the mycelial growth of *Bipolaris sorokiniana* (Manandhar and Tiwari, 2005). The essential oil of *Z. zanthoxyloides* harvested in Burkina Faso showed a high antifungal activity on the five phytopathogenic fungi that cause significant damage to food crops in Burkina Faso and West Africa. This broad spectrum of action against phytopathogenic fungi may be due to the chemical composition of its oil. According to Menut et al. (2000), the composition of the essential oil of the fruit pericarp of *Z. zanthoxyloides* is very diversified (more diversified than the essential oil of its leaves) with 85.50% of monoterpens and a large proportion of oxygenate compounds, including linalool, geranial and (E) -β-ocimen. Previous work has shown that the antifungal potential of an essential oil may be due to its chemical composition (Hmiri, et al. 2011). Thus, previous work has attributed the inhibitory effect of essential oils on fungal growth to the presence of monoterpens (Hmiri, et al. 2011). Monoterpens are the major compounds in the essential oil of *Z. zanthoxyloides* in this study. The class of monoterpens acts on the mitochondrial membrane of fungal cells and thus blocks the physiology of fungal cells (Nazarro et al., 2017; Yoshimura et al., 2010).

These results show that the *Zanthoxylum* genus, which includes more than 549 species (GBIF, 2017) distributed in the tropics and temperate regions, can be an important source of biomolecules for the control of pathogenic fungi. This can be an advantage because several species of this genus whose organs are used to extract the essential oil are also used in food. This is the case of *Z. zanthoxyloides* (Abdou-Bouba, 2009; Dongmo, et al. 2008), *Z. Bungeanum* Maxim. (Gong, et al. 2009), *Z. Leprieurii* Guill. And Perr (Ngoumfo, et al. 2010) and *Z. piperitum* DC. (Lee & Lim, 2008).

The results of this study showed that the essential oil of *Z. zanthoxyloides* does not inhibit the growth of seedlings of the three species studied. On the other hand, a slight stimulation of the germination speed of maize seeds by the essential oil is observed in comparison with the absolute and fungicidal controls ($p = 0.046$). Considering the other growth parameters (stem and root length, seedling weight), there is an improvement in the vigor of the seedlings. This is the case of root and stems length and seedling weight for maize and stem length for cowpea. These results open up huge potential for application of this essential oil in local agriculture because the cost of treating seeds with synthetic fungicides is often beyond the reach of most farmers. This plant is also widespread in southwestern vegetation of Burkina Faso (Ouoba, 2006). This availability of the raw material can allow exploitation of its fruits without risk of extinction of the species.

Conclusion

The research of new bioactive molecules as an alternative to chemical treatments based on synthetic fungicides leads us to explore the potential of our natural resources. In this context, this work was devoted to the study of the antifungal activity of the essential oil of *Z. zanthoxyloides* fruits on the mycelial growth of five fungi responsible for the yield reduction of three main food crops in West Africa. The essential oil of *Z. zanthoxyloides* has been shown to be very active against the five pathogenic fungi with a full inhibition of mycelial growth of *Colletotrichum graminicola* and *Curvularia lunata*. This antifungal activity is mainly due to the chemical composition of the essential oil probably rich in monoterpens. In addition, the essential oil of *Z. zanthoxyloides* did not show toxicity on the seeds and seedlings of the three food crops. This oil has also shown a stimulating effect on the vigor of seedlings. These results open perspectives for vulgarization of this essential oil to fight against food crops fungi because, this specie is widespread in the south-western area of Burkina Faso. This availability of the raw material can allow exploitation of its fruits without risk of extinction of the species. For this reason, we recommend the valorization of the essential oil of this specie to help local farmers. For the future, it will be necessary to domesticate this specie in order to increase the production of the essential oil of this specie.

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