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Research Article

PHYTOTOXICITY AND ANTIFUNGAL ACTIVITIES ASSESSMENT OF TWO TROPICAL PLANTS OILS IN WEST AFRICA

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ARTICLE INFO	ABSTRACT		
Article History: Received 13 th July, 2018 Received in revised form 11 th August, 2018 Accepted 8 th September, 2018 Published online 28 th October, 2018	Food crops such as Maize, Sorghum and Cowpea are widely produced and consumed in West Africa. Unfortunately the production of these food crops faced with several difficulties as well as phytopathogenic fungi which lead to a decrease in production yields and shelf life of foodstuffs. One of the alternatives to chemicals in fighting against these phytopathogenic fungi could be the use of natural substances from local plant species. This investigation has been carried out on the oils of two species of the Africa tropical flora: <i>Ozoroa insignis</i> Del. (Anacardiaceae) and <i>Zanthoxylum zanthoxyloides</i> (Lam.) Zepern. & Timler (Rutaceae). Antifungal tests were carried out on five		
Key Words:	phytopathogenic fungi commonly found on West Africa crops seeds. In addition, phytotoxicity tests were carried out on Maize, Cowpea and Sorghum seeds. The results showed that the oils of both		
Plant Oils, antifungal, phytotoxicity, West Africa	species have antifungal effect varying from one fungus to another and from one oil to another. Both oils have an inhibitory effect on <i>Fusarium</i> genus whose fungi involved in the damping-off. In addition, <i>O. insignis</i> oil showed a high inhibitory effect on <i>Macrophomina</i> genus. Phytotoxicity test has shown that <i>Z. zanthoxyloides</i> oil strongly inhibits <i>Sorghum</i> seeds germination. Finally, both oils		

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have shown a stimulating effect on Maize and Cowpea roots growth.

INTRODUCTION

Maize, Sorghum and Cowpea seeds are mainly used in the diet by people of the West Africa. In 2010, Burkina Faso's Maize and Sorghum seeds production was respectively estimated to 1133450 and 1505543 tons (Josserand, 2013). These data show how important these food crops are strategic in economic issues. Unfortunately the production of these cereals faces several difficulties, including parasitic pressures such as phytopathogenic fungi. The major phytopathogenic fungi faced by farmers are Macrophomina phaseolina, Colletotrichum graminicola, Phoma sorghina, Fusarium moniliform, Fusarium verticiloides, Colletotrichum dematium, Curvularia lunata, Bipolaris maidis (Somda et al., 2008). These fungi reduce vields and limit pre- or post-harvests conservation (Somda et al., 2008). Nowadays, the commonly method used to control phytopathogenic fungi are chemicals (Hmiri et al., 2011) leading to adverse effect on human health and environment (Coleman, 2012). Efforts to find less hazardous pesticides have led to the study of new biological fungicides to control these fungi (Kassi et al., 2014). Thus, one of the alternatives to chemicals that could help control these plant pathogenic fungi

is the use of natural substances from local plant species. Our investigations have been carried out on the oils of two species of the Africa tropical flora: *Ozoroa insignis* Del. (Anacardiaceae), *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler (Rutaceae). These species are fairly common in the Sudano-Guinean zone. Their geographical area extends from Senegal to South Africa (Arbonnier, 2002, Berhaut, 1971)

MATERIALS AND METHOD

Materials

Plant material

The fruits of both plant species were harvested around Bobo-Dioulasso city during the fruiting period of each species (Table 1).

Plant	Fruit type	Oleagino us part	Harvest period		
Zanthoxylum zanthoxyloides	Capsule	seed	November		
Ozoroa insignis	Drupe	Mesocarp	December		

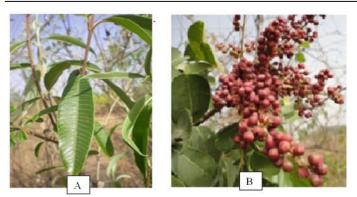


Figure 1 Twigs of O. insignis and Z. zanthoxyloides

Fungi

Antifungal activities were evaluated on six pathogenic fungi isolated from Maize, Cowpea and Sorghum seeds provided by the Phytopathology Laboratory of the Nazi Boni University (Table 2).

Table 2 Pathogenic fungi

Pathogenic fungi	Origin		
Colletotricum dematium (Pers. Ex Fr) Grove	Cowpea (Vigna unguiculata (L.) Walp)		
Colletotrichum graminicola (Ces) Wilson	Sorghum (Sorghum bicolor (L.) Moench)		
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

Seeds harvesting and oils extracting

Seeds harvesting has been achieved around the city of Bobo-Dioulasso in the Dinderosso and Kua Natural Reserves. After drying the seeds, they were crushed with a GM-200 type Retsch grinder. The ground materials obtained were used for the fat extraction. The extraction was done using a soxhlet type extractor according to NF EN ISO 659, 2009 standard. For that, the cartridges (HM1004-33x94mm) were filled before hand with 200g grams of ground material and placed in the extractor column. Petroleum ether (CARLO ERBA REAGENT-SDS), was used as solvent. After 4 hours, the extract (mixture of ether and fat) was evaporated (Buchi ROTAVAPOR R-200) to remove the solvent. The oils free from solvent were stored at 4° C prior using.

Biological tests

Evaluation of antifungal activity of oils

The phytopathogenic fungi received from Plant Pathology Laboratory of Nazi Boni University were transplanted into inclined YMA tubes (Difco® YeastExtract 4g / L, Difco® Malt extract 4g / L, Sucrose 4g / L and Agar 15 g / L) and incubate for seven days at 20°C. To each YMA tube, 20 ml of miliQ water and sterile Tween 20 (one drop of Tween 20 per 100 ml of water) were added. The growth medium upper part was scraped to release the spores. We then proceeded to filtration using a sterile filter. Spore counting was done with the Fuchs-Rosenthal type counting cell. After counting, the conidial suspension was adjusted to 20000 conidia per ml. The antifungal test was carried out in 90 mm diameter petri dishes (Aptaca Italy) by the disk diffusion method. After inoculating the growth media with fungal inocula by surface flooding, the paper disks were impregnated with 20 microliters of oil of each plant species and deposited on the surface of the culture media. Three disks were deposited for each petri dish. The inoculated dishes were sealed with parafilm (Parafilm®, Neemah, Wi54956) and placed in an incubation room under near ultraviolet light for 12 hours and alternated with 12 hours of darkness (Mathur and Kongsdal, 2003). After 7 days incubation, the inhibition zones were measured.

Oils Phytotoxicity evaluation

Phytotoxicity was evaluated on Maize, Cowpea and Sorghum by two treatments at a rate of 100 seeds per treatment. The different treatments are: The water control and oil treatment at the rate of 100 μ l per g of seed. The treated seeds were incubated at room temperature for 24 hours and sown in pots containing sterile fine sand in five replicates of 20 seeds per repetition. After sowing, the pots were placed under a tunnel at room temperature (Mathur and Kongsdal, 2003). The evaluation of phytotoxicity consisted of counting the number of emerged seedlings on the fourth and seventh day after sowing for maize, on fourth and tenth day for sorghum, and fifth and eighth days for cowpeas. Stems height, root length of 20 seedlings of each tested crop (sorghum, maize and cowpea) randomly taken in each replicate were measured for the biomass produced 10 days after sowing.

Data processing and analysis

The Microsoft Excel 2013 spreadsheet was used to enter data and graphs. The XLSTAT 2007 software was used to analyze the data. The comparison of the means was performed using the Student Newman Keuls multiple comparison test at the 5% threshold.

RESULT AND DISCUSSION

Results

Antifungal activity of oils

The inhibitory effects of each oil have varied from one fungus to another after 7 days of incubation. *O. insignis* oil has an inhibitory effect on all tested phytopathogenic fungi except *C. graminicola.* Similarly, *Z. zanthoxyloides* oil has an inhibitory effect on all tested phytopathogenic fungi except *M. phaseolina* and *C. graminicola.* Oils of both species have shown the broadest antifungal spectra by inhibiting more than 50% of the five studied fungi. *O. insignis* oil has shown the best inhibition diameter of 22.23 mm on *M. phaseolina*; As for the *Z. zanthoxyloides* oil, it has shown the best inhibition diameter of 14.33 mm on *F. verticillioides.*

 Table 3 Effect of oils on the mycelial growth of the six

 phytopathogenic fungi

	Inhibition zone (mm) $(n = 3)$			
Phytopathogenic fungi	Ozoroa insignis	Zanthoxylum zanthoxyloides		
Macrophomina phaseolina	22.33 ± 0.58	0		
Colletotrichum graminicola	0	0		
Fusarium moniliforme	15 ± 0.38	14± 0		
Fusarium verticillioides	12.67 ± 0.62	14.33 ± 0.71		
Colletotrichum dematium	15 ± 0.65	$11.33\pm\ 0.58$		

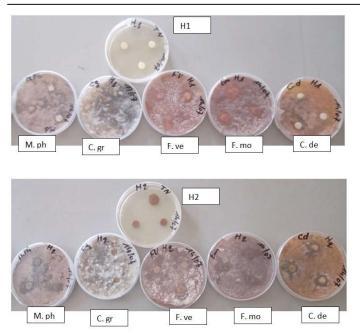


Figure 2 Effects of *Z. zanthoxyloides* and *O. insignis* oils on mycelial growth M. ph: *M. phaseolina*, C. gr: *C. graminicola*, F. ve: *F. verticillioides*, F. mo: *F. moniliforme*, C. de: *C. dematium*, H1: Oil of *Z. zanthoxyloides* without inoculum, H2: Oil of *O. insignis* without inoculums

Phytotoxicity of Z. zanthoxyloides and O. insignis oils on seeds and seedlings

Seedling emergence test

The inhibitory effects of each oil have varied from one culture to another (Table 4, Figure 3, Figure 4): In general, both oils inhibit the germination of Sorghum seeds. Thus, on the fourth and seventh day after incubation, the germination rates of Sorghum seeds treated with oils are significantly lower (at the 5% threshold) than those that have not been treated. In contrast, *O. insignis* oil did not show an inhibitory effect on germination of Maize and Cowpea seeds (Figure 4). The germination rates of the seeds of these two crops did not differ significantly (at a threshold of 5%) from the germination rate observed with the seeds of the water control. On the other hand, it is found that the germination of Maize and Cowpea is inhibited by *Z. zanthoxyloides* oil (Figure 3). In the case of Maize and Cowpea, emergence rates on fourth and seventh days are lower (p <0.05) than germination rates of water control seeds.

Table 4 Average germination rate of Maize, Cowpea and

 Sorghum seeds. The values in the table indicate the number of

 seeds germinated on twenty seeds sown by repetition

Treatment	Sorghum		Maize		Cowpea	
1 reatment	4DAS	10DAS	4 DAS	7 DAS	5 DAS	8 DAS
Water	15a	15a	19.4a	19.4a	14.2a	14.4a
Z. zanthoxyloies oil	1.8b	2.2b	12.6b	14b	6.2b	10.8b
O. insignis oil	6c	7.4c	19.4a	19.6a	15.4a	15.8a
P-value	< 0.0001	< 0.0001	0.004	0.004	0.001	0.016

Effect of both oils on seedling growth

Table 5 presents the results of stem and root length measurements of Sorghum, Maize and Cowpea. *Z.zanthoxyloidses* oil significantly reduces Sorghum seedlings stems and roots growth. For Maize and Cowpea, *Z. zanthoxyloides* oil significantly reduces stem growth. On the other hand, *O. insignis* oil has no inhibitory effect on seedling

stem and root growth of all the three crops. The organs (stems and roots) of seedlings from seeds treated with *O. insignis* oil are longer than those of the water control.

Table 5 Mean values of root and stem length

Treatment	Sorghum		Maize		Cowpea	
	Root	Stem	Root	Stem	Root	Stem
Water control	18.2a	18.2a	18.2a	18.2a	18.2a	18.2a
Z. Zanthoxyloides oil	7.9b	4.73b	27.15b	12.55b	16.8a	10.7b
O. insignis oil	24.00a	8.18a	26.55b	13.2a	17.0a	12.4a
P-value	< 0.0001	< 0.0001	0.015	0.193	0.462	< 0.0001

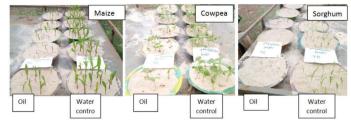


Figure 3 Effect of *Z. zanthoxyloides*oil on Maize, Cowpea and Sorghum seedlings, ten days after sowing.



Figure 4 Effect of *O. insignis*oil on Maize, Cowpea and Sorghum seedlings, ten days after sowing.

DISCUSSION

The results obtained show more or less effective antifungal activity for the two oils against the tested fungi. The overall results have shown an inhibition of mycelial growth with the different oils. The oils had a high antifungal effect on M. phaseolina, Colletotrichum dematium and Fusarium moniliforme for O. insignis and the two species of the Fusarium genus for Z. zanthoxyloides. Several studies have shown the antifungal activity of plant natural substances on phytopathogenic fungi. But most of these works have been focused searching for new antifungal molecules based on essential oils (Ouattara et al., 2018, Ouoba et al., 2018, Gansoré et al., 2018). Seeking natural molecules against phytopathogenic fungi based on fixed oils is not much mentioned in the literature. However, fixed oils can also be used as natural antifungals whose are less harmful to human health (Illah, 2013). The antifungal activity of fixed oils can be attributed to their fatty acids profile. According to some authors, the antifungal activity of fixed oils could be related to the presence of linoleic and linolenic acids (Illah, 2013; Schafferman et al., 1998). Indeed, previous works on the fatty acids profile of O. insignis and Z. zanthoxyloides fixed oils have shown that their oils contain more or less important proportions of linoleic and linolenic acids (Ouattara et al., 2017; Ouattara et al., 2016). The antifungal activity of the two oils being studied here can be related to the presence of these compounds in their oils.

Phytotoxicity assessment of these two oils showed that their inhibitory effects on tested food crop seeds and seedlings

varied from one crop to another. Thus, O. insignis oil has no inhibitory effect on Maize and Cowpea growth parameters. On the other hand, this oil inhibits the germination of Sorghum seeds. These results suggest that this oil can be exploited in the treatment of maize and cowpea seeds in organic farming. In addition, Z. zanthoxyloides oil inhibits the Maize, Cowpea and Sorghum seeds germination. Z. Zanthoxyloides oil has shown a weak inhibition of stem growth. However, except Sorghum, this oil has enhanced the development of Maize and Cowpea root system. An increase in Maize and Cowpea root length was observed with both Z. zanthoxyloides and O. insignis oils. At the same time, these two oils have an inhibitory effect on Fusarium genus, major food crops fungi. This increase in the root length of seedlings treated with oils compared to untreated seedlings could be due to the inhibitory effect of these oils on these two species of Fusarium, fungi that can lead to dampingoff (Lamichhane, 2017). Similar results have been reported in the literature by several authors whose have shown that treating seeds with plant extracts with antifungal properties can increase root vigor (Ouédraogo et al., 2018; Islam, 2012).

CONCLUSION

The present study has highlighted the antifungal potential of oils of *O. insignis* and *Z. zanthoxyloides*, two African tropical plants. Both oils studied have shown a broad antifungal spectrum by inhibiting at least 50% of the fungi tested. These oils have shown activities against *Fusarium* genus which causes enormous damage to food crops such as damping-off. In perspective, other properties of these oils could be investigated in other fields of application. Indeed, the phytotoxicity study of these oils has shown an enhancement of the roots of seedlings of Maize and Cowpea. Subsequent studies will better show the agronomic benefits of the oils of these two plants beyond their antifungal properties.

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Conflict of interest

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

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