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Post-Harvest Fungi of *Vitellaria Paradoxa* and *Parkia Biglosa* in Chad Republic and Bioactivity of Natural Products against some Pathogenic Fungi

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ABSTRACT

In Chad Republic, kernels/grains of Shea butter Nuts butter tree (*Vitellaria paradoxa*) and locust bean (*Parkia biglobosa*) are two Non-Timber Forest Products (NTFP) which offers important revenues to the growers in the production, processing and marketing chain. However, un-identified post-harvest fungi that affect their sanitary quality, marketability and nutritional value are responsible for high post-harvest losses. The objective of the study was to contribute to the management of the post-harvest diseases of Shea butter nuts and locust bean. To achieve this, fungi were isolated from infected grains and their pathogenicity tested. Then, antifungal activity of essential oil (EO) of *Thymus vulgaris* (obtained by hydro distillation) and crude extract of Panax africana (obtained from a naturopath) was carry out by the dispersion method on the agar medium on four pathogenic fungi isolated from the two infected NTFP. Results showed that fungal species frequently associated with Shea nuts and locust bean grains were: *Aspergillus niger* (46%), *Rhizopus nigricans* (17%), *Oidium* sp (22%) and *Cercospora* sp (8%); and *Oidium* sp (55%), *A. niger* (18%), *A. flavus* (18%) and *Cercospora* sp (6%) in *V. paradoxa* and *P. biglobosa* respectively. Pathogenicity test was positive with all species of the genus *Aspergillus* and the species *Oidium* sp. Growth inhibition of the four fungal species tested with essential oil of *T. vulgaris* at 1.5 µl/ml and with crude extract of Panax africana at 120 µg/ml and this was significantly comparable (p<0.05) to the reference fungicide (Terazeb). Biological control of post-harvest diseases of *V. paradoxa* and *P. biglosa* grains with EO of *T. vulgaris* and Panax africana is envisaged for further studies.

Key words: Antifungal activity, Post-Harvest Fungi, *Vitellaria Paradoxa*, *Parkia Biglobosa*, Panax Africana, *Thymus Vulgaris*.

INTRODUCTION

Vitellaria paradoxa C.F. Gaertn (Shea butter Nuts tree) and *Parkia biglobosa* Jacq R.Br. (locust bean) are two plant species endemic to the Sudanian zone of Africa (FAO, 2004). Due to their socio-economic and cultural importance in Chad, their exploitation constitutes an income-generating activity of interest in rural areas, especially for women. All parts of these plants are useful and can be used for human consumption, as well as in pharmacopoeia and for industrial purposes. Mastering their production and rational exploitation can contribute to food security and promote sustainable development for rural people. Today, Shea butter Nuts tree and locust bean sectors are ranked among the priority sectors in Chad and exploitation of these resources has a positive socio-economic impact and the Shea butter Nuts tree sector could be an alternative to oil exploitation (MAI, 2013).

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Due to their long production cycle (15 to 25 years), the Chadian government has therefore undertaken to increase the productivity of these species in order to satisfy the growing domestic and external demand for grains. Shea butter Nuts is classify on the International Union for Conservation of Nature list as endangered species due to bushfires and overexploitation of the grains (Lisan, 2017). Both in the field as well as in post-harvest, trees and grains are subjected to various fungal infections. For example, in Shea butter Nuts, fungi *Fusicladium butyrospermi* and *Pestalozzia heterospora* are responsible of leaf spots and high yield losses (Dudut, 2012). In locust bean, the genus *Phellinus* sp (Basidiomycetes) cause tree dieback and *Cercospora* sp, *Hypoxylon rubiginosum* and *Phyllachora leonensis* were reported on leaves (Eyog et al., 2000). In the best of our knowledge, very little work has been done on post-harvest fungi of Shea butter Nuts nut and locust bean grains in Africa in general and in Chad in particular. However, in the field, rots and moulds present on these products are responsible for grains depreciation and loss of their trade quality.

MATERIALS AND METHODS

Collection and Storage of Samples

Healthy and necrotic nuts of Shea and locust bean (Figure 1) were collected in the Sudanian zone of Chad, specifically in six markets in the provinces of Moyen-Chari (9° 08′ 46″ N, 18° 23′ 03″ E) and Mandoul (8° 54′ 36″ N, 17° 33′ 00″ E). The average annual temperature is 27°C with a minimum of 15.5 and maximum of 39.1°C and the annual relative humidity is 59.7% with a minimum of 29.4% and a maximum of 81.6% (Climate data, 2018). Grains collected were transported to the Phytopathology laboratory of the University of Dschang, Cameroon and stored at 4ºC before fungi isolation.

Fig. 1: Grains contamination of *V. paradoxa* by fungi. (A) entire grains, (B) section of infected grains showing fungal fruiting bodies and (C) section of healthy grains free of fungi.

Isolation and Identification of Fungi

Isolation of fungi associated with infected Shea butter Nuts and locust bean grains was done using the blotting paper and agar medium methods. Isolation on blotting paper consisted of a using a total of 400grains of each plant species. Infected grains were disinfected in a 5% bleach solution for 2 min and rinsed 3 times during 5, 10 and 15 min with sterile distilled water to eliminate disinfectant residues. Then, five to ten disinfected grains were plated in a 90 mm diameter Petri dish containing moistened (with sterile distilled water) filter paper (ISTA, 2001). Incubation program was at lab temperature (22±2°C with alternating 12 h of light and 12 h of darkness for eight days. After that, grains were examined under a magnifying glass (10X) to observe the presence of mycelia filaments (Amadi et al., 2014). Isolation on agar medium took place on potato dextrose agar (PDA) medium. Grains disinfected as previously describe were fragmented and placed in sterile Petri dishes containing 20 ml of PDA medium with 5 fragments per dish in the microbiological hood. Petri dishes were incubated at 21ºC during five days, then the fungal colonies visible on the incubated on fragmented grains were isolated and purified on the same culture medium (Djeugap et al., 2017). Fungi identification was carry out using the classical identification method. Indeed, morphological characters' fungi such as mycelium structure and spore morphology were used for identification by referring to identification keys of fungi (Alexopoulos et al., 1996). Isolation frequency of each fungus was calculated using the following formula: F = (NF÷NT) × 100, where F represents the frequency of occurrence (%) of a fungus, NF is the total number of samples from which a particular fungus was isolated and NT is the total number of samples from which isolations were carried out (Iqbal and Saeed, 2012).

Pathogenicity Test

For pathogenicity, grains were disinfected with 99% ethanol for 5 min and rinsed with sterile distilled water. Then, spores suspension of each purified and identified fungus was calibrated at $2x10^4$ conidia/ml and inoculated on ten healthy grains by dipping the healthy grains (slightly scarified) with 50 ml of spore suspension. The inoculated grains were introduced in Petri dishes and placed in dark boxes and incubated at Lab temperature (22±2ºC) (Umana et al., 2015). Pathogenicity was positive when the inoculated fungus develop disease symptoms on grains or negative otherwise. Infection rate (IR) was calculated as: IR = (number of disease grains/total number of inoculated grains) x 100.

In Vitro Efficacy of Essential Oil of *T. vulgaris* and Panax Africana

Preparation of Essential Oils

Essential oil extraction was through hydro distillation in a Clevenger type apparatus. In fact, 150 g of fresh leaves were placed in a 2000 ml flask, a volume of about 1200 ml of water was added, and the whole was brought to the boil using a heating flask. During hydro distillation, oil-laden vapours passed through a refrigerant column and condensed. Then, oil and water separated by density difference since oil is lighter than water (Nguemtchouin, 2012). Essential oil collected was stored at 4°C in the dark in the presence of anhydrous sodium sulphate (Yaouba et al., 2010).

Inhibition of Radial Growth of Fungi by the Two Natural Products

Antifungal activity of *T. algeriensis* EOs was tested against fungi that are potentially dangerous for consumers because of their ability to produce mycotoxins at 0.25; 0.5; 0.75; 1 and 1.5 µl/ml (Yaouba et al., 2010) and at 1; 15; 30; 60; 120 µg/ml for aqueous extracts of Panax africana. A drop of 1% Tween 80 was added in each product to allow their mixture with the culture medium. Mycelium explants of 5 mm in diameter, were die-cut from a pure seven days old fruiting culture and placed in the centre of the Petri dish. The dishes were incubated for 7 days at room

temperature (Tsopmbeng et al., 2014). The radial growth diameter of each cultured was measured on a daily basis until the mycelia filled the control dishes. Inhibition percentage (IP) of the pathogen by the natural products was obtained using the formula IP = 100 x (A-B)/A (Dohou et al. 2004) where A is the average diameter of the mycelium in the control Petri dishes, B is the average diameter of the mycelium in the presence of essential oils or aqueous extract of Panax africana.

Statistical Analysis

The equivalent concentration of the inhibition of 50 and 90% (EC50 and EC90) of the fungal growth by *T. vulgaris* and African Panax were obtained by transforming inhibition percentages into probits. Statistical analysis was performed using SPSS (Statistical Package for Social Science version 21) software program. The analysis of variance (ANOVA) was performed for each variables collected using the generalized linear model. Means were separated using the Student's smallest significant difference test at 5%.

RESULTS

Infection Rate of Grains in their Collecting Points and Post-Harvest Fungi of Shea Butter Nuts and Locust Bean Grains

Grains of *V. paradoxa* and *P. biglobosa* traded in the different markets of Sarh and Koumra are colonised by several species of post-harvest fungi. Morphological observations reveals that grains of *V. paradoxa* are the most infected compared to *P. biglobosa* irrespective to the locality/market where grains were collected. Infection rates range from 77 to 95% in *V. paradoxa* and from 0.6 to 2.6% in *P. biglobosa* (Table 1). Fungi frequently isolated were *Aspergillus niger* (46.34 %), *Rhizopus nigricans* (17.88 %) and *Oidium sp* (22.76 %) on *V. paradoxa* and *Oidium* sp (54.90 %), *A. flavus* (18.63 %) and *A. niger* (18.63 %) on *P. biglobosa* (Table 2).

Germination, Infection and Seed Vigour Index

In general, germination was very low irrespective to the collecting sites. It varies from 0 to 5%. However, there was significant differences in germination, infection rate and vigour index between the localities (Table 3). Indeed, germination was significantly higher on samples from NSF and no grains from NSB germinate. The infection rates of the seeds vary from 5% (Sarh Kassaï market samples) to 51% (Sarh Bégoue market samples).

V. paradoxa seeds are very recalcitrant and lose their germination capacity very quickly just one week after harvest. For this reason, the determination of germination rate and vigour index on blotting paper was only carried out on *P. biglobosa* seeds. The germination percentages of seeds from two regions are very low or even zero (Table 7). Germination rates were significantly different between localities with $p < 0.05$. Infection rates were higher than germination rates and infected seeds failed to germinate. This rate varies from region to region. The difference in infection percentage is significant with ($F=3.091$ and $\alpha=0.035$).

Pathogenicity of Fungi

Disease symptoms observed on the inoculated grains vary from brown-black spot to brown-white and brown-yellow spot, grains rot. These infected grains were mostly covered with fruiting bodies of the fungi (Figure 2). Pathogenicity test was positive with both Shea butter Nuts and locust bean grains with *Aspergillus niger, Oidium* sp and *A. fumigatus* but negative with *Cercospora* sp 4 days after inoculation (Figure 2). Fungal growth on grains was of *T. algeriensis* EO, from the 4th concentration (1 mg.ml^{-1}). Thyme EO has fungicidal inhibitory activity at 1.5 mg.ml^{-1} on the genus *Aspergillus* while it is fungicidal on *Cercospora* sp at the same concentration.

The concentrations of African Panax were much higher than those of *T. algeriensis* EO to better understand their antifungal activities. Like the EO, African Panax was found to be fungistatic on all 4 fungal species tested. The minimum inhibitory concentration of African Panax was 120 mg.ml^{-1}. At this concentration, the species *Aspergillus niger* proved to be resistant and was only inhibited to 60.78%. This inhibition rate is significantly different ($P<0.05$) from the inhibition percentage of 3 other fungi that were 100% inhibited at the same concentration. The EO inhibitory activity of *T. algeriensis* is accompanied by

Table 1: Grains/kernels infection (%) of *V. paradoxa* and *P. biglobosa* from different collection sites.

Collection sites (markets)	Geographical coordinates of collection sites	Infection rate (%)	
		Vitellaria paradoxa	*Parkia biglobosa*
Koumra Central market	N08°55.416' E017°32.913'	91 ± 6.39^{a}	0.6 ± 1.02^{a}
Sarh Kassaï market	N09°07.997' E01824.133'	77 ± 9.48^{b}	2 ± 0.41^{a}
Sarh Central Market	N09°08.614' E018°23.215'	94 ± 5.55^{a}	1.8 ± 0.33^{a}
Sarh Bégoue market	N09º09.579' E01822.984'	78 ± 9.53^{b}	2.6 ± 0.53^{a}
Sarh Yalnas market	N09°08.436' E018°22.847'	95 ± 4.51^{a}	1.9 ± 0.43^{a}
OGFDT	N08°55.9' E017°33.413'	95 ± 5 .25^{a}	2.6 ± 1.07^{a}

*Means followed by the letter in a given colunm are not significantly different according to Duncan's multiple test at 5%. OGFDT: Organization of women's groups for development in Chad.

Table 2: Frequency of isolation (%) of fungi on *Vitellaria paradoxa* and *Parkia biglosa* grains.

Fungi	*Vitellaria paradoxa* (%)	*Parkia biglobosa* (%)
Aspergillus flavus	/	18.63 ± 0.58^{b} (19)
Aspergillus fumigatus	1.62 ± 0.2^{d} (2)	1.96 ± 00^{c} (2)
Aspergillus niger	46.34 ± 5.23^{a} (57)	18.63 ± 15.84^{b} (19)
Cercospora sp	8.65 ± 1.24^{c} (14)	5.88 ± 4.02^{c} (6)
Oidium sp	22.76 ± 12.91^{b} (28)	54.90 ± 33.14^{a} (56)
Rhizopus nigricans	17.88 ± 8.51^{bc} (22)	/

For a given line, means followed by the same letter are not significantly different according to the 5% Fischer test. Numbers in parentheses represent the number of isolates of each fungal species.

Table 3: Grains germination and infection and vigour index of locust bean (*Parkia biglobosa*) from different collection sites (ISTA method).

Collection sites	Germination (%)	Infection rate (%)	Vigour index
Koumra central market	$1.77 \pm 0.57^{b*}$	30.36 ± 8.78^{b}	19.64 ± 6.29^{b}
Sarh Kassaï market	1.89 ± 1.57^{ab}	5.36 ± 3.57^{d}	5.36 ± 2.71^{c}
Sarh Central market	0.75 ± 0.76^{c}	17.86 ± 6.87^{c}	0.56 ± 0.54^{c}
Sarh Bégoue market	0.0 ± 00^{c}	51.78 ± 7.65^{a}	0.0 ± 00^{c}
Sarh Yalnas market	5.36 ± 2.57^{a}	37.5 ± 12.20^{b}	278.57 ± 50.78^{a}
OGFDT	3.57 ± 2.12^{ab}	14.28 ± 5.43^{c}	253.57 ± 24.305^{a}

*Means followed by the letter in thr colunm are not significantly different according to Duncan's multiple test at 5%. OGFDT: Organization of women's groups for development in Chad.

Table 4: Pathogenicity of the fungi isolated from the two plant species.

Isolated Fungi	*Vitellaria paradoxa*		*Parkia biglobosa*	
	Inoculated seeds	Seeds infection (%)	Inoculated seeds	Seeds infection (%)
Aspergillus flavus	50	100	50	100
Aspergillus fumigatus	50	100	50	100
Aspergillus niger	50	100	50	100
Cercospora sp	50	0	50	0
Oidium sp	50	100	50	100
Rhizopus nigricans	50	100	50	100

Table 6: Effect of African Panax on growth inhibition (%) of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Cercospora* sp.

Concentration ($\mu g.ml^{-1}$)	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*	*Cercospora* sp
Control	00 ± 00^{d}	00 ± 00^{c}	00 ± 00^{e}	00 ± 00^{d}
1	15.88 ± 8.78^{c}	19.61 ± 14.80^{b}	11.37 ± 4.51^{d}	40.78 ± 15.13^{c}
15	25.49 ± 8.98^{b}	27.06 ± 9.90^{b}	12.352 ± 8.34^{cd}	73.53 ± 8.09^{b}
30	30.59 ± 13.17^{b}	31.57 ± 14.95^{b}	19.41 ± 7.67^{cd}	77.25 ± 9.90^{b}
60	37.65 ± 13.71^{b}	33.33 ± 16.98^{b}	24.51 ± 6.72^{c}	86.86 ± 7.36^{b}
120	100 ± 00^{a}	100 ± 00^{a}	60.78 ± 6.79^{b}	100 ± 00^{a}
Terazeb (Fungicide)	100 ± 00^{a}	100 ± 00^{a}	100 ± 00^{a}	100 ± 00^{a}

*Means followed by the letter in a given colunm are not significantly different according to Duncan's multiple test at 5%.

Table 7: Effect of mixtures of pure natural products on growth inhibition (%) of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Cercospora* sp.

Mixture between EO and Panax	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*	*Cercospora* sp
Control	00 ± 00^{c}	00 ± 00^{d}	00 ± 00^{d}	00 ± 00^{d}
C_1EO x C_1AP	31.96 ± 11.31^{b}	21.66 ± 4.66^{c}	22.35 ± 11.34^{c}	53.14 ± 17.12^{c}
C_2EO x C_2AP	25.98 ± 8.54^{c}	33.33 ± 10.74^{b}	40.88 ± 12.71^{b}	78.23 ± 8.55^{b}
C_3EO x C_3AP	32.94 ± 10.43^{b}	47.35 ± 19.95^{b}	48.52 ± 13.99^{b}	83 ± 12.24^{ab}
C_4EO x C_4AP	55.78 ± 19.48^{b}	60.88 ± 22.11^{b}	53.33 ± 17.56^{b}	93.43 ± 6.25^{a}
C_5EO x C_5AP	100 ± 00^{a}	100 ± 00^{a}	80.39 ± 19.90^{a}	100 ± 00^{a}
Terazeb (Fungicide)	100 ± 00^{a}	100 ± 00^{a}	100 ± 00a	100 ± 00^{a}

*Means followed by the letter in the colunm are not significantly different according to Duncan's multiple test at 5%. EO = Essential oil, AP= African panax.

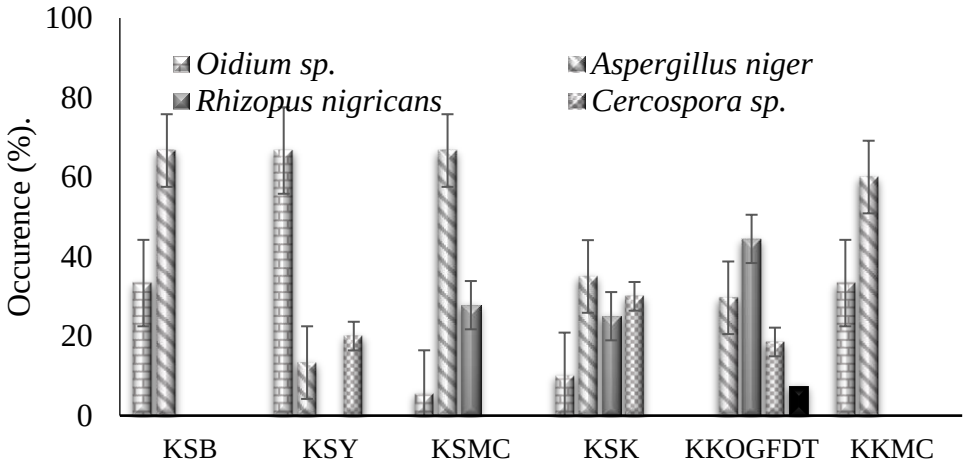

Fig. 2: Occurrence (%) of different fungi identified in *V. paradoxa* according to sample collection localities in the Mandul region (Koumra). Origin of grains: KSB= Sarh Bégoue market, KSY= Sarh Yalnas market, KSMC= Sarh Central market, KSK: Sarh Kassaï market, OGFDT: Organization of women's groups for development in Chad; KSK: shea seeds collected at the Kassaï market in Sarh.

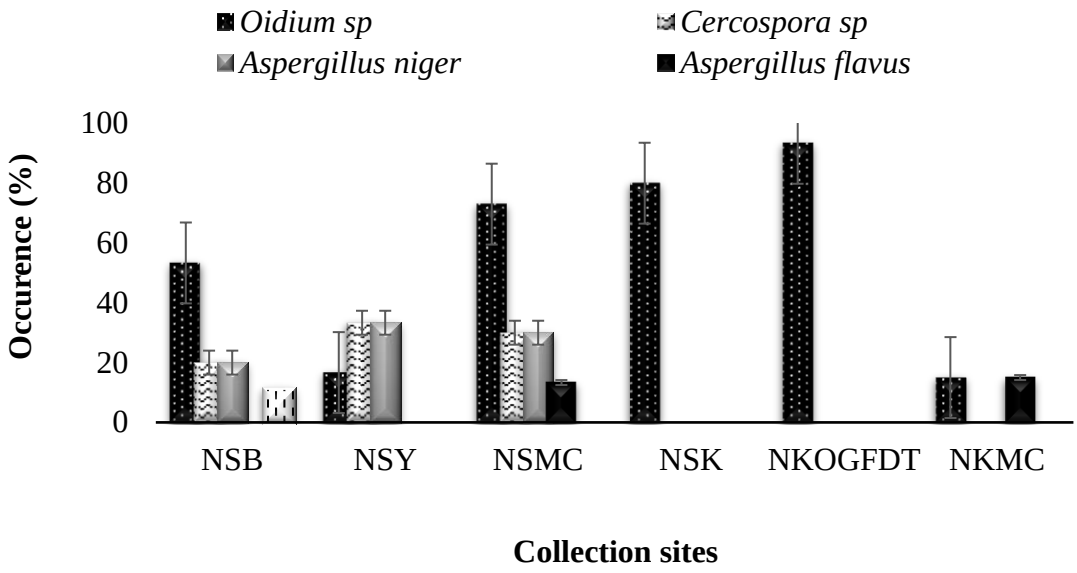

Fig. 3: Occurrence (%) of the different fungi identified in *P. biglobosa* according to the sample collection sites in the Moyen Chari region (Sarh).

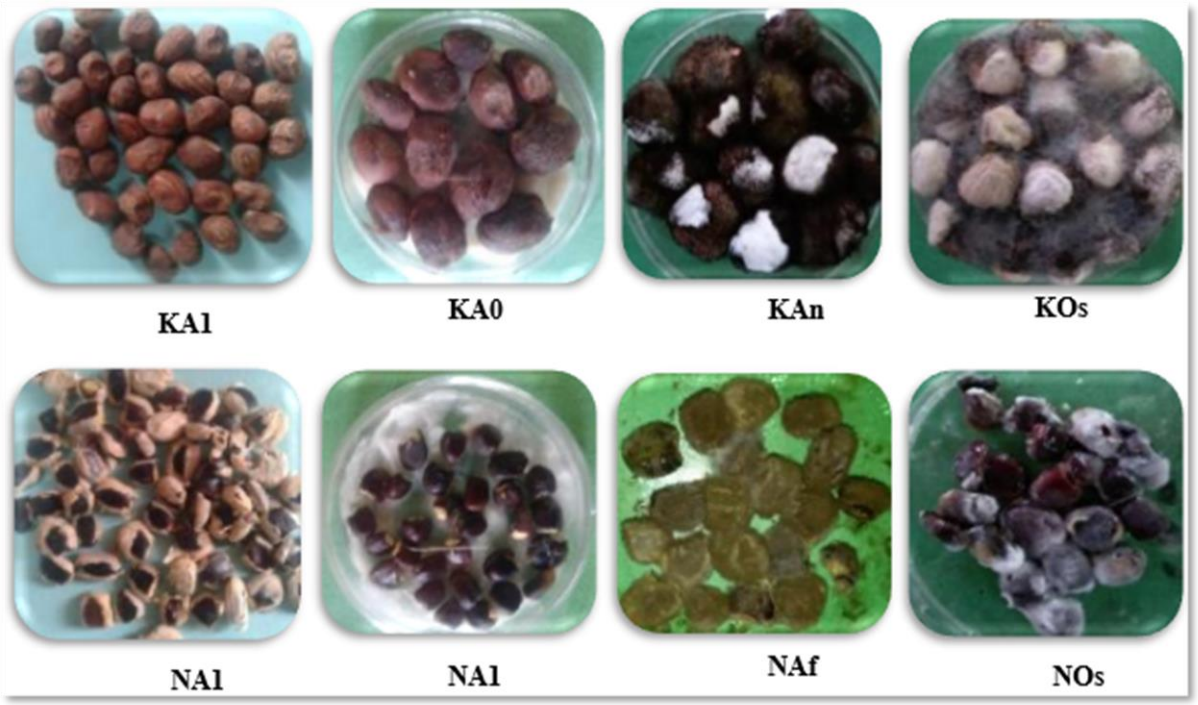

Fig. 4: Pathogenicity test of some isolated fungi on healthy grains of *V. paradoxa* and *P. biglobosa*, 4 days after inoculation.

a progressive discoloration of the normal coloration of all species belonging to the genus *Aspergillus* taking on a whitish colour in the presence of high concentrations. The essential oil of *T. algeriensis* and African Panax exerted fungistatic and fungicidal activity on the different strains tested at 1.5 mg.ml^{-1} in *T. algeriensis* and 120 mg.ml^{-1} in African Panax. However, on the intermediate concentrations, significant differences in inhibition were also observed (Table 5). The species *Aspergillus niger* which was inhibited at 1.5 mg.ml^{-1} of the EO, showed resistance in the presence of African Panax at 120 mg.ml^{-1}. The EO, of *T. algeriensis* showed inhibitory activity at intermediate concentrations different from that of African Panax. The analysis of variance of the EC_{50} and EC_{90} of *T. algeriensis* and African Panax EO reveals that there was a significant difference ($p < 0.05$) in fungitoxicity between the bio fungicides tested (Table 8). In fact, the EC90 of EO of *T. algeriensis* and African Panax was significantly higher against *A. flavus* and *Cercospora* sp respectively.

DISCUSSION

Post-Harvest Fungi Identified

Bare eye observation identified an average infection rate of 89.16% in *V. paradoxa* compared to 1.91% in *P. biglobosa*, which is very low. In all localities, the seeds

Table 5: Effect of essential oil of *Thymus vulgaris* on growth inhibition (%) of *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Cercospora* sp.

Concentration ($\mu l.ml^{-1}$)	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Cercospora* sp
Control	00 ± 00^{e}	00 ± 00^{d}	00 ± 00^{e}	00 ± 00^{c}
0.25	20.19 ± 5.27^{d}	29.27 ± 6.89^{c}	16.27 ± 1.48^{d}	65.49 ± 8.82^{b}
0.5	70.39 ± 3.02^{c}	36.08 ± 4.57^{c}	35.09 ± 7.47^{c}	82.94 ± 7.13^{a}
0.75	84.71 ± 2.76^{bc}	40.39 ± 5.2^{c}	74.11 ± 9.99^{b}	90.00 ± 9.01^{a}
1	87.25 ± 0.91^{b}	80.98 ± 6.3^{b}	88.43 ± 7.59^{b}	100 ± 00^{a}
1.5	100 ± 00^{a}	100 ± 00^{a}	100 ± 00^{a}	100 ± 00^{a}
Terazeb (Fungicide)	100 ± 00^{a}	100 ± 00^{a}	100 ± 00^{a}	100 ± 00^{a}

*Means followed by the letter in a given colunm are not significantly different according to Duncan's multiple test at 5%.

Table 8: Equivalent concentration 50 and 90 (EC_{50} and EC_{90}) values* for the natural products tested.

Biofungicides	Pathogenic fungi	CE_{50}	CE_{90}
Essential oil of *Thymus algeriensis*	*Aspergillus flavus*	4.95 ± 1.27^{b}	21.19 ± 5.47^{a}
($\mu l.ml^{-1}$)	*Aspergillus fumigatus*	4.22 ± 1.15^{b}	15.81 ± 3.80^{b}
	Aspergillus niger	2.46 ± 0.18^{cd}	9.78 ± 3.08^{a}
Aqueous extract of African panax	*Cercospora* sp	0.24 ± 0.07^{d}	7.71 ± 0.26^{cd}
($\mu g.ml^{-1}$)	*Aspergillus flavus*	2.34 ± 1.06^{cd}	4.80 ± 2.31^{d}
	Aspergillus fumigatus	2.28 ± 3.95^{cd}	1.33 ± 0.31^{e}
	Aspergillus niger	1.16 ± 1.01^{d}	2.57 ± 1.45^{d}
	Cercospora sp	7.71 ± 2.26^{a}	26.17 ± 4.76^{a}

*Means followed by the letter in the colunm are not significantly different according to Duncan's multiple test at 5%.

collected showed infection with a non-significant variability at the 5% level. The seeds of *V.* paradoxa show much more surface fungi than the seeds of *P. biglobosa*. This resistance would be due in part to the very hard seed coat of *P. biglobosa* resulting in an opposition to the penetration of parasites and that the fungi that these seeds harbour are more internal parasites. The incision of the seeds of *V. paradoxa* shows that their hilums are filled with the fruiting structures of the parasites. The fungi isolated are species frequently associated with post-harvest and storage products. The species regularly identified on the seeds of two plants are: *Aspergilus niger*, *A. flavus*, *Cercospora* sp, and *Oidium* sp. On *V. paradoxa* and *P. biglobosa*, seeds, these fungi are identified for the first time. The high frequencies of some fungi could be explained by the fact that these species are polyphagous and ubiquitous likely to live on more diverse substrates. The number of species of the isolated fungi (6 species) is less than those obtained by Dongmo et al. (2017), (12 fungal species) on *Ricinodendron heudelotii* and *Garcinia kola* almonds in Western Cameroon and by Nwadiaro et al. (2015) on *P. biglobosa* seeds in Nigeria (17 fungal species). This difference can be explained on the one hand by the higher temperatures in Chad which are not favourable to the development of hygrophilic fungi and on the other hand by the nature of the seeds studied. The genus *Aspergillus* is dominant due to the fact that they are thermo tolerant species whose growth temperature is between 15 and 50°C (Djossou, 2011). These species are responsible for the production of mycotoxins which are products of their secondary metabolism that can develop on the plant in the field or during storage and have toxic potentialities towards humans and animals (Galtier et al., 2006). *Aspergillus flavus* can produce Aflatoxins B1 and B2, cyclopiazonic acid and aspertoxin among others (Amani, 2016). *Aspergillus niger* can produce Achratoxin A (OTA), (Sevastianos et al., 2006). *Aspergillus fumigatus* species can synthesise several highly toxic metabolites such as fumagiline, helvolic acid, gliotoxin, quinone derivatives and ergot-like alkaloids. Mycotoxins are a group of toxic substances with mutagenic, carcinogenic, teratogenic, immunotoxigenic and estrogenic activities (Bar and Bars, 2000). In addition to their mycotoxins, *A. fumigatus*, *A. flavus* and *A. niger* are responsible for serious pathologies in humans and animals when they develop in large numbers: they are called aspergillosis and are diseases due to the direct inhalation of the fungi spores (Djossou, 2011). *Aspergillus niger* was isolated from *Ivingia gabonensis* seeds in Nigeria (Sanyaolu et al., 2014) and from *Ricinodendron heudelotii* and *Garcinia kola* kernels in the western highlands of Cameroon (Dongmo et al., 2017). Powdery mildew is a fungus belonging to the Ascomycetes group, family Erysiphaceae. It is cited by Schnee in 2009 as a fungal disease responsible for crop depreciation in grapes. The genus *Cercospora* belongs to the family *Mycosphaerellaceae* (*Capnodiales*). This genus includes many fungal pathogens causing economically significant damage to a wide variety of woody and herbaceous species, but it can also cause necrotic lesions on flowers, fruits, bracts, seeds and stems (Goodwin et al., 2001; Agrios, 2005). *Cercospora* sp is reported by Nasraoui to be a cercosporin producing fungus which is also a mycotoxin (Nasraoui, 2015). On locust bean *Cercospora* sp has been recorded as causing leaf spots (World Agroforestry Centre). On *P. biglobosa* seeds, *Cercospora* sp and *Oidium* sp were isolated for the first time. The fact that the fungi identified in the two species are almost identical highlights the fact that they are post-harvest fungi. The actors in the Shea butter Nuts sector in Chad are the same as those in the locust bean sector. Once harvested, these seeds undergo more or less the same storage and preservation process. This is a task generally reserved for women and to a lesser extent for children. This is why it is referred to as "women's gold" in sub-Saharan Africa.

Germination, Infection and Seedlings Vigour Index

The infection rate of two seeds is very high, so prompt action is required with proper treatment. Freshly harvested seeds should be well dried and stored in dry and ventilated shelters. It is necessary to provide fungicide treatments, preferably of organic origin, in order not to poison consumers of the by-products. Therefore, the application of

EO, and African Panax as a treatment product are essential for this purpose. The study conducted by Nwadiaro et al. (2015) on locust bean seeds in Nigeria showed that colonization of the seeds by post-harvest fungi significantly reduced the nutrient content of the seeds.

The germination rate of *P. biglobosa* seeds obtained on blotting paper was very low (2.21%) and far from the normal germination rate accepted for the species, which is 75% (World Agroforestry Centre). On the other hand, the infection rate was 26.19%. This can be explained on the one hand by the fact that the fungi associated with the seeds were responsible for the loss of germination capacity of the seeds and on the other hand by the inadequate storage conditions of the seeds which lead to the loss of germination capacity. Seedlings from fungus-affected seeds were less vigorous than normal ones, indicating that fungus attack of the seeds limited the growth of the plants even if they managed to germinate. This was also confirmed by Djeugap et al. (2017) on *Pericopsis alata* seeds in Cameroon.

Pathogenicity

The pathogenicity test was positive on all fungi isolated from two seeds. The isolated fungi are constantly attached to the seeds and develop rapidly in the presence of favourable conditions. This is the case for *Oidium* sp for example, which colonised all seeds within 24 hours. *Aspergillus* spp. are very invasive moulds of post-harvest products, since they are present in air, water and soil. On seeds in storage, their development is rapid and easily observed. The absence of *Cercospora* sp on both seeds shows that it is not a contaminant of crop products but its origin in the seeds would be from the field. The softened appearance of experimentally inoculated seeds is evidence that these fungi are responsible for the deterioration of nutrient content and seed rot.

Antifungal Activity of Essential Oils and African Panax on the Radial Growth of Fungi

Essential oils contain important fungitoxic compounds that can be a renewable source of fungicides. The essence of thyme is often reported to be among the most active essential oils (Nadia et al., 2014). They are composed by aromatic molecules of plant origin with a very large structural diversity. Thymol produced by *T. algeriensis* is known to be a powerful antifungal agents. This species is naturally rich in phenols, especially thymol and carvacrol. These two compounds are characterised by their strong antimicrobial properties (Szentandrássy et al., 2003; Ipek et al., 2005). Phenols have been shown to act through the inactivation of fungal enzymes that contain the SH group in their active site (Zohra et al., 2010). This antifungal power may also be due to the result of synergies between the different constituents of these oils.

The difference in antifungal efficiency between thyme EO, and African Panax can be attributed to their chemical compositions. Indeed, the molecules of *T. algeriensis* have a very broad spectrum of antimicrobial activity. They are naturally present in the essences of most species of *Thymus* spp. The antifungal activity of essential oils can be influenced by several parameters, namely: the method of evaluating the antifungal activity, the choice of plants, the type and molecular structure of the active components, the dose added, the type of microorganisms targeted. The antifungal activity of African Panax is due to the presence of ginsenosides and anthraaquinone contained in ginseng and *Aloe vera* respectively (Lee et al., 2005; Castillo et al., 2010). This is the first time that African Panax is showed to have fungicidal activity against plant pathogens.

Cercospora sp reacted differently in the presence of EO and African Panax than *Aspergillus* spp. The discoloration observed on *Aspergillus* spp. is evidence of the appearance of suffering forms including deformations, which implies a membrane action of EO on the fungi. Ouraïni et al. (2005) reported this fact in their study on the antifungal activity of essential oils of aromatic plants on dermatophytes. The difference in sensitivity of fungal genera to essential oils may be due to certain factors, namely the dose applied and the target species. The same authors had shown that increasing the fungistatic concentration had fungicidal effects on the same fungi. One of the factors influencing the intensity of the antifungal action of EO is the applied dose. This fact confirms the fungicidal effects that we obtained by increasing the inhibitory concentrations of EO and Panax to 2 and 160 $mg.ml^{-1}$ respectively. Magan and Olsen (2004) showed the existence of differences in sensitivity to oil between different species belonging to the same genera and between the various fungal structures of the same genus: spores, sclerotia and mycelial fragments. Therefore, in the presence of African Panax, *Aspergillus niger* behaved differently from the other fungi and was only inhibited at 60%, whereas the other fungi were inhibited at 100% at the same concentration (120 $mg.ml^{-1}$).

Thyme essential oil and African Panax showed fungistatic and fungicidal effects on the fungi tested. After 7 days of incubation on the unsupplemented media of the extracts. There was a resumption of mycelial growth of the genus *Aspergillus* (*A. niger, A. flavus, A. fumigatus*) from the fragments taken on media supplemented with Thyme essential oil (fungistatic effect) whereas the species *Cercospora* sp *did* not grow on the new medium (fungicidal effect) at the final concentration (1.5 $mg.ml^{-1}$) African Panax showed a fungistatic effect on the different fungi tested. By increasing the concentration of the essential oil from 1.5 to 2 $mg.ml^{-1}$ and the aqueous extract of African Panax from 120 to 160 $mg.ml^{-1}$; the biofungicides proved to be fungicidal except for African Panax on the species *Aspergillus niger* which showed a fungistatic effect.

Conclusion

The study showed that *V. paradoxa* and *P. biglobosa* (two edible NTFP) harbour a diversity of fungal species among which *Oidium* sp, *Aspergillus niger, A. flavus* and *Rhizopus nigricans* were the most frequent. The species *Aspergillus flavus, A. niger* and *A. fumigatus, R. nigricans* and *Oidium* sp are pathogenic to Shea butter Nuts and dwarf seeds on which they cause necrosis of tissues and rots. These fungi are therefore responsible for the post-harvest deterioration and low germination rate of these plant species. The essential oil of *Thymus algeriensis* showed stronger antifungal activity than African Panax at 120 $mg.ml^{-1}$. This study is a significant contribution to the knowledge of fungal pathogens of *V. paradoxa* and *P. biglobosa* seeds and this is the basis for the development of an alternative approach to the chemical control of post-harvest diseases of these two NTFPs in Chad.

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REFERENCES

Agrios GN. (2005). Plant pathology. Fifth edition. Elsevier Academic Press, California, USA, 316p.

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). *Introductory mycology* (No. Ed. 4). John Wiley and Sons.

Amadi, J. E., Nwaokike, P., Olahan, G. S., & Garuba, T. (2014). Isolation and identification of fungi involved in the post-harvest spoilage of guava (Psidium guajava) in Awka metropolis. *Int. J. Eng*, *4*(10), 7-12.

Amani L. (2016). Mycotoxins and mycotoxigenic fungi in commercialized sorghum grains in Tunisia: Incidence and ecophysiological profiles. PhD Thesis, Institut Supérieur de Biotechnologie de Monastir, Tunisia, 225p.

Bars Le, J., & Le Bars, P. (2000). Mycotoxigenesis in Grains Application to Mycotoxic Prevention in Coffee. In *Coffee Biotechnology and Quality: Proceedings of the 3rd International Seminar on Biotechnology in the Coffee Agro-Industry, Londrina, Brazil* (pp. 355-368). Springer Netherlands.

Castillo, S., Navarro, D., Zapata, P. J., Guillén, F., Valero, D., Serrano, M., & Martínez-Romero, D. (2010). Antifungal efficacy of Aloe vera in vitro and its use as a preharvest treatment to maintain postharvest table grape quality. *Postharvest Biology and technology*, *57*(3), 183-188.

Djeugap, F. J., Nzuta, C., Temgoua, L. F., Kenmogne, G., & Tekam, P. M. (2017). Champignons pathogènes associés aux semences de Pericopsis elata et effet des substrats sur la germination, la croissance et l'infection des plantules au Cameroun. *Revue Scientifique et Technique Forêt et Environnement du Bassin du Congo-RIFFEAC*, *8*, 19-27.

Djossou, O. (2011). Post-harvest mycoflora of robusta coffee and use of lactic acid bacteria for the control of mycotoxigenic moulds and Ochratoxin A. *PhD thesis*, Paul Cezanne University, France, 153p.

Dohou N., Yamani, K., Badoc, A., and Douira, A. (2004). Antifungal activity of *Thymelea lythroides* extracts on three rice fungi. *Bulletin Sociaux Pharmaceutique* de Bordeaux, *143*(3), 31-38.

Dongmo, G. Z., Djeugap, J. F., Fenohi, N., Kenfack, N. D., Takuete, R., & Teguefouet, P. (2017). Contribution to the identification of post-harvest fungi associated with Ricinodendron heudelotii and Garcinia kola kernels collected in the Western Highlands of Cameroon. *International Journal of Biological and Chemical Sciences*, *11*(4), 1840-1850.

Dudut, O. (2012). Butters: Shea butter Nuts (*Bytyrospermum parkii*), cocoa (*Theobroma cacao* L.) kohum (*Garcinia indica*) and illipé (*Shorea stenoptera*). PhD thesis in Pharmacy, University of Nantes, France, 128p.

Eyog, M. O., Gandé, G. O., and Dossou, B. (2000). Forest genetic resources programme in sub-Saharan Africa. Food Woody Species Network. Minutes of the first meeting of the Network 11-13, CNSF Ouagadougou, Burkina Faso, 241p.

FAO (Food and Agriculture Organization of the United Nations). (2004). International Workshop on the Processing, Valorization and Trade of Shea butter Nuts in Africa, © FAO and CFC, 332p.

Galtier, P., Loiseau, N., Oswald, I. P., & Puel, O. (2006). Toxicologie des mycotoxines: dangers et risques en alimentation humaine et animale. *Bulletin de l'Académie vétérinaire de France*, *159*(1), 5-13.

Goodwin, S. B., Dunkle, L. D., & Zismann, V. L. (2001). Phylogenetic analysis of Cercospora and Mycosphaerella based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology*, *91*(7), 648-658.

Ipek, E., Zeytinoglu, H., Okay, S., Tuylu, B. A., Kurkcuoglu, M., & Baser, K. H. C. (2005). Genotoxicity and antigenotoxicity of Origanum oil and carvacrol evaluated by Ames Salmonella/ microsomal test. *Food Chemistry*, *93*(3), 551-556.

Iqbal, N., & Saeed, S. (2012). Isolation of mango quick decline fungi from mango bark beetle, Hypocryphalus mangiferae S. (Coleoptera: Scolytidae). *The Journal of Animal Science*, *22*, 644-648.

ISTA (International Seed Testing Association). (2001). International Rules for Seed Testing, Rules. Seed sciences Technologies, 335p.

Lee, T. K., Johnke, R. M., Allison, R. R., O'Brien, K. F., & Dobbs Jr, L. J. (2005). Radioprotective potential of ginseng. *Mutagenesis*, *20*(4), 237-243.

Lisan, B. (2017). Agroforestry in a dry tropical climate: *technical document for agroforestry parks in a dry tropical climate.* Version V1.0, 149p.

Magan, N., & Olsen, M. (Eds.). (2004). *Mycotoxins in food: detection and control*. Woodhead Publishing.

Ministry of Agriculture and Irrigation (MAI). (2013). Plan de développement de l'agriculture au Tchad. Rapport d'activité, 58p.

Nadia Z., Farah, A., & Bahhou, J. (2014). Chemical analysis and antibacterial activity of essential oils from three species of Thymus: Thymus zygis, T. algeriensis and T. bleicherianus. *Bull. Société R. Sci. Liège*, *83*, 118-132.

Nasraoui, B. (2015). Pathogenic fungi and pseudo fungi of cultivated plants. *Mycology book*, INAT publication, 199p.

Nguemtchouin, M. M. G. (2012). Formulation of insecticide powders by adsorption of essential oils of *Xylopia aethiopica* and *Ocimum gratissimum* on modified Cameroonian clays. PhD Thesis, Ecole Nationale Supérieure de chimie de Montpellier, France, 293p.

Nwadiaro, P. O., Wuyep, P. A., Ogbonna, A. I., Nwaukwu, I. A., & Nwanokwai, M. (2015). Mycoflora of Stored Parkia Biglobosa (JACQ) R. BR. EX G. DON (Locust Bean) Seeds from Markets in Jos, Nigeria and Changes in their Nutritional Composition.

Ouraïni, D., Agoumi, A., Ismaili-Alaoui, M., Alaoui, K., Cherrah, Y., Amrani, M., & Belabbas, M. A. (2005). Study of the activity of essential oils from aromatic plants antifungal properties in the different stages of development of dermatophytes. *Phytotherapy*, *4*, 147-157.

Adeniyi, S. A., Adedotun, A. A., & Akinniyi, O. (2014). The effects of post harvest mycodeterioration on the proximate composition of irvingia gabonensis seeds. *International Journal of Phytopathology*, *3*(1), 41-48.

Schnee, S. (2009). Resistance factors to powdery mildew (*Erysiphe neccator* Schewein.) in grapevine (*Vitis vinifera* L.). *Doctoral thesis PhD;* University of Neuchatel; Switzerland, 144p.

Sevastianos, R., Zaouia, N., Salih, G., Tantaoui-Elaraki, A., Lamrani, K., Cheheb, M., ... & Ismaili-Alaoui, M. (2006). Characterization of filamentous fungi isolated from Moroccan olive and olive cake: Toxinogenic potential of Aspergillus strains. *Molecular nutrition & food research*, *50*(6), 500-506.

Szentandrássy, N., Szentesi, P., Magyar, J., Nánási, P. P., & Csernoch, L. (2003). Effect of thymol on kinetic properties of Ca and K currents in rat skeletal muscle. *BMC pharmacology*, *3*(1), 1-8.

Tsopmbeng G. N., Pitagor, M. C. J., Appolinaire, L. J., Aoudou, Y., Joseph, D. F., & Ajong, F. D. (2014). Evaluation of antifungal activities of plant extracts against Phytophthora colocasiae, causal agent of late blight of taro (Colocasia esculenta (L.) Schott). *Journal of Applied Biosciences*, *81*, 7221-7232.

Umana, E. J., Akwaji, P. I., Markson, A. A., & Udo, S. E. (2015). Gmelina arborea Roxb: associated mycoflora and diseases in Cross River State, Nigeria. *Global J Sci Front Res*, *15*, 1-11.

Yaouba, A., Léopold, T. N., Michel, J. D. P., Xavier, E. F., & Moses, M. C. (2010). Antifungal properties of essential oils and some constituents to reduce foodborne pathogen. *Yeast Fungal Res*, *1*(1), 1-8.

Zohra M., BACHIK, S., & BELKAROUBE, N. (2010). Potentiel antifongique et antiaflatoxinogène des huiles essentielles d'une plante endémique Thymus fontanesii Boiss. et Reut. *Les technologies de laboratoire*, *5*(19).