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Research Article

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 *Vitellaria paradoxa* and *Parkia biglobosa* are two Edible Non-Timber Forest Products (ENTFP) with high economic value. These products are colonized by un-identified post-harvest fungi that are responsible for high post-harvest losses. The objective of the study was to contribute to the management of the post-harvest diseases of kernels of *V. paradoxa* and *P. Biglosa* through natural products. To achieve this, post-harvest fungi were isolated from infected kernels and their pathogenicity tested. Then, antifungal activity of Essential Oil (EO) of *Thymus algeriensis* and crude extract of African panaxia was carry out by the dispersion method on the agar medium on four pathogenic fungi isolated from the two infected ENTFP. Results showed that *V. paradoxa* kernels were highly infected (77-95%) compared to *P. biglobosa* (0.6-2.6%). Fungal species frequently associated with *V. paradoxa* and *P. Biglobosa* kernels 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 the species belonging to the genus *Aspergillus* and with *Oidium* sp. Essential oil of *T. vulgaris* at 1.5 µl/ml and the crude extract of African panaxia at 120 µg/ml totally inhibited the growth of the four potential mycotoxigenic fungi tested; their efficacy were significantly comparable ($p<0.05$) to the reference fungicide (Terazeb). *In vivo* control of post-harvest diseases with these two natural products is being carry out.

Keywords: Biological activity; Post-harvest fungi; *Vitellaria paradoxa*; *Parkia biglobosa*; African panaxia; *Thymus algeriensis*

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 [1]. 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 [2]. 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 classifying on the International Union for Conservation of Nature list as endangered species due to bush fires and overexploitation of the grains [3]. Both in the field as well as in post-harvest, trees and grains are subjected to various fungal infections. For example, in Shea but-

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ter Nuts, fungi *Fusicladium butyrospermi* and *Pestalozzia heterospora* are responsible of leaf spots and high yield losses [4]. In locust bean, the genus *Phellinus* sp (Basidiomycetes) cause tree dieback and *Cercospora* sp, *Hypoxylon rubiginosum* and *Phyllachora leonensis* were reported on leaves [5]. 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.

Material and Methods

Collection Sites and Storage of Samples

Healthy and necrotic kernels of *V. paradoxa* and grains of *P. biglobosa* (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, 2021). Grains collected were transported to the Phytopathology laboratory of the University of Dschang, Cameroon and stored at 4C° before fungi isolation.

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 400 kernels/grains of each plant species. Infected kernels 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 placed in a 90 mm diameter Petri dish containing moistened (with sterile distilled water) filter paper [6]. Incubation was at lab temperature (22 ± 2˚C) with alternating 12h of light and 12h of darkness for eight days. After that, kernels were examined under a magnifying glass (10X) to observe the presence of mycelia filaments [7]. 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 20ml of PDA medium with 5 fragments per dish in the microbiological hood. Petri dishes were incubated at 21°C during five days, and then the fungal colonies visible on the incubated on fragmented grains were isolated and purified on the same culture medium [8]. 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 [9,10]. 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 [11].

Pathogenicity Test

Healthy kernels of *V. paradoxa* and grains of *P. biglobosa* were selected and disinfected with 95% ethanol for 5 min and rinsed with sterile distilled water. Then, spores suspension of each purified and identified fungus was calibrated at 2 x 10^4 conidia/ml and inoculated on 50 healthy kernels of each plant species by dipping the healthy kernels in 50 ml of spore suspension. The inoculated kernels/grains were introduced in Petri dishes and placed in dark boxes and incubated at Lab temperature (22 ± 2ºC) [12]. Pathogenicity was positive when the inoculated fungus develops disease symptoms on kernels or negative otherwise.

In Vitro Efficacy of Essential oil of *T. algeriensis* and African Panaxia

Preparation of natural products: Essential oil was obtained by extraction through the hydro distillation method 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 [13]. Essential oil collected was stored at 4°C in the dark in the presence of anhydrous sodium sulphate [14]. African panaxia locally called Panaxia was obtained from a well-known naturopath. It is a natural African product made up of a mixture of plants (30% of *Panax ginseng* roots, 25% of *Panax quinquifolium*, 20% of *Aloe vera*, 20% of *Ocimum gratissimum* and water).

Inhibition of Radial Growth of Fungi by the Two Natural Products

Antifungal activity of *T. algeriensis* essential oil (EO) 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 [14] and at 1; 15; 30; 60; 120 µg/ml for aqueous extracts of Panaxia. 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 [15]. 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=100x (A-B)/A 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 Panaxia. The toxicity (fungicidal or fungistatic effect) of these natural products was then evaluated by transferring the mycelia plug which the growth was totally inhibited on a newly prepared PDA (without EO or panaxia). The activity of the product was qualified as fungistatic if there was resumption of the fungal growth and as fungicidal in the opposite case.

Statistical Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Science version 21) software program. The Analysis of Variance (ANOVA) was performed for each variable collected using the generalized linear model. Means were separated using the Student's smallest significant difference test at 5%. Linear regression equations that relate inhibition of mycelial growth and spore germination to logarithmic EO concentrations were used to determine EC50 and EC 90 concentrations (50% and 90% equivalent concentration).

Results

Kernels/Grains Infection and Occurrence of Post-Harvest Fungi of *V. paradoxa* and *P. biglobosa*

Kernels of *V. paradoxa* and grains of *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). The occurrence of *A. niger* and *Oidium* sp was higher in all the collection sites for *V. paradoxa* (Figure 1) and *P. biglobosa* respectively (Figure 2). Fungal colonization of *P. biglobosa* grains from Sarh Kassaï market and the Organization of women's groups for development in Chad were less diversecompared to others collection sites. In fact, samples from these two sites were colonised only with *Oidium* sp (Figure 2). The cross section of infected grains of *V. paradoxa* showed visible fruiting bodies of the fungi (Figure 3).

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

Collection sites	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 same letter in a column are not significantly different according to Student test at 5%. OGFDT: Organization of women's groups for development in Chad.

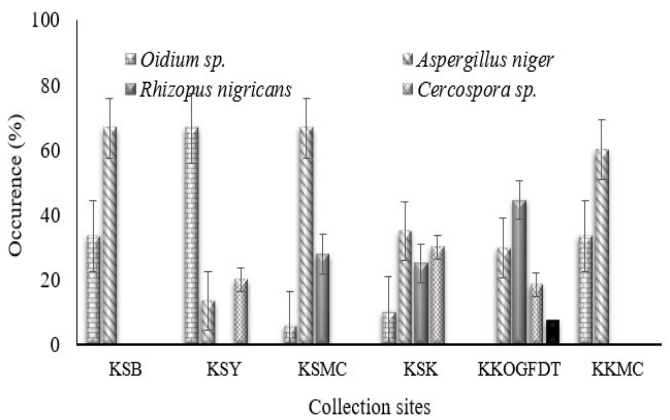

Figure 1: Occurrence (%) of different fungi identified in *V. paradoxa* according to sample collection localities in the Mandul region (Koumra).

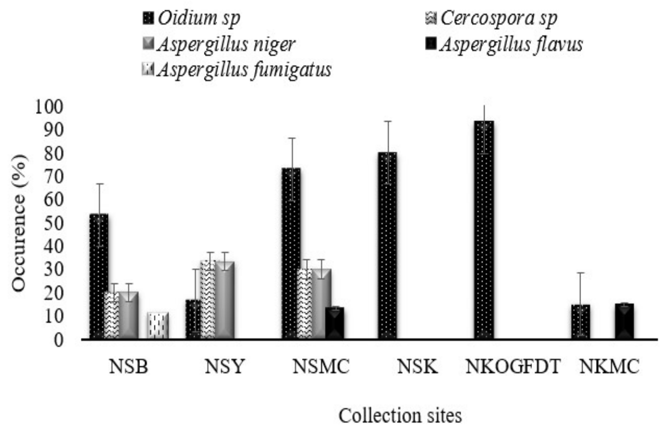

Figure 2: Occurrence (%) of the different fungi identified in *P. biglobosa* according to the sample collection sites in the Moyen Chari region (Sarh).

Figure 3: Contamination of *V. paradoxa*kernels by fungi. **(A)** entire grains, **(B)** section of infected grains showing fungal fruiting bodies and **(C)** section of healthy grains free of fungi.

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

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 column, means followed by the same letter are not significantly different according to student test at 5%. Numbers in parentheses represent the number of isolates of each fungal species.

Pathogenicity of Fungi

Disease symptoms observed on the inoculated kernels of *V. paradoxa* and grains of *P. biglobosa* are black for *A. niger* and *A. fumigatus*, and greenish for *A. flavus*. The species *R. nigricnas* developed rot of kernels/grains. Fruiting bodies of the fungi were visible on infected organs, 4 days after inoculation (Figure 4). In fact, pathogenicity test was positive with both Shea butter nuts (*V. paradoxa*) and locust bean grains (*P. biglobosa*) with *Aspergillus niger*, *Oidium* sp and *A. fumigatus* but negative with *Cercospora* sp. Microscopic features of the fungal species that were positive to pathogenicity test were characteristic of the fungal species inoculated. Negative control (kernels/grains not inoculated) incubated under the same conditions was free of fungi.

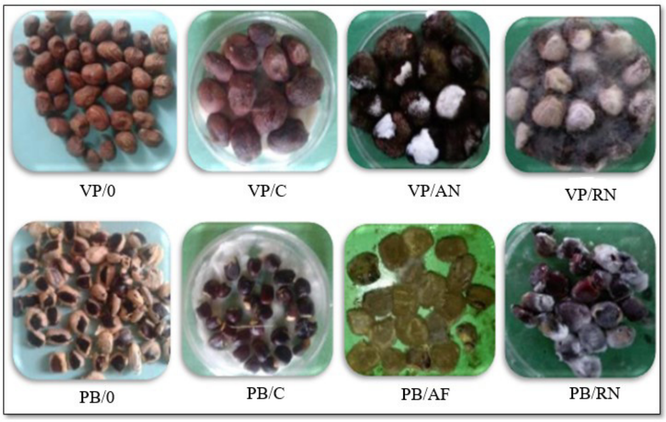

Figure 4: Pathogenicity test of some isolated fungi on healthy kernels of *V. Paradoxa* (VP) and *P. Biglobosa* (PB), 4 days after inoculation. VP/0 = non-inoculated kernels of VP, PB/0 = non-inoculated kernels of PB. VP/C, VP/AN and VP/RN = VP kernels inoculated with *Cercospora* sp, *A. niger* and R. nigricans respectively. AF= *A. flavus*.

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

The EO of *T. algeriensis* showed different degrees of antifungal activity against *A. fumigatus*, *A. flavus, A. niger* and *Cercospora* sp (Table 3). It was observed a total growth inhibition (100%) of *Cercospora* sp at 1 µl/ml by the EO of *T. algeriensis* while at 1.5 µl/ml the inhibition was total with all the fungi tested. This efficiency was significantly comparable to the reference synthetic fungicide. The effect of this EO was fungicidal to all the fungi at 1.5 µl/ml. These four pathogenic fungi tested are potential mycotoxinogenic agents of post-harvest food stuffs. Like with the EO of *T. algeriensis*, the growth inhibition of the pathogen decrease with concentrations of Panaxia. Apart of *A. niger*, the growth of the other pathogenic fungi was totally inhibited by the aqueous extract of Panaxia at 120 µg/ml and the extract was fungitoxic for these three pathogen at the same concentration. This efficacy was significantly comparable ($p<0.05$) to the chemical (Terazeb). At this concentration, the growth inhibition of *Aspergillus niger* was 60.78% which was significantly different ($p>0.05$) to the reference fungicide (Table 4). The aqueous extract of Panaxia was fungistatic on *A. flavus*, *A. fumigatus* and *Cercospora* sp at 120 µg/ml. The mixture between EO of *T. algeriensis* and aqueous extract of panaxia don't significantly improve the efficiency of the pure natural products. However, their biological activities were higher than the activity of the aqueous extract of Panaxia for all the pathogens and at all the concentrations tested (Table 5). The analysis of variance of the EC_{50} and EC_{90} of *T. algeriensis* EO and Panaxia extract reveals that there was a significant difference ($p<0.05$) in the fungitoxicity between the two natural products tested (Table 6). In fact, the EC50 values were significantly higher with the EO of *T. algeriensis* on *A. flavus* (5.95 µl/ml) and with the Panaxia extract on *Cercospora* sp (7.71 µl/ml). The EC90 was also significantly higher against *A. flavus* (21.19 µl/ml) in presence of *T. algeriensis* EO and *Cercospora* sp (26.17 µg/ml) in Panaxia extract.

Table 3: Effect of essential oil of *Thymus algeriensis* on growth inhibition (%) of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Cercospora* sp.

Concentration (µl/ml)	*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 Student test at 5%.

Table 4: Effect of African panaxia on growth inhibition (%) of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Cercospora* sp.

Concentration (µg/ml)	*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 Student test at 5%.

Table 5: Effect of mixtures of pure natural products on growth inhibition (%) of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Cercospora* sp.

Mixture between EO and Panaxia	*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 a column are not significantly different according to Student test at 5%. EO = Essential oil, AP= African panaxia. C1,…, C5 =first to fith concentration of the EO or AP.

Table 6: 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 (µl/ml)	*Aspergillus flavus*	4.95 ± 1.27[b]	21.19 ± 5.47[a]
	Aspergillus fumigatus	4.22 ± 1.15[b]	15.81 ± 3.80[b]
	Aspergillus niger	2.46 ± 0.18[cd]	9.78 ± 3.08[a]
	Cercospora sp	0.24 ± 0.07[d]	7.71 ± 0.26[cd]
Aqueous extract of African panaxia (µg/ml)	*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 column are not significantly different according to Student test at 5%.

Discussion

Infection Rate and Post-Harvest Fungi of Kernels of *V. paradoxa* and Grains of *P. biglobosa*

The infection rate of kernels of *V. paradoxa* (89.16%) was very high compared to grains of *P. biglobosa* (1.97%) in all collection sites. This may be due in part to the very hard grains coat of *P. biglobosa* resulting in an opposition to the penetration of parasites (fungi). The incision of the grains 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 foodstuffs products. Most species identified (*Aspergilus niger*, *A. flavus*, *Cercospora* sp, and *Oidium* sp) are reported for the first time on *V. paradoxa* kernels and *P. biglobosa* grains. The high frequency of some fungi could be explained by the fact that these species are polyphagous and ubiquitous likely to live on more diverse substrates. This is in accordance with Dongmo et al. 2017 [16] findings who identified many of these fungal species on Ricinodendron heudelotii grains and *Garcinia kola* kernels in the West region of Cameroon. This difference on species isolated between these products could be explained by the differences in the agro ecological zones (higher temperatures in Chad and low temperature in western highland zones of Cameroon). In fact, temperature is one of important climatic factor which influences fungal growth. Most of the parasitic fungi rapidly develop on moist environment [17]. The presence of potential mycotoxigenic species like Aspergillus spp and *Cercospora* sp shows that consumers should be very careful and aware of all the risk related to consumption of foodstuffs with mycotoxins [18-20]. Mycotoxins are a group of toxic substances with mutagenic, carcinogenic, teratogenic, immunotoxigenic and estrogenic activities [21].

Antifungal Activity of Essential Oils of *T. algeriensis* and Aqueous Extract of African Panaxia 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 [22]. They are composed by aromatic molecules of plant origin with a very large structural diversity. Thymol produced by *T. algeriensis* is known to have higher antifungal properties. This species is naturally rich in phenols, especially thymol and carvacrol. These two compounds are characterised by their strong antimicrobial properties [23]. Phenols have been shown to act through the inactivation of fungal enzymes that contain the SH group in their active site [24]. This antifungal power may also be due to the result of synergies between the different constituents of these oils. The efficiency of Panaxia aqueous extract to control fungal diseases was previously reported against *Phytophthora magakarya* (cocoa black pod) [25]. It was normal that difference on the antifungal efficiency exist between thyme EO, and Panaxia based to the differences in their chemical compositions. Indeed, thymol founded in *T. algeriensis* essential oil has a very broad spectrum of antimicrobial activity. However, the antifungal activity of essential oils can be influenced by several factors such as: the method of extraction, the plant family, the molecular structure of the active components, the dose/concentration, the targeted microorganisms, etc. Moreover, the presence of ginsenosides and anthraaquinone in *Panax ginseng* and *Aloe vera* respectively which are ingredients of African panaxia aqueous extract could be responsible to their antifungal activities [26,27]. This is the first time that Panaxia is showed to have fungicidal activity against potential mycotoxigenic fungi of foodstuffs products. *Cercospora* sp reacted differently in the presence of EO and Panaxia 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. Ouraïnil et al. 2005 [28] 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. Magan and Olsen 2004 [29] 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 Panaxia, *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 µg/ml). 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 µl/ml) African Panax showed a fungistatic effect on the different fungi tested.

Conclusion

The study showed that *V. paradoxa* kernels and *P. biglobosa* grains 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 were pathogenic to edible kernels and grains of these two plants on which they cause tissues necrosis and rots. The essential oil of *Thymus algeriensis* showed fungicidal effect at 1.5 µl/ml on all the four pathogenic fungi tested (*Aspergillus flavus*, *A. niger*, *A. fumigatus* and *Cercospora* sp) while aqueous extract of African panaxia was fungitoxic on *Aspergillus flavus*, *A. fumigatus* and *Cercospora* sp at 120 µg/ml. This study is a significant contribution to the understanding of pathogenic fungi of *V. paradoxa* kernels and *P. biglobosa* grains and highlight the biological activity of essential oil of *T. algeriensis* and aqueous extract of African panaxia against their post-harvest fungi. It is therefore, the basis for the development of an alternative approach to chemical control of potential post-harvest mycotoxigenic fungi of these two edible non-timber forest products in Chad.

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