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Chemical Composition and Efficacy of *Ocimum basilicum* L. Essential Oil against Stored Rice (*Oryza sativa* L.) Molds Sold in Ngaoundere (Cameroon)

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Authors' contributions

This work was carried out in collaboration among all authors. All members contributed in designing the study. Authors NDN, SFSN and TMNB wrote the protocol and managed the analyses of the study. Author ATS performed the statistical analysis together with authors NDN, SFSN and TMNB who wrote the first draft of the manuscript. Author LNT supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

The aimed of this study to evaluate the antifungal activity of essential oils of *Ocimum basilicum* L. on some fungi isolated from commercial rice sold in Cameroon. Fungal isolates were primarily identified based on morphological characteristics, while representative isolates were identified using PCR-based methods. Essential oils were extracted by hydro-distillation, chemical composition was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) and antifungal activity against isolated fungi were assessed using the micro-atmospheric method. The percentage of frequency and abundance varied with the sample analyzed and the fungi detected.

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A total of 85 fungal isolates of eight genera including *Aspergillus, Fusarium, Penicillium, Rhizopus, Cladosporum, Mucor Alternaria* and *Acremonium* in decreasing order of predominance were identified. The most fungal species identified were *Aspergillus flavus, A. fumgatis, A. niger, A. carbonarius, Fusarium oxysporum, F. graminarum, F. moniliforme, Penicillium citrinum, P. expansum*, *Rhizopus oryzea, Cladosporum sp, Mucor hiemalis, Alternaria solani, Acremonium murorum.* More than 61% (*A. flavus, A. niger, P. citrinum*) and 44% (*M. hiemalis, A. flavus, A. niger*) of analyzed samples contain respectively aflatoxin B1, AFB1 (0 - 17.3 µg/kg) and ochratoxin A, OTA (0 - 5.2 µg/kg). Citrinin was not detected. The main compounds identified in *O. basilicum* EO are eugenol (30.6), linalool (29.5), cineole (14.4), Terpinen-4-ol (5.6), 2-norpinen (4.1), Cadinen (3.3), Limonen (2.4) and (E)-*β*-Ocimene (2.2). The *O. basilicum* EO exhibit antifungal activity with the MIC range from 750-2000 ppm. This activity varies with the strains and the concentration of EO. The increased prevalence of mycotoxigenic fungi in rice, a highly consumed food grain in Africa, poses serious health concerns to the general public. The *O. basilicum* EO can therefore be used as alternatives to synthetic pesticide in rice storage.

Keywords: Ocimum basilicum; essential oil; rice; molds; mycotoxins; Ngaoundere; Cameroon.

1. INTRODUCTION

Filamentous fungi are widely distributed in environment and are frequent contaminants of food and animal feed [1]. The most common species of fungi isolated from food belong to genera *Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, Mucor, Rhizopus, Eurotium* and *Emericella* [1,2,3,4] . Species of genera *Aspergillus, Fusarium* and *Penicillium* are known to be contaminants of agricultural produce whether stored or still in the farm with the capacity to develop at low water activity value [1,3, 4,5] . These species are usually found in or on cereal grains and cereal products like rice [3,4] . Rice (*Oryza sativa* L.) is one of the leading cereal crops and a staple food for about 50% of the world's population with its consumption which increase significantly in Africa within the last few decades [3,5]. However, inadequately dried hay, under faulty storage not only accelerates the deterioration but also improve mold infestation [2,3]. Fungi spoilage is responsible for yield reduction and great economic damages throughout the world. It has been reported that the growth of molds in cereals on farms and during the storage period could cause about 50 to 80% of the losses in tropical conditions [6]. Field losses due to fungal spoilage in rice production may amount 5–10% or even more in the developing countries [7]. The presence of some species during the storage is responsible for the production *in situ* of mycotoxins including aflatoxins, ochratoxin A, citrinin, patulin, fumonisins, zearalenone, etc. [1,3,5,8]. They are responsible for the chronic toxicity like cytotoxicity, hepatotoxicity, neurotoxicity, teratogenicity, mutagenicity, and cancerogenity which can provoke acute and chronic effects in man and animals ranging from disorders of central nervous, cardiovascular, pulmonary and intestinal tract systems to death [1,8].

In order to inhibit fungal growth, and therefore reduce the production of mycotoxins on stored food, several methods have been developed and described in the literature and included the use of pesticides and synthetic fungicides, etc. [9]. Synthetic fungicides have effectively controlled plant diseases for a number of years [2]. They are usually applied, with or without combination with some physical methods. However, growing concern about the health and environmental effects of fungicides coupled with the multiple resistance to known antifungal drugs with high mortality rate have been reported [2,10]. This is currently pushing consumers towards a high demand for natural products and foods free of chemicals, preservatives and synthetic additives.

The genus *Ocimum*, belonging to the Lamiaceae family, has long been acclaimed for its diversity [11,12]. *Ocimum* comprises more than 30 species of herbs and shrubs from the tropical and subtropical regions of Asia, Africa, Central and South America, but the main center of diversity appears to be Africa. Among this, *Ocimum basilicum* collectively called basil is empirically used in folk medicine [9,10,12]. Different authors have described *O. basilicum* for the cough, inflammation, dyspepsia, headaches, worms, cardiotonic, abdominal pains, anti-diarrheal, acne, diabetes, eye diseases, dizziness and insomnia, etc. [9,12]. Similarly, some authors reported that essential oils obtained from the flower, seed, bark, stem and leaf of a plant could be a source of biologically active components responsible for the antifungal,

antibacterial, antiradical, anti-mycotoxigenic and antioxidant activities [2,9,11]. The *O. basilicum* EO's exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants [11]. On the other hand, the antimicrobial activity of EO is strain dependent and this vary with the taxonomy and their ecology [2]. In Ngaoundere, rice is widely consumed by the population and also represent a significant part of diets in Cameroon, due to their availability. To the best of our knowledge, antifungal activities of *O. basilicum* EO's against rice fungi have not yet been reported. Similarly, studies on fungal and mycotoxin contamination of rice are yet undocumented. The present work was undertaken with the main objective to investigate the chemical composition of the *O. basilicum* EO's and their antifungal activities.

2. MATERIALS AND METHODS

2.1 Plant Material

Whole mature plant of *O. basilicum* L. was harvested in Mbouda (5°38' North and 10°15' East) in the West Region of Cameroon. These plants were harvested in September 2018 and were identified in the National Herbarium in Yaounde -Cameroon.

2.2 Extraction and Chemical Composition Analyses of Essential Oils

Essential oil of plant material was extracted by the hydrodistillation method using Clevenger-type apparatus. Essential oil obtained was analyzed by gas chromatography and gas chromatography coupled with mass spectrometry (GC/MS). Gas chromatography was done in a Varian CP-3380 GC with flame ionization detector (FID) fitted with a fused silica capillary column (30 m x 0.25 mm coated with DB5, film thickness 0.25 µm). The operating conditions were: Injection temperature: 200°C; detection temperature: 200°C; temperature program 50–200°C at 5°C/min; carrier gas nitrogen, with a flow rate of 1mL/min. GC-MS analyses were performed using a Hewlett-Packard apparatus equipped with an HP1 fused silica column (30m x 0.25mm, film thickness 0.25 m) and interfaced with a quadrupole detector (GC quadrupole MS system, model 5970). Column temperature was programmed from 70–200°C at 10°C/min; injector temperature was 200°C. Helium was used as carrier gas at a flow rate of 0.6 mL/min. The mass spectrometer was operated at 70eV. The operating conditions were: temperature programming: 70 to 200°C at 10°C/min; Injection temperature: 200°C; Flow rate of vector gas (helium): 0.6 mL/min, injection volume: 0.1 µL of essential oil solution diluted at 10% in hexane. Compounds were identified by comparing the calculated retention indices and the mass spectrum with the literature.

2.3 Rice Sampling

Eighteen commercial rice samples of different brands were randomly collected from vendors in the suburban and rural market of Ngaoundere district during the month of July of 2019. One kilogram of each sample was collected aseptically in sterile, leak-proof plastic packaging (stomacher bags), labelled and immediately transported to the laboratory for further analysis.

2.4 Water Content

The water content of the rice samples was obtained using method described by AOAC [13], while, water activity was measured using an electronic hygrometer.

2.5 Microbial Characterization, Isolation and Identification of Molds

The method described by Pitt and Hocking [14] was used. After cleaning the surface of the rice grains in a 1% aqueous solution of NaOCl, they were rinsed three times with sterile distilled water for a total of 15 minutes. Ten grains thus cleaned were placed in Petri dishes containing Sabouraud culture medium, supplemented with chloramphenicol (60µg/mL). Incubation was carried out at 25 ± 2°C for 5 to 7 days, and visual observations were made every 24 hours.

The contamination rate of rice grains was calculated as follows:

$$\%\ \mathrm{GC} = \left(\frac{\mathrm{NCG}}{\mathrm{NSW}}\right) * 100$$

Where, GC: Percentage of contaminated grain, NCG: Number of contaminated grains at the end of the incubation period. NSW: Total number of grains sown

The fungal frequency (FF) of each species was calculated using the following formula:

%FF = number of isolates of a fungal species detected in a sample/total number of fungal isolates detected in a sample.

The fungal mycelia obtained were isolated and purified.

2.6 Identification of Molds

2.6.1 Phenotypical characterization

The isolated strains were purified using the successive dilution method described by Raper and Fennel [15]. The identification of isolated mold strains was carried out on the basis of morphological characteristics. The morphological identification keys and the laboratory guide for the identification of different mold species were used for mold identification. The identity was confirm using molecular based-methods,

2.6.2 DNA extraction from moulds, PCR amplification and sequencing

Purified fungal colonies were removed from the Petri dishes under aseptic conditions, using sterile distilled water with 0.1% Tween 80. Then the extraction of DNA from the moulds was done using FastDNA Spin Kit (MP Biomedical, Fisher Scientific SAS, France) according to the manufacturer's instructions. Purified DNA samples were quantified using a Nanodrop ND-1000 Spectrophotometer apparatus (Thermo Scientific, France) and stored at –20°C until use.

For species level identifications, a fragment of a specific region of the 28S rDNA called the D2 region, was amplified while using the following universaleukaryotic primers: forward, U1f GC (5' - CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GTG AAA TTG TTG AAA GGG AA – 3') and reverse, U2r (5' - GAC TCC TTG GTC CGT GTT – 3'). PCR was carried out in a final volume of 50 µL containing: 1.5 mM of $MgCl_2$, 5 µL of $MgCl_2$-free 10xTaq reaction buffer (Promega), all the deoxyribonucleotide triphosphate (dNTPs) at 200 µM, 0.2 µM of each primer, 2 µL of extracted DNA (≈ 30 ng) and 1.25 Units of Taq DNA polymerase (Promega). The amplification conditions were an initial denaturation for 3 min at 94ºC, 30 cycles of heating at 94ºC for 45 sec, cooling at 50ºC for 50 sec, heating at 72ºC for 90 sec and a final extension of 5 min at 72°C.

PCR products purity were verified by electrophoresis by loading 5 µL on 2% TAE agarose gels with 100 pb molecular weight ladder. Gels were stained and photographed as mentioned above. PCR products were purified of DNA from low melting agarose gel, with the Wizard PCR temperature Preps DNA purification system. Then were re-amplified under the same conditions but in this case using primers without GC-clamp and then sent for sequencing at GATC Biotech (Germany). The sequences of the 28S rDNA obtained were compared with those of the database available at NCBI GenBank (National Center for Biotechnology Information databases) using the BLAST program to determine the closest known sequences.

2.6.3 Determination of mycotoxins in different samples of rice

The dry rice samples were frozen at -80°C, then 20 g of this sample was placed in a "mixer blender" (Perten Laboratory Mill 3600) containing 100 mL of the extraction solvent (methanol + 3% bicarbonate solution, 50+50). The mixture was homogenized for 2 min at maximum speed. The resulting mixture was centrifuged in a refrigerated centrifuge at 3000 rpm for 30 min, and 25 mL of the supernatant was recovered. Subsequently, in 30 mL of PH 7.3 phosphate buffer (PBS), 10 mL of the extract was diluted, and the sample was placed in an immunoaffinity column (Ochraprep®, Rhône Diagnostics, Scotland). In the end, the toxin was released by elution from the column with methanol, as it allows the destruction of the antigen/antibody bonds by modifying the structure of the antibodies. The methanol was evaporated under a nitrogen stream at 70°C, and the residue was taken up by 1 mL of the mobile phase (water/acetonitrile/acetic acid, 51: 48: 1, v/v/v) in a vial for HPLC analysis. Optimal detection conditions for each toxin are obtained by applying the following excitation and fluorescence emission parameters: 365 and 440 nm for AFB1; 333 and 460 nm for OTA; 331 and 500 nm for CIT. The operating conditions are as follows: 100 µL injection loop, C18 reversed phase HPLC column, ODS granulometry 5 µm with identical precolumn, thermostatted at 35°C, isocratic flow rate of 1mL/min. The contents are calculated from a calibration curve derived from the standards.

2.7 Statistical Analysis

The different tests were carried out in triplicate and results were expressed as means ± standard deviation. The data were subjected to analysis of variance (ANOVA), differences between means were tested using the Duncan Multiple Range tests and multivariate analysis. All analysis was performed using XLStat^{TM} v. 2016.02 software (Addinsoft, Paris, France). The differences

were considered significant for a p-value $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Water Content, Water Activity of Rice Grain and Temperature of Storage Room

The temperature of the storage room, water content and water activity of the rice samples were measured, and the results are shown in Table 1. The values obtained vary from 23.5 – 27. 5°C, 0.6 - 0.7 and 10.5 - 18.0% respectively for temperature, water activity and water contain. There are no significant differences between these measured values (P>0.05) with regard to the room temperature and water activity except the water content where we observe significant differences between the samples (P <0.05).

Table 1. Water content, water activity of rice grains and storage temperature

Codes	Temperature	Water activity	Water contains (%)
SAKC1	26.0 ± 1.2^{bc}	0.68 ± 0.04^{a}	13.8 ± 0.2^{fg}
SAKQ1	24.5 ± 0.6^{ab}	0.64 ± 0.05^{a}	10.8 ± 0.2^{a}
SAMG1	24.0 ± 1.1^{ab}	0.65 ± 0.08^{a}	13.2 ± 0.2^{ef}
SCAP1	25.5 ± 1.5^{abc}	0.64 ± 0.06^{a}	12.9 ± 0.1^{e}
SDIJ1	26.0 ± 0.4^{bc}	0.66 ± 0.04^{a}	10.5 ± 0.6^{a}
SEMN1	25.5 ± 0.6^{abc}	0.65 ± 0.07^{a}	14.6 ± 0.6^{gh}
SEMO2	27.0 ± 0.5^{c}	0.65 ± 0.07^{a}	16.8 ± 0.3^{j}
SLUL1	25.5 ± 1.3^{abc}	0.64 ± 0.05^{a}	17.8 ± 0.2^{k}
SLUS1	24.5 ± 0.7^{ab}	0.66 ± 0.08^{a}	11.9 ± 0.1^{bcd}
SMAH1	24.5 ± 0.8^{ab}	0.65 ± 0.07^{a}	12.7 ± 0.4^{de}
SMÉK1	26.0 ± 1.2^{bc}	0.64 ± 0.06^{a}	14.9 ± 0.1^{h}
SMÉR1	26.0 ± 1.1^{bc}	0.67 ± 0.09^{a}	12.9 ± 0.1^{e}
SNED1	25.5 ± 0.6^{abc}	0.67 ± 0.04^{a}	11.9 ± 0.1^{bc}
SONI1	25.5 ± 0.8^{abc}	0.64 ± 0.06^{a}	11.7 ± 0.4^{b}
SPAA1	24.5 ± 0.7^{ab}	0.65 ± 0.06^{a}	15.8 ± 0.3^{i}
SPRE1	24.0 ± 1.2^{ab}	0.67 ± 0.01^{a}	12.6 ± 0.5^{cde}
STAM1	26.0 ± 1.3^{bc}	0.63 ± 0.04^{a}	12.7 ± 0.5^{de}
SWEF1	23.5 ± 0.7^{a}	0.69 ± 0.06^{a}	11.7 ± 0.5^{b}

Values in each column with different superscripts are significantly different

Table 2. Microbial characteristics and mycotoxins (OTA, AFB1, CIT) contains of rice samples

Codes	Number of samples	Contamination (%)	OTA (µg/Kg)	AFB1 (µg/Kg)	CIT (µg/Kg)
SPAA1	4	100	0	0	n.d
SAKC1	5	10	0	7.3	n.d
SNED1	4	100	0	3.3	n.d
SPRE1	6	30	0	13.1	n.d
SWEF1	3	80	0.1	4.1	n.d
SAMG1	7	30	0	0	n.d
SMAH1	4	10	0.3	0	n.d
SONI1	6	20	1.1	3.8	n.d
SDIJ1	4	30	0	5.1	n.d
SMÉK1	6	30	0	0	n.d
SLUL1	3	10	0.2	0.5	n.d
STAM1	4	20	0	17.3	n.d
SEMN1	3	100	3.9	0.4	n.d
SEMO2	3	40	5.2	9.7	n.d
SCAP1	7	30	0	0	n.d
SAKQ1	7	30	2.5	1.3	n.d
SMÉR1	5	50	0	0	n.d
SLUS1	4	10	0	0.4	n.d
References[a]	/	/	3	5	2

n: number of isolates identified in the samples; maximum values recommended by the European Commission N° 1881/2006; AFB1: aflatoxins B1, OTA: ochratoxin A, CIT: citrinin

The value of water content and temperature obtained in this study is slightly high than this reported by authors with the maximum values of 18.0% and 27.5°C respectively [4]. The rice samples sold and collected in this study are mainly imported products. They face many difficulties, especially during storage [4]. The parameters involved are, among others, the storage temperature, the relative humidity of the surrounding environment and especially the storage time. In addition, these different parameters vary with the geo-climatic conditions (climate, reliefs, etc.) of the sampling site, the storage silos, and even the conditions of analysis [3,8]. Several authors report the positive effect of the water content of rice and room temperature on the growth of fungi.

The literature data show that storage temperature generally influences fungal growth in rice seeds, and can also modify the chemical composition of rice; a storage temperature of 15 °C would be appropriate to limit losses in rice quality [3]. Rice, at all stages of production is known to be susceptible to attack from many pathogenic fungi.

3.2 Contamination and Fungal Identification

The rate of mold contamination was evaluated and the data is reported in Table 2. All the samples analyzed are contaminated and the level of contamination varies with the sample. Contamination rates range from 10 to 100% and more than 27% of the rice samples collected had more than half of the seeds contaminated by mold. Globally, an average of 5 fungal species per sample were detected. Representative isolates (n = 85) from each sample were selected and identification were performed using morphological and molecular techniques (Table 3). The DNA extraction performance verification gel shows intense bands of high molecular weights (16 kb). These bands would correspond to microbial genomic DNA including that of molds. The appearance of the bands obtained reflects a sufficient quantity of DNA for the performance of the PCR (Fig. 1). Otherwise, the results of PCR amplification of the 28s gene of mold rDNA show single, intense bands between 250-298 bp, which corresponds to the expected size of 260 bp for molds (Fig. 1). Eight fungal genera (n = 8) and 14 fungal species were identified in the rice sold in Ngaoundere town. The genera *Aspergillus, Fusarium* and *Penicillium* are mainly represented with percentages of 51%, 30% and 12% respectively. The fungal species involved are *A. flavus, A. fumigatus, A. niger, A. carbonarius, F. oxysporum, F. graminarum, F. moniliforme, P. citrinum, P. expansum* with varying percentages. The identification at the species level was confirmed using molecular approach. In addition, this approach allowed us to identify other minority species belonging to the genera *Rhizopus, Cladosporum, Mucor, Alternaria* and *Acremonium* in this study.

Several studies on fungal contamination of rice in storage and those having undergone prolonged transportation have been made worldwide [3,4]. Authors have reported the presence of *Aspergillus, Penicillium Fusarium Rhizopus,*

Table 3. Fungal frequency and identities at species level of isolates by Morphological and molecular approach

Genera	Proportion (%)	Morphological	Proportion (%)	Molecular	PI
Aspergillus	46	*A. flavus*	24	*A. flavus*	100
		A. fumgatis	9	*A. fumgatis*	99.9
		A. niger	7	*A. niger*	99.9
		A. carbonarius	6	*A. carbonarius*	100
Fusarium	25	*F. oxysporum*	19	*F. oxysporum*	99.9
		F. graminarum	8	*F. graminarum*	99.9
		F. moniliforme	7	*F. moniliforme*	99.9
Penicillium	12	*P. citrinum*	7	*P. citrinum*	99.9
		P. expansum	5	*P.expansum*	100
Rhizopus	11	*R. oryzea*	11	*R. oryzea*	100
Cladosporum	1	*Cladosporum* sp	1	*Cladosporum* sp	99.9
Mucor	1	*Mucor* sp	1	*M. hiemalis*	99.9
Alternaria	1	nd	1	*A. solani*	99.9
Acremonium	1	nd	1	*A. murorum*	99.9

nd: not detected; P: proportion

Cladosporum and *Mucor sp,* species in different rice samples around the world [1,3,4]. They are mostly implicated in spoilage of rice [3,4,8]. Similarly, it has been reported in the literature that *A. flavus* specie was the major contaminant of 34 rice's samples [8]. Among the species identified, some are able to produce a range of mycotoxin including: *A. versicolor, A. fumigatus, A. carbonarius, F. graminerium, F. moniliforme, A. solani* and *P. citrinum*. It is however important to emphasize that the presence of toxigenic strains does not automatically imply the presence of mycotoxins, certain conditions must be met for them to be effectively produced [3]. A species may be known to be toxigenic, but the strains or isolates do not produce mycotoxins. Surekha et al. [3] showed that 83% of *A. flavus* and 50% of *A. ochraceus* isolates from rice produce AFB1 and OTA, . It was therefore necessary to detect the presence of mycotoxins in different rice samples.

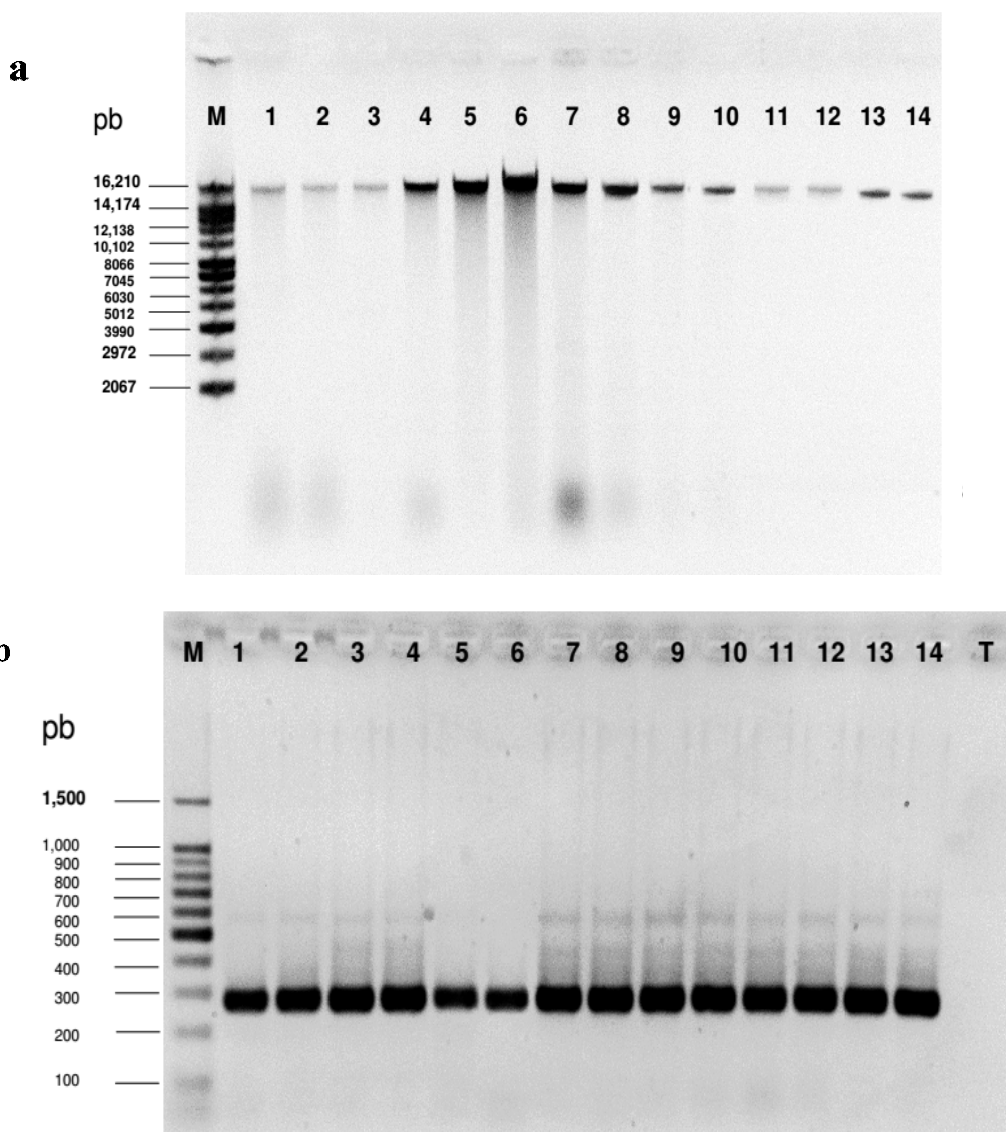

Fig. 1. Photo of gel for verification of mold DNA extraction performance (a), and verification of mold DNA amplification (b) 1: A. flavus; 2: A. fumigatus; 3: A. niger; 4: A. carbonarius; 5: F. oxysporum; 6: F. moniliforme; 7: F. graminearum; 8: P. citrinum; 9: P. expansum; 10: R. oryzea; 11: Cladosporum sp.; 12: Mucor sp.; 13: Alternaria sp.; 14: Acremonium sp

3.3 Mycotoxins

The mycotoxin content including aflatoxin B1 (AFB1), ochratoxin (OTA) and citrinin (CIT) were measured (Table 2). We observed that more than 61% (11/18) and 44% (8/18) of analyzed samples contain respectively AFB1 (values range from 0 - 17.3 µg/Kg) and OTA (values range from 0 - 5.2 µg/Kg). In addition, 33.3% (6/18) was co-contaminated with AFB1 and OTA while, 27.8% (5/18) were free-contaminated with AFB1 and OTA. The CIT was not detected in this study. According to the recommendations of the European Commission N ° 1881/2006, the maximum limit values are set at 5 µg/kg (AFB1) and 2 µg/kg (OTA). This study revealed that 37.5% (3/8) and 36.4% (4/11) of samples respectively contains mycotoxins OTA and AFB1 with values above the recommended one, this shows that some rice of the Ngaoundere locality present a high risk for human consumption. Among these rice mycotoxins, aflatoxin B1, (AFB1), fumonisin B1 and ochratoxin A are the most toxic for human health [8]. Several authors reported the presence of aflatoxin and ochratoxin in rice samples [1,5]. Regard to the same study, data show that *Aspergillus* specie's were dominant fungi on rice. The high levels can be explained by the fact that mycotoxins are reduced in white rice due to treatments such as milling and polishing. Iqbal et al. [16] found an incidence of 29% and 46% and an average OTA content of 8.5 µg / kg and 7.84 µg / kg, respectively in samples of white rice and in samples of brown rice collected in Pakistan. Regarding AFB1, Iqbal et al. [16] found that 15 of 28 (54%) and 14 of 34 (41%) of brown rice and white rice samples, respectively, were positive for AFB1. The average levels found were 8.91 µg / kg and 7.70 µg / kg, respectively for brown rice and white rice. Reiter et al. [17] found AFB1 contents varying from 0.45 to 9.86 µg / kg in samples of short grain rice, long grain rice, brown rice, basmati rice, as well as puffed rice from Austria.

3.4 Chemical Composition of *Ocimum basilicum* Essential Oils

The EO extraction yield of *O. basilicum* was 0.56%.This yield can be compared to those obtained by Sajjadi [11] who found a yield of 0.67% after hydrodistillation of *O. basilicum* from Iran. The chemical composition of *O. basilicum* EO's was analyzed by GC/MS (Table 4). This analysis revealed the presence of 13 compounds. Of these compounds, we note the presence of monoterpenes (60.7%), among which with oxygenates monoterpens (54.7%) were found in highest proportion. Also, aromatic compounds (30.6%) and sesquiterpenes (8.7%) were also detected. The majority compounds in this essence are eugenol (30.6%), linalool (29.5%), cineole (14.4%). The essential oil of *Ocimum basilicum* was the subject of former studies. It was previously reported [18] that the oil of *O. basilicum* contained linalool (69%), eugenol (10%), (E)-α-bergamotene (3%) and thymol (2%). Linalool (45.7%), eugenol (13.4%), methyl eugenol (9.57%) and fenchyl alcohol (3.64%) were reported to be the main components of the previously analysed materials [19]. The differences on yields extraction and chemical composition observed between these results may be due to parameters such as: the pre-treatment of the plant material before distillation (drying, grinding), the hydrodistillation time, the storage time of the plant material after harvest, the place of harvest, the geographic origin of the plant, the collection period and the climate [20].

3.5 Antifungal Activity

The antifungal activity of *O. basilucum* EO's was also evaluated in this study. The mycotoxinogenic fungal strains selected on the basis of the literature were tested and the different Minimum Inhibitory Concentration (MIC) values obtained are illustrated in Table 5. The MIC values varies with the tested fungi. The data obtained show that the essential oil of *O. basilicum* is active on the selected fungal isolates with MIC values ranging from 750-2000 ppm. Among the nine species tested, *A. fumigatis, A. niger, P. citrinum* were the most sensitive strain because it has the lowest MIC values while, *A. flavus* the most resistant strain with the highest MIC value. Neveen et al. [10] reported that the MIC value of *O. basilicum* against *A. flavus* was 1000 ppm. The extent of inhibition was widely dependent upon the composition and the concentration of EOs. The inhibitory effect of the oils increased as oil concentration increased. The antifungal activities of these oils might be attributable to the main compounds that they contain. A large variety of EOs and many of their majority compounds, mainly mono and sesquiterpenes have been shown to have antifungal activities. Generally, the EOs possessing the strongest antifungal activities against food borne pathogens contain a high percentage of phenolic compounds such as carvacrol, linalool, eugenol, isoeugenol and thymol [9].

The inhibitory mechanisms vary and depend on these compounds. They may include enzyme inhibition, loss of function by the oxidized compounds through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins. The consequence include damage to membrane integrity, which could affect pH homeostasis and equilibrium of inorganic ion [9].

Table 4. Toxigenic potential of fungi colonized in stored rice

Sample code	Molds	Mycotoxins (µg/Kg)	
		OTA	AFB1
SPAA1	*R. oryzea, M. hiemalis, A. solani, A. murorum*	0	0
SAKC1	*R. oryzea*, *A. niger*	0	7.3
SNED1	*A. niger, P. citrinum*	0	3.3
SPRE1	*A. niger*, *M. hiemalis*	0	13.1
SWEF1	*R. oryzea*, *A. flavus*	0.1	4.1
SAMG1	*R. oryzea*	0	0
STAM1	*A. niger, A. flavus*	0.3	0
SONI1	*A. niger, R. oryzea*	1.1	3.8
SDIJ1	*A. flavus*	0	5.1
SLUL1	*A. niger, A. flavus, A. fumigatus, P. citrinum, F. solani*	0.2	0.5
STAM1	*A. flavus*	0	17.3
SEMN1	*M. hiemalis*, *A. flavus*	3.9	0.4
SEMO2	*A. flavus, A. niger*	5.2	9.7
SCAP1	*R. oryzea*	0	0
SAKQ1	*A. flavus, A. niger*	2.5	1.3
SLUS1	*Cladosporum* sp	0	0.4

Table 5. Chemical composition of *O. basilucum* essential oil

No	Retention index	Compounds	Proportions (%)
1	1365	Eugenol	30.6
2	1102	Linalol	29.5
3	1033	1.8-cineole	14.4
4	1181	Terpinen-4-ol	5.6
5	1439	2-norpinen	4.1
6	1624	Cadinen	3.3
7	1030	Limonen	2.4
8	1047	(E)-*β*-Ocimene	2.2
9	1194	*α*-terpineol	2
10	1148	Camphor	1.6
11	1092	Fenchone	1.6
12	979	*β*-pinène	1.4
13	1546	*α*-humulène	1.3

Table 6. Minimal inhibitor concentration (MIC) of *O. basilicum* for each isolate

Species (n=isolates)	Samples	MIC (PPM)
A. flavus (3)	STAM1, STAM1, SAKQ1	1500-2000
A. fumigatis (2)	SLUL1	750-1000
A. niger (3)	STAM1, SONI1, SAKQ1	750-1000
A. carbonarius (2)	SDIJ1, SMÉK1	1000-1250
F. oxysporum (2)	SMEK1, SMER1	1250-1500
F. graminarum (1)	SMER1	750-1000
F. moniliforme (1)	SMER1	1000
P. citrinum (2)	SNED1, SLUL1	750-1000
P. expansum (2)	SMEK1	1250-1500

4. CONCLUSION

The results of this study demonstrated that, some rice sold in Cameroon represent a human health risk for consumers based on the levels of mycotoxins contained. On the other hand *O. basilicum* essential oils from Cameron can also serve as alternative means to prevent or control fungal attack and the presence of mycotoxin in stored rice and upon foodstuff. However, the strength of these properties depends on the plant and fungal species, concentration of the testing oil and the testing conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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