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Prevention of Ochratoxin A (OTA) Production in Coffee Beans Using Natural Antifungal Derived from *Solanum indicum* L. Green Berries

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Abstract This study was aimed to find out an alternative to synthetic fungicides used in the control of fungi development in crops including coffee. The antifungal was extracted from green berries of *Solanum indicum* L. and its effect on the behavior of three *Aspergillus* species Ochratoxin A (OTA) producers (*Aspergillus niger*, *Aspergillus carbonarius* and *Aspergillus ochraceus*) was evaluated on a coffee-agar medium. The results show a reduction of growth with the increase of the antifungal content in the medium. Indeed, on the medium without antifungal, the growth rate has reached 90 mm within only 3 days of incubation for the three strains tested, while no growth was observed for *A. niger* and *A.carbonarius* from 8% of antifungal in the medium and for *A. ochraceus*, at 10% of antifungal in the medium even after 7 days of incubation. However, the total inhibition of OTA was observed from 8% of antifungal in the medium for these three strains of *Aspergillus*. Indeed, the amounts of OTA produced on the coffee-agar medium without antifungal were 0.005, 1.63 and 0.108 µg/mL respectively for *A. niger*, *A. carbonarius* and *A. ochraceus*, while from 8% of antifungal fraction in the medium, no detectable OTA amount was observed for the three strains tested after 7 days of incubation. The phytochemical screening of this antifungal fraction revealed compounds including flavonoids, carotenoids and saponins. These results suggest the use of this antifungal fraction as an alternative to synthetic fungicides for the control of fungi development and OTA production in coffee beans.

Keywords: *coffee beans, Solanum indicum L., green berries, Aspergillus, Ochratoxin A, antifungal*

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1. Introduction

Coffee is the most widely commercialized tropical product on the international market [1]. With global consumption forecast at a record 163.2 million bags, exports are expected up in response to strong demand according to United State Department of Agriculture [2]. Thus, world coffee production for 2018/2019 is forecast 11.4 million bags higher than the previous year at a record 171.2 million according to the USDA's "Coffee: World Markets and Trade" report, published in June 2018. In developing countries including Côte d'Ivoire, although consumption of coffee is less than that of the developing countries, this product is one of the raw material supporting the economy. Indeed, in Africa, the quantity of the variety Robusta which is coffee more produced was around 7.2 million bags of 60 kg according to [3]. Unfortunately, coffee as many vegetables products is highly susceptible to fungal contamination in various stages of growth and processing and in different local climates [4]. The occurrence of these fungi isolated from coffee cherries has been reported by several authors [5,6]. According to [4], *A. niger* was predominated (66.41%), followed by *A. carbonarius* (14.50%). The prevalence of these *Aspergillus* species was also observed in other studies [7,8]. This contamination by fungi leads to a putrefaction of food that involves any physicochemical change and makes food unacceptable for human consumption [9]. Some of these fungi are capable of producing mycotoxins. The main mycotoxin found in coffee is Ochratoxin A (OTA) [10,11]. It is produced by molds of the genera *Penicillium* in moderately cold regions and by the genera *Aspergillus* in tropical regions [12]. According to [13], *A. carbonarius*, *A. ochraceus* and *A. niger* were the main species OTA producers isolated. These authors showed that 100% of *A. carbonarius* and *A. ochraceus* strains isolated were potential OTA producers. However, although *A. niger* was more isolated than *A. carbonarius* and *A. ochraceus*, only 2.4% to 3.8% of strains tested were capable of producing OTA. The

isolate, originally described as *A. ochraceus*, from which ochratoxin A was first discovered in South Africa, is now the type strain of *A. westerdijkiae* [14] and this species is now recognized as a major producer of ochratoxin A. The natural occurrence of OTA in green coffee beans has been reported by several authors in concentrations from 0.2 to 360 $\mu g \cdot kg^{-1}$ [15]. This mycotoxin produced by these toxigenic fungi has been shown to exhibit nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties [16,17]. The International Agency for Research on Cancer (IARC) has classified OTA as a possible human carcinogen (Group 2B) based on sufficient evidence for carcinogenicity in experimental animal studies and inadequate evidence in humans [18]. Furthermore, this contamination of food by fungi leads to the degradation of the nutritional quality.

Thus, in order to avoid an impact of this mycotoxin on human health, the European Union has set maximum levels of 5 µg/kg for OTA in roasted coffee and of 10 µg/kg for soluble coffee [19]. This maximum limit for OTA in coffee could affect international trade for producer countries. Therefore, solutions to avoid coffee cherries contamination by OTA must be found. For many years now, it has been clear that the most effective means to prevent contamination of crops by mycotoxins is to avoid growth of mycotoxigenic fungi [20]. The primary method of control is good manufacturing practice as described and shown by [21]. However, the zero risks of contamination is difficult to be achieved. Thus, many famers use chemical fungicides to prevent crops contamination by these fungi. However, requirements regarding the safety of these formulations were improved due to the toxicological risks [22]. Furthermore, the general public demands a reduced use of chemical preservatives and additives in food and feed [23]. Therefore, the use of natural substances capable of inhibiting fungi development as well as mycotoxins production is of great importance. Our preliminary investigations have shown that the berries of *Solanum indicum* L. a wild plant consumed by rural populations in Côte d'Ivoire seem not to be infected by microorganisms despite the ecological conditions which are favorable for their development [24]. This plant species belongs to the genus *Solanum* and the family of *Solanaceae* with more than 1,700 species. This species is an erect plant of 0.30 to 1.5 meters in height. The leaves are ovate, 3.5 to 15 centimeters long, and 2.5 to 8 centimeters wide. The leaves in the branchlets are much smaller. The unripe fruit is green while, the color of the ripe fruit varies from yellow to red [25]. They are rounded; about 0.8 to 1.5 centimeters in diameter. The berries of this plant which are called in Côte d'Ivoire "Gnangnan" are used for nutritional and culinary purposes in many parts of Africa as they contain appreciable amounts of starch, calcium, vitamin A, ascorbic acid and phosphate [26]. In addition to components mentioned above, these berries have been shown to contain polyphenols [25] and steroidal glycerides [27]. However, the use of this species has not limited to food. Indeed, *Solanum indicum* L. seeds, roots, leaves and berries are used therapeutically for asthma, dry cough, chronic febrile afflictions and in dysuria. The berries have been suggested useful in leucoderma, pruritis and bronchistis and they have been claimed in folk medicine to have an antihypertensive effect [28]. In West Africa, the uses are not based on scientific studies but rather on empirical practices. Whether these berries are effective in treating any of these diseases, their use as food and medicine indicates that they have been ingested by humans for quite a while at many doses.

Our recent activities carried out have shown that the extract of the green berries of this plant has an inhibitory effect on growth and mycotoxins production by fungi such as *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus nidulans* [29].

From this background, we can move forward to explore the use of these green berries of *Solanum indicum* against growth of strains of *Aspergillus* OTA producers and their ability of producing OTA in coffee for further contribution for the search for alternative in chemical fungicides.

2. Material and Methods

2.1. Material

2.1.1. Biological Material

In this study, green berries of *Solanum indicum* L. ("Gnangnan" berries) were used. These berries have been collected from rural zones of the central part of Côte d'Ivoire where they are found in abundance. In addition to these berries, *Coffea canephora* P., variety Robusta green coffee beans which are more produced in Côte d'Ivoire were used. Three *Aspergillus* species tested capable of producing Ochratoxin A (OTA) (*Aspergillus niger* PN01, *Aspergillus carbonarius* PC62 and *Aspergillus ochraceus* PO22) from the laboratory of Biochemistry and Food Sciences, UFR Biosciences, University Félix Houphouet-Boigny (Côte d'Ivoire) were also used. These *Aspergillus* species were identified by the laboratory of Parasitology-Mycology of Pasteur Institute of Abidjan (Côte d'Ivoire).

2.2. Methods

2.2.1. Medium Preparation

The culture medium used in this study was the coffee-agar medium (CAM) prepared using green coffee powder of *Coffea canephora* P., variety Robusta tested free of OTA. This medium was used to bring us closer to field conditions. A quantity of 30 g of finely ground green coffee beans was added to 1 L of distilled water. The whole was homogenized for 1 h. To the resulting coffee suspension obtained, a quantity of 15 g of agar was added. The resulting coffee-agar medium was sterilized by autoclaving at 120 °C for 20 min.

2.2.2. Berries Extract Preparation

The berries extract preparation was done according to the method used by [29]. This extraction consisted in grounding the green berries of *Solanum indicum* and 30 g of the obtained homogenate were added to 150 mL of ethanol 70 % (v/v). The mixture was boiled in water bath at 80°C for 1 h under gentle stirring. The resulting mixture was centrifuged at 1500 rpm for 5 min. The supernatant was then filtered through Whatman paper and the filtrate

obtained was evaporated to dryness under Fume Hood. This drying was made by a ventilation system. The residue obtained was dissolved into 15 ml of boiled distilled water and shaken until total dissolution. In order to purify the homogenate obtained and used the fraction containing the antifungal compounds, the method of purification by ethyl acetate was used. This purification of the extract was made by adding to the homogenate obtained, 15 ml of ethyl acetate (v/v). The resulting mixture was shaken during 1 min. and centrifuged at 2000 rpm for 10 min. Aqueous and ethyl acetate phases were obtained. The ethyl acetate phase was recovered into a new tube. This purification was done three times. The aqueous solution obtained was dried under Fume Hood. The residues obtained was dissolved into 15 ml of distilled water and then filtrated onto 0.20µm cutoff membranes to eliminate residues which were not dissolved and eventual contaminants. This aqueous fraction containing the antifungal compounds was used for the in vitro control of fungal growth and OTA production.

2.2.3. Phytochemical Studies of the Antifungal Fraction of Berries Extract

The antifungal fraction obtained was subjected to various qualitative tests for the identification of constituents like flavonoids, alkaloids, saponins, glycosides and carotenoids. Different methods were used for the identification of each constituent.

- The test for flavonoids used was that of [30] which consisted in adding to 1 mL of the antimicrobial fraction in a test tube, a few chop of 1 % NH3 solution. The appearance of yellow coloration shows the presence of flavonoids compound.
- For the alkaloids, the test used was that of [31] which consisted in adding to 1 mL of the antifungal fraction, a quantity of 2 mL of Drangendroff's reagent. The appearance of a turbid orange color shows the presence of alkaloids. The carotenoids were also identified.
- For these carotenoids, the test used was that of [32] which consisted in drying under Fume Hood, 1 mL of the antifungal fraction containing in a test tube.

A quantity of 10 mL of chloroform was then added to the residue obtained and the whole was shaken vigorously. The resulting mixture was filtered and 85% sulphuric acid was added. The appearance of a blue color at the interface shows the presence of carotenoids.

- For the saponins, the test of [33] was used. This test consisted in shaking vigorously 10 mL of the antifungal fraction and then sat it for 10 min. The appearance of a stable froth shows the presence of saponins.
- For the glycosides, the test used was that of [34] which consisted in adding to 1 mL of the antifungal fraction, 1 mL of FeCl3 reagent (mixture of 1 volume of 5% FeCl3 solution + 99 volume of glacial acetic acid) and a few drops of concentrated H2SO4. The appearance of a greenish blue color within few minutes shows the presence of glycosides.

2.2.4. Preparation of the Tested Strains

The *Aspergillus* strains were sprayed onto the Czapeck Yeast Extract Agar (CYA) for 3 days. The different suspensions of spores were then prepared by scraping the conidiospores into 10 mL of sterilized distillated water and filtered onto sterilized Miracloth (Filter composed of rayon polyester with a pore size of 22 µm and an acrylic binder). The conidia concentration of each strain was determined by counting them in a hemacytometer and appropriate dilution was made to obtain a concentration of 10^6 spores/mL. These suspensions of 10^6 spores/mL were used for the tests of inhibition of fungal growth and OTA production [35].

2.2.5. Monitoring of Fungal Growth

The fraction containing the antifungal compounds was added (v/v) to the coffee-agar medium (CAM) to obtain mediums with different contents of 1%, 2%, 4%, 6%, 8% and 10%. Each medium was put into a Petri dish and after solidification, 10µL of the *Aspergillus* conidia suspension were put aseptically in the center of this medium. The medium without antifungal fraction was also inoculated. For each antifungal content in the medium, three Petri dishes were used. All the inoculated mediums were incubated at 30°C. The growth rate was determined by measuring the diameter of the colony after 7 day of incubation according to the method of [36]. The tests were done in triplicate. The measurement of the diameter was made using a graduated ruler.

2.2.6. Preparation of Standard OTA and Analysis

For the standard solutions preparation, a commercially bought CGA (Certified good for analysis) were dissolved in polar-solvent (ethanol) and standard OTA solutions with concentrations of 0.026, 0.11 and 0.15 µg/mL were prepared and analyzed. This analysis was done by high-pressure liquid chromatography (HPLC) with a fluorescence detector FL3000 (excitation wavelength 332nm, emission wavelength 466nm). The HPLC column used was a C18 Sorbox SB-48 (5 µm, 4.6 × 150 mm) (Agilent technologies, USA). A total of 80 µL of each sample was injected. The mobile phase consisted of acetonitrile/acetic acid 0.2% (41:59). The flow rate was 1 $mL \cdot min^{-1}$.

The retention time for the detection of OTA was around 16 min. The detection limit was 0.0003 $\mu g \cdot g^{-1}$. The chromatograms obtained were used to determine the calibration curve.

2.2.7. Analysis of OTA Produced by Fungi

After monitoring of the growth, the test for OTA production by fungi was carried out by using the method of [37]. On each medium containing antifungal fraction at different contents, three agar plugs were removed from different points of the colony after these 7 days of incubation and extracted with 1 mL methanol. The extracts were filtered and analyzed by high-pressure liquid chromatography (HPLC) with a fluorescence detector FL3000 in the chromatographic conditions as those described above.

OTA produced by strains of *Aspergillus* on the coffee-agar medium without antifungal fraction was also evaluated in order to determine the potential toxigenic of these strains on this medium. The chromatograms which peak appears at the retention time of the chromatogram of the OTA standard solution were identified as OTA produced by the *Aspergillus* strains tested.

2.2.8. Statistical Analysis

The statistical analysis of data was done by Analysis of Variance (ANOVA) using 5% level of significance. The statistical package used is IBM SPSS Statistics version 20. Tukey's Multiple Comparison test was used to identify these differences.

3. Results

The evaluation of the effect of the antifungal fraction of *Solanum indicum* L. berries extract on growth of the three strains of *Aspergillus* tested reveled that with increases in this antifungal fraction content in the medium, there was less fungal growth (Figure 1). The mean of growth rate after 7 days of incubation which was 90 mm on the medium without antifungal fraction decreased to reach the values of 89.5, 80.6, 58.5, 25.9, 0 and 0 mm for *A. niger*, 89.2, 81, 52.4, 23.1, 0 and 0 mm for *A. carbonarius*, and 89.6, 88, 64.4, 40.7, 19.8 and 0 mm for *A. ochraceus* respectively on the medium at 1%, 2%, 4%, 6%, 8% and 10% of antifungal fraction. The absence of growth was noted from 8% of antifungal fraction in the medium for *A. niger* and *A. carbonarius*, while for *A. ochraceus*, the total inhibition was observed at 10% of antifungal fraction in the medium. However, whatever the *Aspergillus* strain tested, a dose dependent inhibition of the growth was observed with the increase of the antifungal fraction content in the medium. Indeed, it was noted that inhibition of the three strains proliferation was influenced significantly ($P < 0.05$; Table 1) by the antifungal fraction content in the medium. This reduction of growth started already on the medium at 1% of antifungal fraction (Table 1). However, from 0 to 1 % of antifungal fraction in the medium, no significant difference between the growths rate was observed for *A. niger* and *A. carbonarius* ($P>0.05$). For *A. ochraceus*, no significant difference between the growths rate was observed from 0 to 2 % of antifungal fraction in the medium ($P>0.05$). Significant reduction of fungal growth was observed at concentration of 3% of antifungal in the medium for *A. niger* and *A. carbonarius* while for *A. ochraceus*, significant reduction of fungal growth was observed from 4% of antifungal in the medium.

The tests for OTA production showed also a dose dependent inhibition of OTA production with the increase of the antifungal fraction content in the medium for the three strains of *Aspergillus* tested (Table 2). The amounts of OTA produced were determined using the calibration curve obtained by analyzing three solutions of OTA standard (Figure 2) which concentration were 0.026, 0.11 and 0.15 µg/mL. The calibration curve equation was $y = 920808x$ (Figure 3).The amounts of OTA produced on the medium without antifungal fraction which were 0.0054, 1.63 and 0.108 µg/mL (Figure 4, Table 2) respectively for *A; niger*, *A. carbonarius* and *A. ochraceus*. decreased significantly with the increase of the antifungal fraction content in the medium to reach undetectable values from 8% of antifungal fraction in the medium for all these strains of *Aspergillus* ($P<0.05$, Table 2). It is also noted that, although there was growth of *A. ochraceus* on the medium at 8% of antifungal fraction in the medium, the test for OTA production didn't show detectable amount of this mycotoxin.

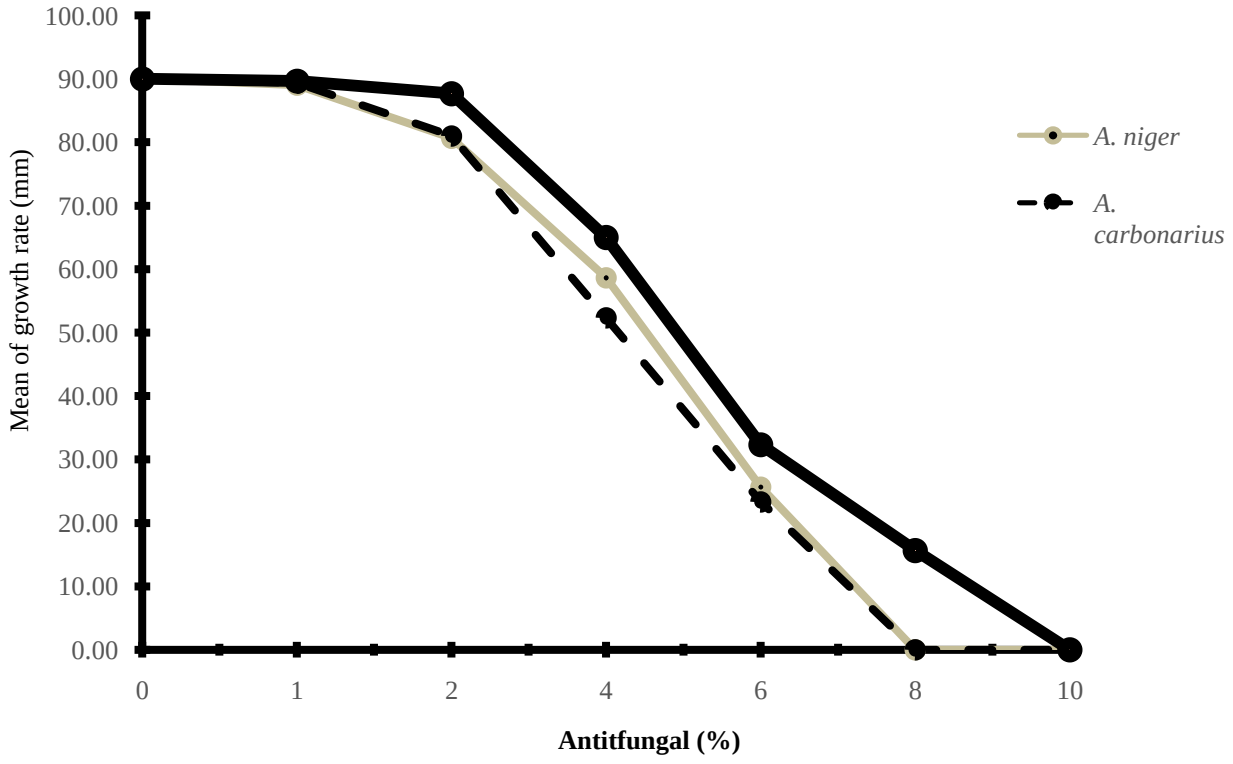

Figure 1. Effect of the antifungal fraction of *Solanum indicum* L. green berries extract on the growth of *A. niger, A. carbonarius and A. ochraceus* after 7 days of incubation

Table 1. Dose-dependent effect of the antifungal fraction of *Solanum indicum* L green berries on fungal growth

Homogeneus subsets						
Tukey HSD				**Growth rate (mm)**		
Antifungal content in the medium		**Subset for alpha = 0.05**				
A. niger	Medium without antifungal fraction	90.00				
	Medium at 1% of antifungal fraction	89.00 ± 1.00				
	Medium at 2% of antifungal fraction		80.67 ± 0.58			
	Medium at 4% of antifungal fraction			58.67 ± 0.58		
	Medium at 6% of antifungal fraction				25.67 ± 1.15	
	Medium at 8% of antifungal fraction					0.00
	Medium at 10% of antifungal fraction					0.00
	Significance	**0.81**	**1.00**	**1.00**	**1.00**	**0.81**
A. carbonarius	Medium without antifungal fraction	90.00				
	Medium at 1% of antifungal fraction	89.33 ± 0.58				
	Medium at 2% of antifungal fraction		81.00 ± 1.00			
	Medium at 4% of antifungal fraction			52.33 ± 0.58		
	Medium at 6% of antifungal fraction				23.33 ± 0.58	
	Medium at 8% of antifungal fraction					0.00
	Medium at 10% of antifungal fraction					0.00
	Significance	**0.81**	**1.00**	**1.00**	**1.00**	**0.81**
A. ochraceus	Medium without antifungal fraction	90.00				
	Medium at 1% of antifungal fraction	89.67 ± 0.58				
	Medium at 2% of antifungal fraction	87.67 ± 0.58				
	Medium at 4% of antifungal fraction		65.00 ± 1.00			
	Medium at 6% of antifungal fraction			32.33 ± 058		
	Medium at 8% of antifungal fraction				15.67 ± 0.58	
	Medium at 10% of antifungal fraction					0.00
	Significance	**0.79**	**1.00**	**1.00**	**1.00**	**1.00**

n.d.: Not detected
Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Table 2. Dose-dependent effect of the antifungal fraction of *Solanum indicum* L. green berries on OTA production by fungi

Homogeneus subsets					
Tukey HSD				**Amounts of OTA (µg/mL)**	
Antifungal content in the medium		**Subset for alpha = 0.05**			
A. niger	Medium without antifungal fraction	0.0054 ± 0.0002			
	Medium at 1% of antifungal fraction	0.0054 ± 0.0002			
	Medium at 2% of antifungal fraction	0.0053 ± 0.0001			
	Medium at 4% of antifungal fraction		0.004 ± 0.0001		
	Medium at 6% of antifungal fraction		0.0035 ± 0.0001		
	Medium at 8% of antifungal fraction			n.d.	
	Medium at 10% of antifungal fraction			n.d.	
	Significance	0.79	0.81		
A. carbonarius	Medium without antifungal fraction	1.63 ± 0.01			
	Medium at 1% of antifungal fraction	1.62 ± 0.01			
	Medium at 2% of antifungal fraction	1.62 ± 0.01			
	Medium at 4% of antifungal fraction		1.2 ± 0.1		
	Medium at 6% of antifungal fraction			0.5 ± 0.1	
	Medium at 8% of antifungal fraction				n.d.
	Medium at 10% of antifungal fraction				n.d.
	Significance	0.79	1.00	1.00	
A. ochraceus	Medium without antifungal fraction	0.108 ± 0.001			
	Medium at 1% of antifungal fraction	0.108 ± 0.001			
	Medium at 2% of antifungal fraction	0.105 ± 0.001			
	Medium at 4% of antifungal fraction		0.059 ± 0.01		
	Medium at 6% of antifungal fraction			0.04 ± 0.01	
	Medium at 8% of antifungal fraction				n.d.
	Medium at 10% of antifungal fraction				n.d.
	Significance	0.79	1.00	1.00	

n.d.: not detected
Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

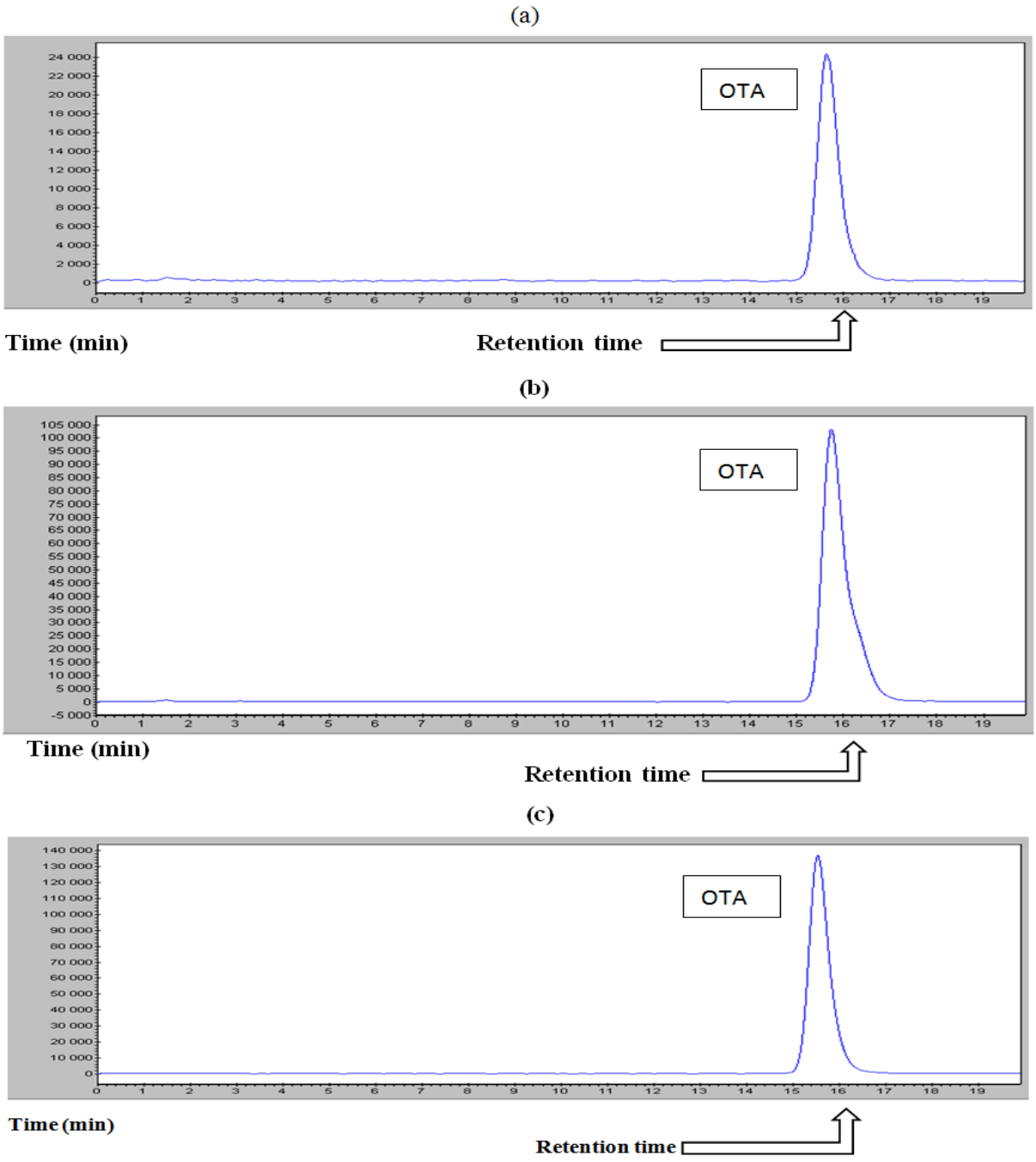

Figure 2. Chromatograms of OTA standard: **(a)**: 0.026µg/mL; **(b):** 0.11µg/mL; **(c):** 0.15µg/mL

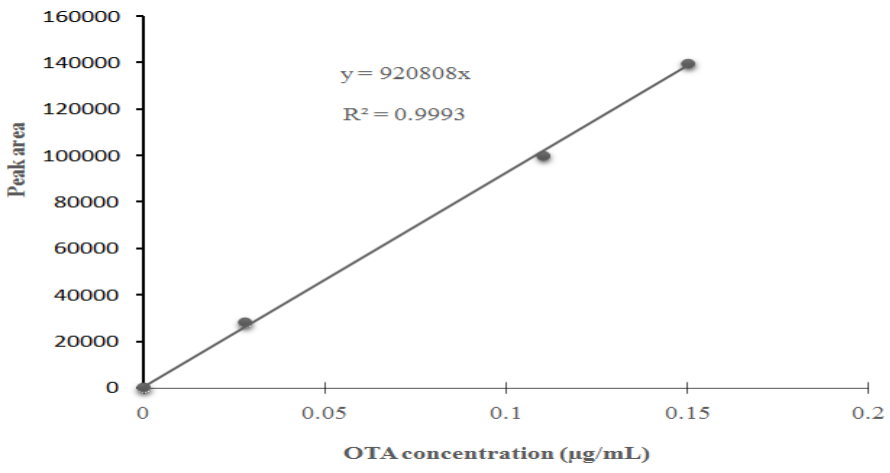

Figure 3. Calibration curve of standard OTA

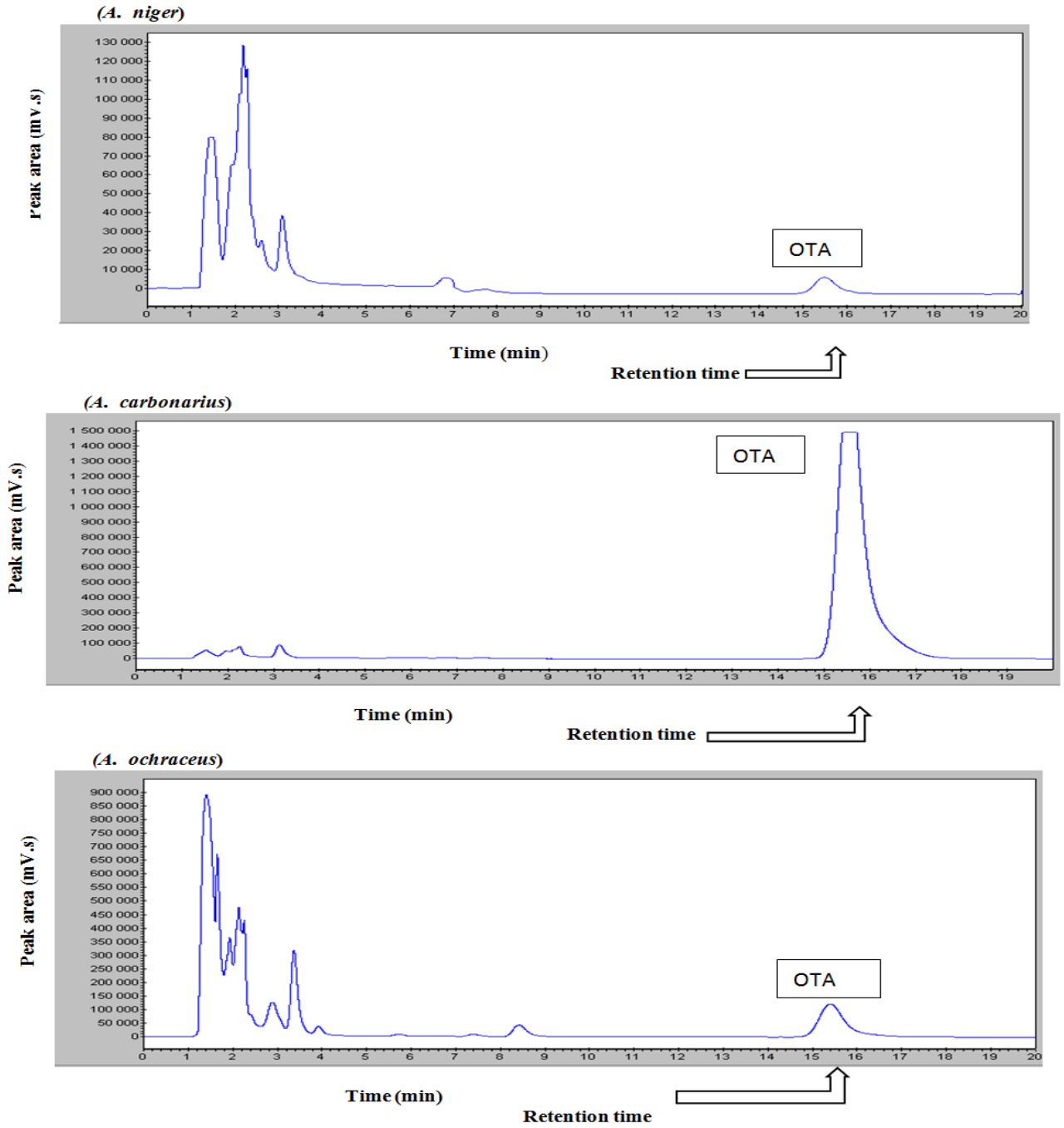

Figure 4. Chromatograms of OTA produced after 7 days of incubation by the three strains of *Aspergillus* on coffee-agar medium without antifungal

The phytochemical analysis of several parameters yielded strongly positive results for flavonoids, saponins, and carotenoids. The test for glycosides gave slightly positive results, and alkaloids could not be detected at all (Table 3).

Table 3. Phytochemical analysis of the antifungal fraction of *Solanum indicum* L. green berries extract

Phytochemical tests	Antifungal fraction
Test for flavonoids	++
Test for alkaloids	--
Test for saponins	++
Test for glycosides	+
Test for carotenoids	++

++ = strongly present, + = slightly present, -- = absence.

4. Discussion

In this study, the antifungal fraction of *Solanum indicum* L. green berries exhibited a significant inhibition on the growth of the three strains of *Aspergillus* tested. Total inhibition of growth of these strains was observed. The inhibition of fungal growth by the antifungal fraction of *S. indicum* berries have also been shown in previous studies [29]. These authors have shown that this antifungal fraction exhibited an inhibitory effect on growth of *A. fumigatus*, *A. nidulans* and *A. flavus*. However, the total inhibition of growth of these *Aspergillus* strains was observed from 1% of antifungal fraction in the culture medium while in the present study, the total inhibition of growth of the strains tested was observed from 8% of antifungal fraction in the culture medium. This difference

of results obtained could be explained by intrinsic factors such as the type of *Aspergillus* species tested and also by the culture medium used. Indeed, in the previous study, the culture medium used was Czapek yeast extract agar (synthetic medium), while in the present study, the medium used was the coffee-agar medium (natural medium or complex medium). The inhibitory effect of the antifungal fraction could be explained by its constituents. Indeed, the phytochemical analysis of the antifungal fraction showed the presence of flavonoids, carotenoids, glycosides and saponins. The antimicrobial effect of such compounds has been shown in previous studies. It has been shown that the antimicrobial properties of propolis have been attributed to its high flavonoids content and in particular the presence of the flavonoids such as galangin and pinocembrin [38]. It has also been shown that quercetin, one of the constituents of flavonoids caused an increase in permeability of the inner microbial membrane and a dissipation of the membrane potential [39]. These authors have also demonstrated that the flavonoids such as quercetin and naringenin significantly inhibited microorganism's motility.

Some investigations on anthocyanin showed also that, these constituents have potential antimicrobial activities [40]. In addition to its inhibitory effect on growth, the antifungal fraction of *S. indicum* green berries extract caused the reduction of the ability of producing OTA of the strain tested. Indeed, the more the antifungal fraction content in medium was high, the less OTA was produced by the *Aspergillus* strains tested. This reduction of OTA production was already started on the medium at 4% of the antifungal fraction. The total inhibition of OTA production was observed from 8% of the antifungal fraction in the medium for all the *Aspergillus* strains tested. It is noted that although there was growth of *A. ochraceus* on the medium at 8% of antifungal fraction, the test for OTA production didn't show detectable amount of this mycotoxin. It means that fungal growth doesn't lead always to mycotoxins production. These results confirmed those obtained by [41] who showed that fungal development gives little indication of OTA production.

5. Conclusion

This study highlights the discovery of natural substances for the search for alternative in chemical fungicides. For many years now, these berries are eaten in many African countries without any toxic effect noted. Therefore, to avoid the toxicological risks for the environment and for human being, due to the use of chemical fungicides and to reduce the use of chemical preservatives and additives in food and feed, *Solanum indicum* L. green berries extract can be used. This inhibitory effect of these berries could be due to the compounds found in the antifungal fraction. Further research must be carried out on the compounds of this antifungal fraction in order to confirm this fact.

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Competing Interest

The authors do not declare any conflict of interest.

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