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Full Length Research Paper

The use of *Lycopersicum esculentum* Mill. leaves extract against the survival of fungi

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In this study, the antifungal fraction of *Lycopersicum esculentum* Mill. leaves extract was evaluated for its effect against biomass and cell viability of three strains of *Aspergillus* (*Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus nidulans*). The results obtained show a reduction of the RNA concentration representing the biomass with the increase of the antifungal fraction content in the medium. Indeed, the mean of the RNA concentrations in the medium without antifungal fraction were about 113, 83 and 69 times those observed in the medium at 1% of antifungal fraction, respectively for *A. nidulans*, *A. fumigatus* and *A. flavus*. This reduction of the biomass was related to a reduction of the surviving cells with the increase of the antifungal fraction content in the medium. Indeed, the percentage in reduction of Alamar blue indicating cell viability which was 100% in the medium without antifungal fraction for the three strains tested decreased to reach the values of 2.40, 2.67 and 3.52% in the medium at 1%, respectively, for *A. nidulans, A. fumigatus* and *A. flavus* after five days of incubation. Thus, *L. esculentum* leaves extract exhibit a real inhibitory effect against fungi with an ability of killing them.

Key words: *Lycopersicum esculentum*, antifungal fraction, *Aspergillus*, biomass, cell viability.

INTRODUCTION

Fungi are major spoilage agents of crops, foods and feed-stuffs. Indeed, according to Akande et al. (2006), energy, crude protein and crude fat contents of moldy maize may reduce up to 5, 7 and 63%, respectively. Raju and Rao (2004) have reported previously that mold growth reduces all amino acids in diet, particularly lysine and arginine. In addition to this degradation of the nutritional quality of the products infected, some fungi species are capable of producing mycotoxins. These mycotoxins are secondary metabolites produced by fungi which mostly belong to the *Aspergillus, Penicillium* and *Fusarium* genera found in both animal feedstuffs and human foods (Steyn, 1995; Binder et al., 2007). These naturally occurring poisons can have acute or chronic effects on humans and animals and they were recently defined as a major food safety concern (Kuiper-Goodman, 2004). In order to protect health of consumers from mycotoxins ingestion, 77 countries have currently imposed regulatory limits for mycotoxins. This can results in undue economic burden on growers. Thus, in addition to this threat to human health, mycotoxins can cause great economic loss.

The Food and Agriculture Organization of the United Nations (FAO) has estimated a worldwide loss of about one billion metric tons of foodstuff per year as a result of

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mycotoxins (Choudhary and Kumari, 2010). Innovative technologies are urgently needed to reduce the risks of mycotoxin in food and feed. For many years now, it has been clear that the most effective means to prevent contamination of food by mycotoxins is to avoid growth of mycotoxigenic fungi (Bullerman, 1977).

The primary method of control is the use of chemical fungicides. However, they have become less favored by regulators due to the toxicological risks (Directive 91/414/CEE of the EU, 1991). Also, some of these chemical fungicides do not kill the fungi. They simply inhibit growth for a period of days or weeks (Rouabhi, 2010). Furthermore, the general public demands a reduced use of chemical preservatives and additives in food and feed (Brul, and Coote, 1999). Therefore, the use of natural substances capable of inhibiting fungi development and killing them is of a great importance. Indeed, several plants are known to possess antimicrobial activities (Gould, 1996; Friedman et al., 2002).

Among these plants, there is *Lycopersicum esculentum* Mill. (tomato), the most important *Solanaceae* crop grown throughout the world and the second most important vegetable crop in the world in terms of consumption per capita and recognized as a highly valuable and nutritious food (Rick, 1980). Its leaves contain the steroidal glycoalkaloid (solanine) known to possess antimicrobial properties (Hui et al., 2001). Its concentration is about 1 mM (Figen, 2006). In folk medicine, the leaves extract is used to treat mycosis. No toxicity of this glycoalkaloid (solanine) by contact and inhalation was shown up to now. The toxicity noted was by ingestion in opposite to many chemical fungicides such as pyraclo-strobin and methyl bromide used in agriculture which cause irritation and other toxicological effects by contact and by inhalation even at low concentrations (Iowa Depart-ment of Public Health, 2008). The toxicity by ingestion of the solanine contained in *L. esculentum* leaves is observed at concentrations above 200 mg/kg of leaves. In plants, the glycoalkaloids serve as phytoanticipins, providing the plant with a preexisting chemical barrier against a broad range of pathogens (Sandrock and Vanetten, 1998; Hoagland, 2009).

Thus, this study was carried out to evaluate the effect of *L. esculentum* leaves extract on the survival of fungi in order to contribute to the search for alternative natural fungicides in modern fungicides which inhibit fungi without killing them and which cause many toxicological effects on health and environment.

MATERIALS AND METHODS

Materials

In this study, *L. esculentum* Mill. leaves were used. The culture medium used was the Czapeck Yeast Extract (CY). Three *Aspergillus* strains (*Aspergillus flavus* K220fl, *Aspergillus nidulans* K217ni and *Aspergillus fumigatus* K320fu from the laboratory of Mycology of Pasteur Institute of Cocody-Abidjan (Ivory Coast) were also used.

Methods

Leaves extract preparation

L. esculentum leaves were dried in the shelter of the sun. These dried leaves were grinded and 30 g of the obtained homogenate were added to 150 mL of 100% ethanol. The mixture was boiled in water bath at 80°C for 1 h under gentle stirring. The resulting mixture was centrifuged at 2000 rpm for 10 min. The supernatant was then filtered through Whatman paper (Kouadio et al., 2011). The resulting solution was evaporated to dryness under Fume Hood. 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. The resulting mixture was shaken for 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. To the remaining aqueous phase, 15 mL of ethyl acetate were added again, shaken and centrifuged as described above. This purification was done three times. The three ethyl acetate phases were put into the same tube and the aqueous phase into another tube and then, these two solutions obtained were dried under Fume Hood. The residues of the aqueous and ethyl acetate phases were dissolved respectively into 15 mL of distilled water and 15 ml of ethyl acetate.

The resulting solutions were then filtrated separately onto 0.20 µm cutoff membranes to eliminate residues which were not dissolved and eventual contaminants. These aqueous and ethyl acetate fractions were evaluated for their antifungal activities. The fraction containing the antifungal compounds was used to evaluate its effect on proliferation and fungi survival.

Evaluation of the antifungal activities of the fractions obtained after purification of the leaves extract by the ethyl acetate method

Each *Aspergillus* suspension of 10^6 spores/mL was sprayed onto the Czapeck Yeast Extract Agar (CYA) medium by inundation. A disc of 1 cm of diameter was impregnated with 100 µl of each frac-tion of the extract and put onto the medium inoculated. Each medium with impregnated disc was incubated at 30°C for *A. flavus* and 37°C for *A. fumigatus* and *A. nidulans*. The disc around which any fungal growth was observed was identified as the disc impregnated with the fraction containing the antifungal compounds.

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 Mira cloth.

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. A quantity of 1 mL of this spore's suspension at 10^6 pores /mL was inoculated into liquid medium of Czapeck yeast extract (CY) of 150 ml for 18 h at 37°C for *A. fumigatus* and *A. nidulans* and 30°C for *A. flavus* under shaking at 250 rpm to obtain the microbial ball. This microbial ball was re-suspended into a new liquid medium of CY of 50 mL. Then, 2 mL of the ball suspension was put into different tubes aseptically (CLSI, 1999). Then, into each tube, the antifungal fraction was added to obtain concentrations of 0.05, 0.1, 0.5 and 1%. Medium without antifungal fraction was used as control. For each concentration, 3 tubes were used. Then, all the tubes were incubated also at 37°C for *A. fumigatus* and *A. nidulans* and 30°C for *A. flavus* under shaking

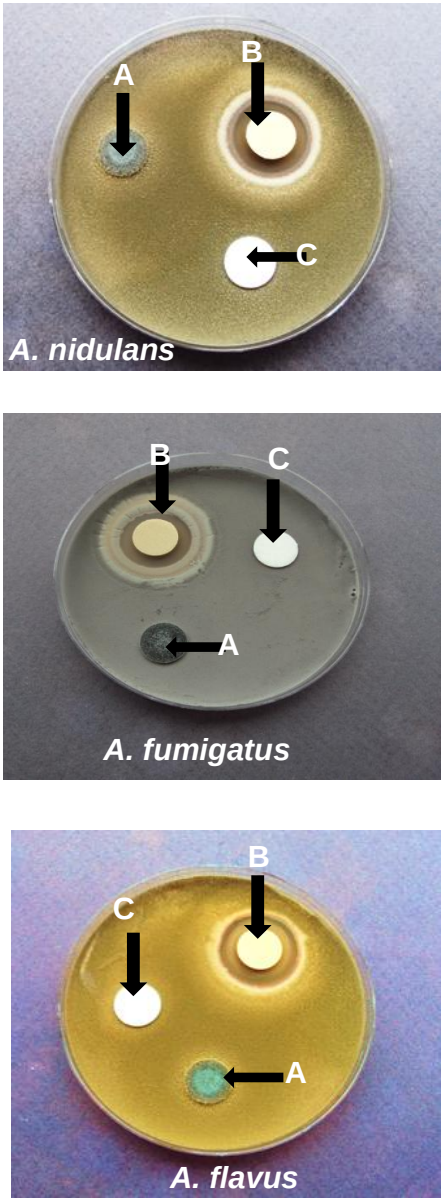

Figure 1. Inhibitory effect of (A): ethyl acetate fraction, (B): aqueous fraction of *L. esculentum* leaves extract and (C): ethyl acetate on fungi growth.

at 250 rpm. The microbial ball obtained after the incubation time was used for the RNA analysis and for the bioassay analysis (test for the determination of the percentage in reduction of Alamar blue).

Extraction and determination of RNA concentration

After 24 h of incubation, the RNA was extracted. For this RNA extraction, the method of Sánchez-Rodríguez et al. (2008) was used. The culture was put onto a sterilized Mira cloth filter Buchner funnel to separate the medium. The ball was removed from the Mira cloth and put into a tube. In each tube, 250 µl of 0.5 mm Zirconium/Silica beads and 1 ml of Trizol reagent were added. The resulting mixture obtained was homogenized into a Mini-Beadbeater at 4°C at maximum speed for 2.5 min.

It was then incubated at room temperature for 20 min. A quantity of 200 µl of chloroform was added to the mixture obtained, shaken vigorously for 20 s and centrifuged at 11600 rpm for 10 min at 4°C. The supernatant obtained was transferred into new microfuge tube. To this supernatant, 500 µl of isopropanol were added and mixed by inversion. It was incubated at room temperature for 10 min and centrifuged at 11600 rpm for 10 min at 4°C. Then, the supernatant obtained was removed and the pellet was washed with 1 mL of 70% ethanol made with DEPC-treated water. The mixture obtained was centrifuged again at 11600 rpm for 5 min at 4°C and the supernatant was removed. The resulting pellet was dried at room temperature for 10 min, re-suspended into 50 µl DEPC-water. After extraction according to the method described above, the purified RNA obtained was quickly put onto ice. The RNA concentration was determined by spectrophotometry at a wavelength of 600 nm. The electrophoresis of the RNA was then done on Formaldehyde Agarose Gel to show the RNA bands.

Bioassay analysis

The experiment was conducted over a span of 5 days. After each 24 h of incubation, 700 µl of liquid medium of CY and 300 µl of Alamar blue reagent were added into each tube. The final concentration of the Alamar blue reagent into each test-tube was 10%. Then, the microbial ball with the Alamar blue reagent was incubated at 37°C for 4 h. A liquid medium of CY without the microbial ball but containing Alamar blue reagent was also incubated.

After this incubation time, 100 µl of each suspension was put into separate wells of a micro-plate and the absorbance was monitored at 570 nm using 600 nm as a reference wavelength in an apparatus Bio-Teck ELISA.

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.

RESULTS

The results show that the antifungal compounds derived from *L. esculentum* Mill. leaves extract are water-soluble compounds. Indeed, no fungal growth was observed around the discs impregnated with the aqueous fraction (Figure 1). With the increases in this antifungal fraction content in the medium, a decrease of RNA concentration representing the biomass in a dose-dependent manner was noted. Indeed, the RNA concentrations which were 3709.50, 38.74.067 and 4758.27 µg/mL in the medium without antifungal fraction, respectively, for *A. nidulans*, *A. fumigatus* and *A. flavus* decreased to reach respectively, the values of 32.80, 46.867 and 69.07 µg/mL in the medium at 1% of antifungal fraction (Figure 2). It was noted that the reduction of the biomass was influenced significantly by antifungal fraction content in the medium (Table 1). The electrophoresis of the RNA showed an absence of RNA bands in the medium at 0.5 and 1% of

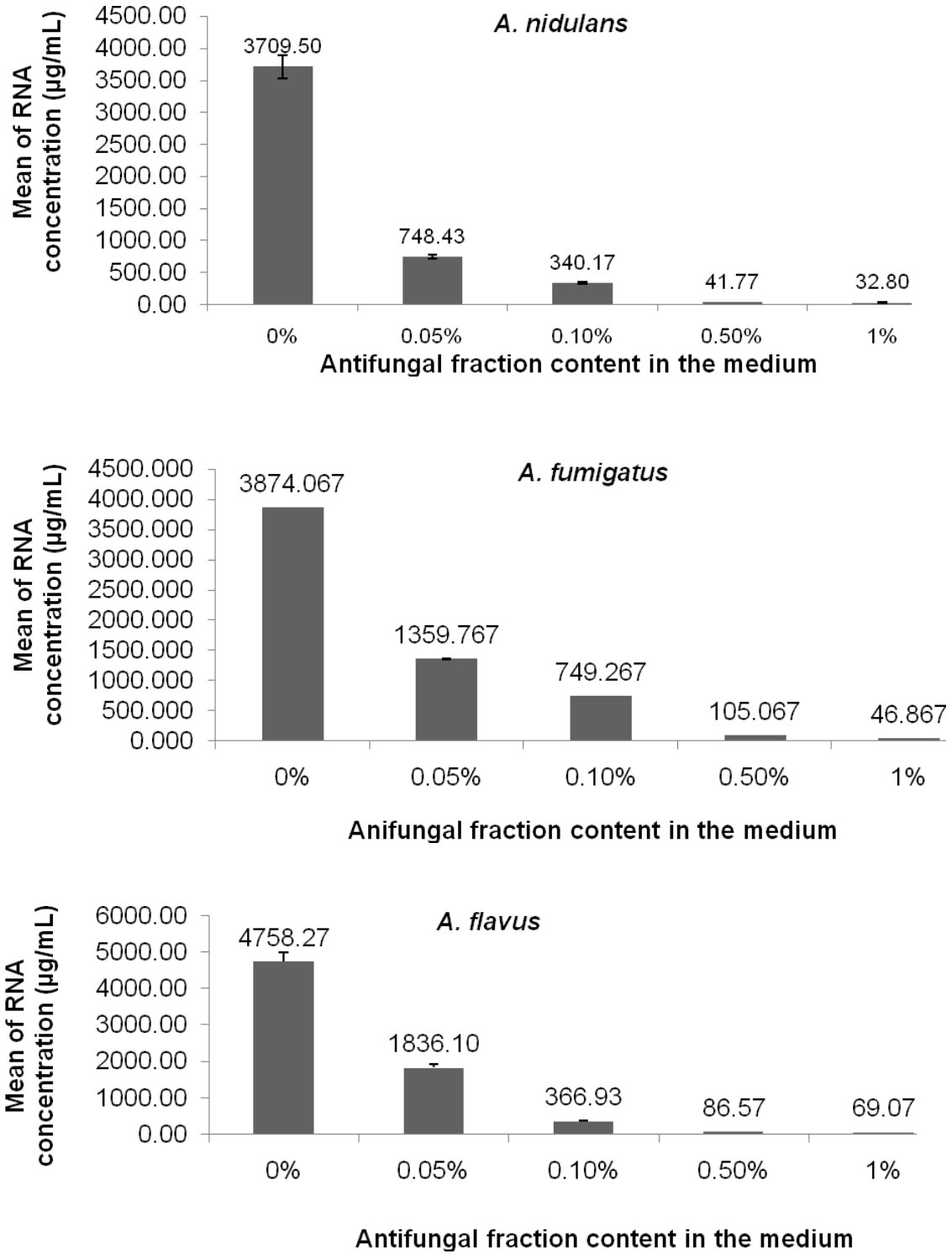

Figure 2. Effect of the antifungal fraction of *L. esculentum* leaves extract on RNA concentration of *A. fumigatus*, *A. nidulans* and *A. flavus*.

the antifungal fraction for *A. nidulans*, while for *A. fumugatus* and *A. flavus*, this absence of RNA bands was observed in the medium at 1% of the antifungal fraction (Figure 3). The reduction of the biomass when the antifungal fraction content in the medium increased was related to a decrease of the percentage of reduced

Table 1. Dose-dependent effect of the antifungal fraction of *Lycopersicum esculentum* leaves extract on biomass of *A. nidulans*, *A. fumigatus* and *A. flavus*.

Fungi	Antifungal fraction content in the medium	N	RNA concentration (µg/mL)				
			Subset for alpha = 0.05				
			1	2	3	4	5
Aspergillus nidulans	Medium at 1% of antifungal fraction	3	32.800				
	Medium at 0.5% of antifungal fraction	3		41.767			
	Medium at 0.1% of antifungal fraction	3			340.167		
	Medium at 0.05% of antifungal fraction	3				748.433	
	Medium without antifungal fraction	3					3709.500
	Significance		1.000	1.000	1.000	1.000	1.000
Aspergillus fumigatus	Medium at 1% of antifungal fraction	3	46.867				
	Medium at 0.5% of antifungal fraction	3		105.067			
	Medium at 0.1% of antifungal fraction	3			749.267		
	Medium at 0.05% of antifungal fraction	3				1359.767	
	Medium without antifungal fraction	3					3874.067
	Significance		1.000	1.000	1.000	1.000	1.000
Aspergillus flavus	Medium at 1% of antifungal fraction	3	69.067				
	Medium at 0.5% of antifungal fraction	3		86.567			
	Medium at 0.1% of antifungal fraction	3			366.933		
	Medium at 0.05% of antifungal fraction	3				1836.100	
	Medium without antifungal fraction	3					4758.267
	Significance		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Used harmonic mean sample size = 3.000. Homogeneous subsets, Tukey HSD

Alamar blue indicating the decreasing of surviving cells for the three strains tested. Indeed, the more the Alamar blue reagent was reduced, the more the percentage of surviving cells was high. The percentage in reduction of Alamar blue which was 100% in the medium without antifungal fraction for the three strains tested was decreased to reach the values of 5.76, 6.16 and 6.73% in the medium at 1% of the antifungal fraction after one day of incubation, respectively for *A. nidulans*, *A. fumigatus* and *A. flavus* (Figure 4).

It was noted that the reduction of the surviving cells was influenced significantly by the antifungal fraction content in medium (Table 2). This reduction of the surviving cells was also influenced significantly by the incubation time (Table 3A, B, C). Indeed, from the first day to the fifth day of incubation, the percentage in reduction of Alamar blue indicating surviving cells, decreased to reach the values of 2.409, 2.678 and 3.52% in the medium at 1% of antifungal fraction, respectively for *A. nidulans*, *A. fumigatus* and *A. flavus* (Table 3A, B, C). However, from the fourth to the fifth day of incubation (Table 3A, B, C), no significance difference was observed between the percentages in reduction of Alamar blue whatever the *Aspergillus* species tested.

DISCUSSION

In this study, the effect of the antifungal fraction of *L. esculentum* leaves extract on the biomass and the survival of *A. nidulans*, *A. fumigatus* and *A. flavus* was recorded. A significant reduction of the biomass of the three strains of *Aspergillus* tested was noted with the increase of the antifungal fraction content in the medium. This antifungal fraction exhibited a significant inhibition on proliferation of the three *Aspergillus* strains with a dose-dependent manner. The highest reduction of the biomass was observed in the medium containing 1% of the antifungal fraction which was the highest antifungal fraction content in the medium tested. These results confirm those obtained previously by Hui et al. (2001) which showed that the glycoalkaloid contained in *L. esculentum* leaves possess antifungal properties. Other previous studies showed also the antifungal activities of the glycoalkaloids.

Indeed, they have shown that the glycoalkaloids inhibited conidia germination (Fewell and Roddick, 1997). In addition to molds, the inhibitory effect of the glycoalkaloids on yeasts has also been shown (Wang et al., 2000). This could explain why in plants, the glycoalkaloids serve

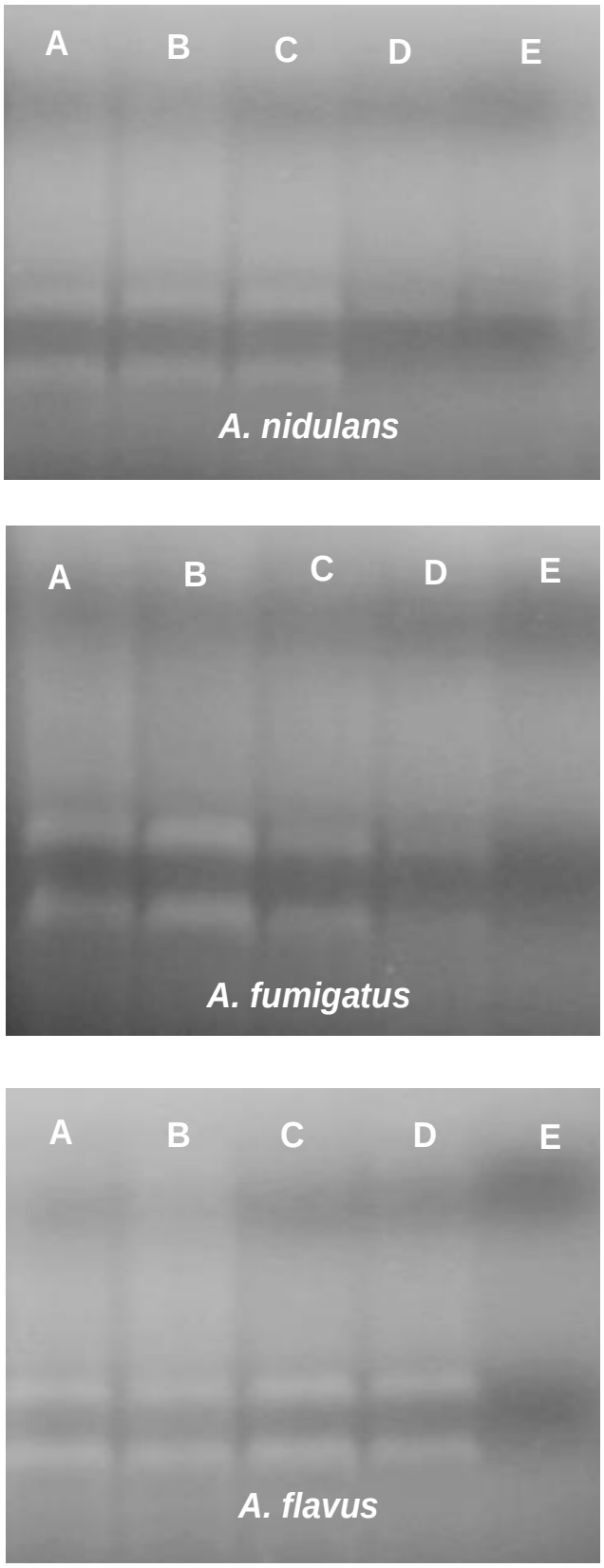

Figure 3. Electrophoresis of the RNA of *A. nidulans*, *A. fumigatus* and *A. flavus* grown in medium at different concentrations of antifungal fraction of *L. esculentum* leaves extract (A: 0%, B: 0.05%, C: 0.1%, D: 0.5%, E: 1%).

as phytoanticipins, providing the plant with a preexisting chemical barrier against a broad range of pathogens (Sandrock et al., 1998; Hoagland, 2009). At this concentration of 1% of antifungal fraction content in the medium, the RNA of the strains tested seems to be damaged as an absence of RNA bands was observed on the Formaldehyde Agarose Gel Electrophoresis. These results could confirm those obtained by Hoagland in 2009 which showed that the glycoalkaloids antifungal activity is believed to be their interaction with sterols in plant pathogen membranes, causing a loss of the membrane integrity and cell lysis. This loss of the membranes due to the glycoalkaloids effect was also shown by Steel and Drysdale (1988) and Keukens et al. (1995). Indeed, these authors have shown that the glycoalkaloids act via disruption of membranes, followed by the leakage of electrolytes and depolarization of the membrane potential. This reduction of the biomass when the extract content increased in the medium could be explained by the death of the fungi cells. Indeed, the more the antifungal fraction content in medium was high, the less the Alamar blue reagent was reduced. This less reduction of the Alamar blue reagent indicating a low rate of surviving cells was observed also in the medium at 1% of the antifungal fraction. This reduction of surviving cells decreased during the incubation time and became stable from the fourth day of incubation. The reduction of surviving cells was observed already in the medium at 0.05% of the antifungal fraction. This indicates that the minimum killing concentration could be at this value.

Conclusion

Regarding the obtained results, we can conclude that the antifungal compounds of *L. esculentum* leaves could be proposed as an effective and powerful antifungal agent against fungi proliferation with an ability of killing them. It highlights the discovery of natural substances for the research in alternative chemical fungicides that inhibit fungal growth without killing them. The highest inhibition of fungi proliferation and the lowest percentages of reduced Alamar blue reagent indicating the lowest rates of viable cells were observed in the medium at 1% of antifungal fraction. At this concentration, the RNA of the strains tested was damaged. These results suggest the use of the antifungal fraction of *L. esculentum* leaves extract at the concentration of 1% or above as a natural fungicide in alternative chemical fungicides which cause environmental and health risks. This antifungal fraction of *L. esculentum* leaves extract could also be used for the prevention of the rottenness of crops.

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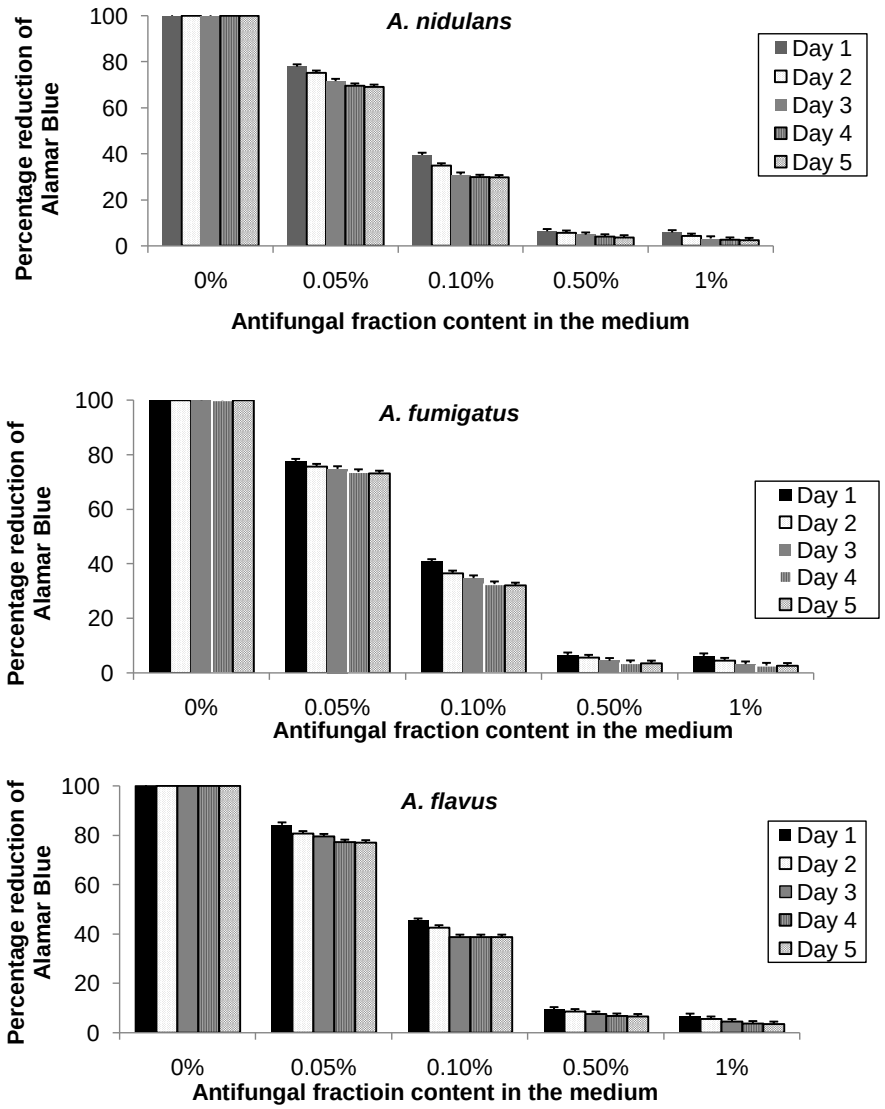

Figure 4. Effect of the antifungal fraction of *L. esculentum* leaves extract on percentage in reduction of Alamar blue of *A. nidulans*, *A. fumigatus* and *A. flavus*.

Table 2A. Dose-dependent effect of the antifungal fraction of *Lycopersicum esculentum* leaves extract on cell viability of *A. nidulans* during 5 days of incubation.

(I) Antifungal fraction content in the medium		Mean difference (I-J)	Multiple comparisons		95% Confidence interval	
			Std. Error	Significance	Lower bound	Upper bound
Medium without antifungal fraction VS	Medium at 0.05% antifungal fraction	21.4196* - 30.0948*	0.02044 - 0.2834	0.000	20.4868 - 30.0276	22.3524 - 30.1621
	Media at 0.1% antifungal fraction	59.8507* - 69.4374*	0.02044 - 0.2834	0.000	58.9180 - 69.3701	60.7835 - 69.5047
	Medium at 0.5% antifungal fraction	95.5928* - 93.0335*	0.02044 - 0.2834	0.000	92.1007 - 95.5255	93.9663 - 95.6601

Table 2A. Contd.

	Medium at 1% antifungal fraction	93.5266* - 96.7568*	0.02044 - 0.2834	0.000	92.5939 - 96.6896	94.4594 - 96.8241
Medium at 0.05 % antifungal fraction VS	Medium at 0.1% antifungal fraction	38.4312*- 39.3425*	0.02044 - 0.2834	0.000	37.4984 - 39.2753	39.3639 - 39.4098
	Medium at 0.5% antifungal fraction	71.6100*- 65.4980*	0.02044 - 0.2834	0.000	70.6812 - 65.4307	72.5467 - 65.5653
	Mediium at 1% antifungal fraction	72.1071* - 66.6620*	0.02044 - 0.2834	0.000	71.1743 - 66.5947	73.0398 - 66.7293
Medium at 0.1 % antifungal fraction VS	Medium at 0.5% antifungal fraction	33.1828* - 26.1554*	0.02044 - 0.2834	0.000	32.2500 - 26.0881	34.1155 - 26.2227
	Medium at 1% antifungal fraction	33.6759* - 27.3194*	0.02044 - 0.2834	0.000	32.7431 - 27.2522	34.6087 - 27.3867
Media at 0.5 % antifungal fraction VS	Medium at 1% antifungal fraction	0.4931 - 1.1640*	0.02044 - 0.2834	0.454 - 0.000	- 0.4397 - 1.0967	1.4259 - 1.2313

*The mean difference is significant at the 0.05 level. Dependent Variable: Percentage in reduction of Alamar blue; Tukey HSD .

Table 2B. Dose-dependent effect of the antifungal fraction of *Lycopersicum esculentum* leaves extract on cell viability of *A. fumigatus* during 5 days of incubation.

(I) Antifungal fraction content in the medium		Mean difference (I-J)	Multiple comparisons		95% Confidence interval	
			Std. Error	Significance	Lower bound	Upper bound
Medium without antifungal fraction VS	Medium at 0.05% antifungal fraction	21.801* - 25.762*	0.2569 - 0.0500	0.000	22.6472 - 25.5981	22.6472 - 25.9276
	Media at 0.1% antifungal fraction	58.568* - 66.882*	0.2569 - 0.0500	0.000	57.7225 - 66.7174	59.4138 - 67.0469
	Medium at 0.5% antifungal fraction	92.793* - 95.444*	0.2569 - 0.0500	0.000	91.9470 - 95.2793	93.6384 - 95.6088
	Medium at 1% antifungal fraction	93.119* - 96.342*	0.2569 - 0.0500	0.000	92.2735 - 96.1774	93.96487 - 96.5069
Medium at 0.05% antifungal fraction VS	Medium at 0.1% antifungal fraction	36.766* - 41.119*	0.2569 - 0.0500	0.000	35.9209 - 40.9545	37.6123 - 41.2839
	Medium at 0.5% antifungal fraction	70.991* - 69.681*	0.2569 - 0.0500	0.000	70.14549 - 69.5164	71.83685 - 69.8459

Table 2B. Contd.

	Mediium at 1% antifungal fraction	71.317* - 70.579*	0.2569 - 0.0500	0.000	70.471930 - 70.4145	72.1633 - 70.7440
Medium at 0.1% antifungal fraction VS	Medium at 0.5% antifungal fraction	34.224* - 28.562*	0.2569 - 0.0500	0.000	33.3789 - 28.3972	35.07026 - 28.7267
	Medium at 1% antifungal fraction	34.551* - 29.460*	0.2569 - 0.0500	0.000	33.7053 - 29.2953	35.3967 - 29.6248
Media at 0.5% antifungal fraction VS	Medium at 1% antifungal fraction	0.326 - 0.898*	0.2569 - 0.0500	0.000	0. 5112 - 0.7333	1.1721 - 1.0628

*The mean difference is significant at the 0.05 level. Dependent Variable: Percentage in reduction of Alamar blue; Tukey HSD.

Table 2C. Dose-dependent effect of the antifungal fraction of *Lycopersicum esculentum* leaves extract on cell viability of *A. flavus* during five days of incubation.

(I) Antifungal fraction content in the medium		Mean difference (I-J)	Multiple comparisons		95% Confidence interval	
			Std. Error	Significance	Lower bound	Upper bound
Medium without antifungal fraction VS	Medium at 0.05% antifungal fraction	15.1988* - 22.1671*	0.18729 - 0.035066	0.000	14.5827 - 22.05168	15.8149 - 22.2825
	Media at 0.1% antifungal fraction	54.1416* -60.5126*	0.18729 - 0.035066	0.000	53.5255 - 60.3972	54.75781 - 60.62807
	Medium at 0.5% antifungal fraction	90.0539* - 92.6700*	0.18729 - 0.035066	0.000	89.4378 - 92.5581	90.67016 - 92.7888
	Medium at 1% antifungal fraction	92.6886* - 95.73*	0.18721953280	0.000	92.0724 - 95.614780	93.3047 - 95.8456
Medium at 0.05 % antifungal fraction VS	Medium at 0.1% antifungal fraction	38.9428* - 38.3456*	0.18729 - 0.035066	0.000	38.3270 - 38.2302	39.5589 - 38.4610
	Medium at 0.5% antifungal fraction	74.85513* - 70.5064*	0.18729 - 0.035066	0.000	74.2390 - 70.3910	75.4713 - 70.6218
	Mediium at 1% antifungal fraction	77.4897* - 73.5631*	0.18729 - 0.035066	0.000	76.8736 - 73.4480	78.1059 - 73.6785
Medium at 0.1 % antifungal fraction VS	Medium at 0.5% antifungal fraction	35.9123* - 32.1608*	0.18729 - 0.035066	0.000	35.2961 - 32.0454	36.5285 - 32.2762
	Medium at 1% antifungal fraction	38.5469* - 35.21752*	0.18729 - 0.035066	0.000	37.9308 - 35.1021	39.1630 - 35.3329

Table 2C. Contd.

Media at 0.5 % antifungal fraction VS	Medium at 1% antifungal fraction	2.6346* - 3.0567*	0.18729 - 0.035066	0.000	2.0185 - 2.9413	- 89.4378 - 3.1721

*The mean difference is significant at the 0.05 level.Dependent variable: Percentage in reduction of Alamar blue; Tukey HSD.

Table 3A. Effect of incubation time on cell viability of *A. nidulans* grown in the medium at 0, 0.05, 0.1, 0.5 and 1% of antifungal fraction of *Lycopersicum esculentum* leaves extract.

Antifungal fraction content in the medium	N	Percentage in reduction of Alamar blue				
		Subset for alpha = 0.05				
		1	2	3	4	5
Medium at 0.05% of antifungal fraction for day5	3	69.0717304233				
Medium at 0.05% of antifungal fraction for day4	3	69.5753138133				
Medium at 0.05% of antifungal fraction for day3	3		71.5617302400			
Medium at 0.05% of antifungal fraction for day2	3			75.1718948033		
Medium at 0.05% of antifungal fraction for day1	3				77.8694301567	
Significance		0.720	1.000	1.000	1.000	
Medium at 0.1 % of antifungal fraction for day5	3	29.7291762700				
Medium at 0.1 % of antifungal fraction for day4	3	29.8681066267				
Medium at 0.1% of antifungal fraction for day3	3		30.8487519167			
Medium at 0.1% of antifungal fraction for day2	3			34.8483130467		
Medium at 0.1% of antifungal fraction for day1	3				39.4382560667	
Significance		0.949	1.000	1.000	1.000	
Medium at 0.5% of antifungal fraction for day5	3	3.5737370427				
Medium at 0.5% of antifungal fraction for day4	3		3.9670709717			
Medium at 0.5% of antifungal fraction for day3	3			4.7637355548		
Medium at 0.5% of antifungal fraction for day2	3				5.5975273043	
Medium at 0.5% of antifungal fraction for day1	3					6.2554838797
Significance		1.000	1.000	1.000	1.000	1.000
Medium at 1% of antifungal fraction for day5	3	2.4097170990				
Medium at 1% of antifungal fraction for day4	3	2.6163437327				
Medium at 1% of antifungal fraction for day3	3		3.0996235989			
Medium at 1% of antifungal fraction for day2	3			4.2515685323		
Medium at 1% of antifungal fraction for day1	3				5.7623609830	
Significance		0.324	1.000	1.000	1.000	

Means for groups in homogeneous subsets are displayed. Used harmonic mean sample size = 3.000. Homogeneous subsets; Tukey HSD.

Table 3B. Effect of incubation time on cell viability of *A. fumigatus* grown in the medium at 0, 0.05, 0.1, 0.5 and 1% of antifungal fraction of *Lycopersicum esculentum* leaves extract.

Antifungal fraction content in the medium	N	Percentage in reduction of Alamar blue			
		Subset for alpha = 0.05			
		1	2	3	4
Medium at 0.05% of antifungal fraction for day 5	3	73.2573294133			
Medium at 0.05% of antifungal fraction for day 4	3	73.6647853533			
Medium at 0.05% of antifungal fraction for day 3	3		74.7965475767		
Medium at 0.05% of antifungal fraction for day 2	3			75.6569177933	
Medium at 0.05% of antifungal fraction for day 1	3				77.4828032867
Significance		0.209	1.000	1.000	1.000
Medium at 0.1% of antifungal fraction for day 5	3	32.1381074000			
Medium at 0.1% of antifungal fraction for day 4	3	32.5552233567			
Medium at 0.1% of antifungal fraction for day 3	3		34.7644540500		
Medium at 0.1% of antifungal fraction for day 2	3			36.5230586300	
Medium at 0.1% of antifungal fraction for day 1	3				40.7162083600
Significance		.108	1.000	1.000	1.000
Medium at 0.5% of antifungal fraction for day 5	3	3.5761280427			
Medium at 0.5% of antifungal fraction for day 4	3	3.5927265687			
Medium at 0.5% of antifungal fraction for day 3	3		4.4613932053		
Medium at 0.5% of antifungal fraction for day 2	3			5.6274128230	
Medium at 0.5% of antifungal fraction for day 1	3				6.4916272387
Significance		1.000	1.000	1.000	1.000
Medium at 1% of antifungal fraction for day 5	3	2.6780559420			
Medium at 1% of antifungal fraction for day 4	3	2.7287387120			
Medium at 1% of antifungal fraction for day 3	3	3.2176108570			
Medium at 1% of antifungal fraction for day 2	3		4.5155355080		
Medium at 1% of antifungal fraction for day 1	3			6.1651937720	
Significance		.314	1.000	1.000	

Means for groups in homogeneous subsets are displayed. Used harmonic mean sample size = 3.000. Homogeneous subsets; Tukey HSD.

Table 3C. Effect of incubation time on cell viability of *A. flavus* grown in the medium at 0, 0.05, 0.1, 0.5 and 1% of antifungal fraction of *Lycopersicum esculentum* leaves extract.

Antifungal fraction content in the medium	N	Percentage in reduction of Alamar blue			
		Subset for alpha = 0.05			
		1	2	3	4
Medium at 0.05% of antifungal fraction for day5	3	77.0843321800			
Medium at 0.05% of antifungal fraction for day4	3	77.2493259700			
Medium at 0.05% of antifungal fraction for day3	3		79.5297225333		
Medium at 0.05% of antifungal fraction for day2	3			80.6908602333	
Medium at 0.05 % of antifungal fraction for day1	3				84.2198344067
Significance		0.914	1.000	1.000	1.000
Medium at 0.1% of antifungal fraction for day5	3	38.7350845967			
Medium at 0.1% of antifungal fraction for day4	3	38.7387530667			
Medium at 0.1% of antifungal fraction for day3	3	38.7436490667			
Medium at 0.1% of antifungal fraction for day2	3		42.5539374833		
Medium at 0.1% of antifungal fraction for day1	3			45.2770163700	
Significance		1.000	1.000	1.000	
Medium at 0.5% of antifungal fraction for day5	3	6.5779239933			
Medium at 0.5% of antifungal fraction for day4	3	6.7810436607			
Medium at 0.5% of antifungal fraction for day3	3		7.5743709903		

Table 3C. Contd.

Medium at 0.5% of antifungal fraction for day2	3			8.5759988667	
Medium at 0.5% of antifungal fraction for day1	3				9.3647047713
Significance		.557	1.000	1.000	1.000
Medium at 1% of antifungal fraction for day5	3	3.5212326953			
Medium at 1% of antifungal fraction for day4	3	3.7308440643			
Medium at 1% of antifungal fraction for day3	3		4.5108440430		
Medium at 1% of antifungal fraction for day2	3			5.5617323067	
Medium at 1% of antifungal fraction for day1	3				6.7300857280
Significance		0.760	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Used harmonic mean sample size = 3.000. Homogeneous subsets; Tukey HSD.

for the identification of the strains of *Aspergillus* used in this study.

REFERENCES

Akande KE, Abubakar MM, Adegbnola TA, Bogoro SE (2006). Nutritional and health implications of mycotoxins in animal feeds: a review. Park. J. Nutr. 5(5):398-403.

Binder EM, Tan LM, Chin LJ, Handl J, Richard J (2007). Worldwide occurrence of mycotoxins in commodities feeds and feed ingredients. Anim. Feed Sci. Technol. 137:265-282.

Brul S, Coote P (1999). Preservative agents in foods: Mode of action and microbial resistance mechanisms. Int. J. Food Microbiol. 50:1-17.

Bullerman LB (1977). Incidence and control of mycotoxins producing molds in domestic and imported cheeses. Ann. Nutr. Food 31:435-446.

Choudhary AK, Kumari P (2010). Management of mycotoxin contamination in pre-harvest and post-harvest crops: present status and future prospects. J. Phytol. 2(7):37-52.

Clinical and Laboratory standards Institute (1999). Methods for determining bactericidal activity of antimicrobial agent: approved guideline, Document M26-A, Wayne, PA: CLSI.

Fewell AM, Roddick JG (1997). Potato glycoalkaloid impairment of fungal development. Mycol. Rev. 101:597-603.

Figen M (2006). Saponins versus plant fungal pathogens. J. Cell Mol. Biol. 5:13-17.

Friedman M, Henika PR, Mandrell RE (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica. J. Food Protect. 65, 1545-1560.

Gould GW (1996). Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J. Food Protect. pp. 82-86.

Hoagland RE (2009). Toxicity of tomatine and tomatidine on weeds, crops and phytopathogenetic fungi. Allelopathy J. 23(2):425-436.

Hui YH, Smith R, David GS (2001). Foodborne Disease Handbook, vol. 3, Plant Toixcants, CRC; 2nd édition, (ISBN 082470343X), p. 99.

Iowa Department of Public Health (2008). Acute Pesticide Poisoning Associated with Pyraclostrobin Fungicide - Iowa.

Keukens EA, de Vrije T, Van den Boom C, de Waard P, Plasman HH, Thiel F, Chupin V, Jongen WM, de Kruijff B (1995). Molecular basis of glycoalkaloid induced membrane disruption. Biochim. Biophys. Acta. 1240:216-228.

Kouadio AI, Oulahal N, Nguyen Thi P, Adt I, Degraeve P (2011). Study of the antimicrobial activities of Solanumindicumssp. Distichum (Schumach. and Thonning 1827) fruits (“gnangnan” berries) from a tropical humid zone (Côte d’Ivoire).Int. J. Biol. Chem. Sci. 5(3):1190-1200.

Kuiper-Goodman T (2004). Risk assessment and risk management of mycotoxins in food. In: Mycotoxins in food Detection and control, Magan N. and Olsen M. (eds). Cambridge, England: Woodhead Publishing Limited, pp. 3-31.

Raju MVLN, Rao RSV (2004). Aflatoxins in poultry diet: natural ways for counteraction. Proc. Annual Conference, Compound livestock feed manufacturers Association, Mumbai, September 13 & 14.

Rick CM (1980). Tomato. In Hybridization of Crop Plants (ed. W. R. Fehr and H. H. Hadley. American Society of Agronomy, Madison, USA, pp. 669-680.

Rouabhi R (2010). Introduction and Toxicology of Fungicides, Fungicides, Odile Carisse (Ed.), ISBN: 978-953-307-266-1. Intech.

Sánchez-Rodríguez A, Portal O, Rojas LE, Ocaña B, Mendoza M, Acosta M, Jiménez E, Höfte M (2008). An efficient method for the extraction of high-quality fungal total RNA to study the Mycosphaerellafijiensis-Musa spp. Interaction. Mol. Biotechnol. 40(3):299-305.

Sandrock RW, Vanetten HD (1998). Fungal sensitivity to and enzymatic degradation of the phytoanticipinα-tomatine.Phytopathol. 88:137-143.

Steel CC, Drysdale RB (1988). Electrolyte leakage from plant and fungal tissues and disruption of liposome membranes by α-tomatine. Phytochem. 27:1025-1030.

Steyn PS (1995). Mycotoxins, general view, chemistry and structure. Toxicol. Lett. 82:843-851.

Wang LD, Guo DA, Yuan L, He QH, Hu YQ, Tu PF, Zheng JH (2000). Antifungal effect of three natural products on the genetic substance of Saccharomyces cerevisiae GL7 and Prototheca wickerhamii. Yao Xue Xue Bao 35(11):860-863.