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Prevention of Crops Contamination by Fungi and Mycotoxins Using Natural Substances Derived from *Lycopersiconesculentum* Mill. Leaves

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Abstract *Lycopersiconesculentum* Mill.(tomato) leaves are known to possess antimicrobial activities. In this study, the aqueous fraction containing the antifungal compounds obtained after purification of these leaves extract was evaluated for its effect on proliferation and mycotoxins production by three *Aspergillus*strains (*Aspergillusfumigatus*, *Aspergillusflavus* and *Aspergillusnidulans*). This antifungal fraction exhibited a significant inhibition on growth of the three *Aspergillus*strains with a dose-dependent manner. Indeed, the radial growth was 90 mm on the medium without antifungal fraction for these three strains, while on the medium at 0.05%, 0.1%, 0.5% and 1% of antifungal fraction, it was respectively 55.67, 40, 20 and 0 mm for *A nidulans*, 70.67, 50, 24.33 and 0 mm for *A. fumigatus* and 85.33, 54.67, 24.33 and 0 mm for *A. flavus* after 7 days of incubation. The absence of conidia germination was also observed only on the medium at 1 % of antifungal fraction for the three strains. This reduction of the growth of the strains when the antifungal fraction content in the medium increased, led to the reduction of mycotoxins production. Indeed, the mycotoxins analysis by Thin Layer Chromatography showed any spots of sterigmatocistin and Gliotoxinin the medium at 0.5% and 1% of antifungal fraction respectively for *A. nidulans and A. fumigatus*, while for the other concentrations tested, spots of these mycotoxins were observed. For *A. flavus*, the absence ofthe spot of aflatoxinB1 was observed only in the medium at 1% of antifungal fraction. These results suggest the use of *Lycopersiconesculentum* leaves extract as a natural fungicide in alternative in chemical fungicides which cause environmental risks.

Keywords: *Lycopersiconexculentum, antifungal fraction, Aspergillus, radial growth, mycotoxins*

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1. Introduction

The preservation of food quality is a major concern around the world because of the infection by natural contaminants such as fungi that crops and processed products are subjected during harvest and post-harvest treatments [1]. This development of fungi on food leads to loss in nutritional quality [2,3]. This fact could be one of the causes of the insufficient diet in the world and mainly in developing countries where climatic conditions are favorable for the development of these microorganisms. According to [4], malnutrition is the greatest single threat to the world's public health. In addition to this threat caused by the loss in nutritional quality, some fungi species are capable of producing mycotoxins, secondary metabolites produced by fungi which mostly belong to the *Aspergillus*, *Penicillium*and *Fusarium*genera found in both animal feedstuffs and human foods [5,6]. These toxins have been detected in various food commodities from many parts of the world [7]. According to the United Nation's Food and Agriculture Organization (FAO), approximately, 25 % of world grain supply is contaminated with mycotoxins. 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 [8]. They have been shown to possess carcinogenic, hepatotoxic, and teratogenicproperties [9]. In order to protect health of consumers from mycotoxins ingestion, 77 countries have currently imposed regulatory limits for mycotoxins. The establishment of regulatory limits may vary in each country depending on level of exposure and sociological, political and economic factors. This can results in undue economic burden on growers. Thus, in addition to this threat to human health, mycotoxins can cause great economic loss in both the livestock industry and aquaculture due to the impact of mycotoxins contamination on international trade in commodities [10,11,12]. 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 mycotoxins contamination [13]. 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 [14]. The primary method of control is the use of chemical fungicides. However, they have become less favored by regulators due to the toxicological risks [15].

Therefore, the use of natural substances capable of inhibiting fungi development including mycotoxigenic fungi is of a great importance. As it is known, the nature has provided abundant plants for all living creatures, and there are some parts of some of them which are less explored

Indeed, although tomato (*Lycopersiconesculentum* Mill.) is 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 [16], its leaves are less used. These leaves contain a glycoalkaloid (solanine) known to possess antimicrobial properties [17]. The content of this glycoalkaloid is about 0.03 to 0.08 mg/100 g in ripe fruit, while in unripe fruits, it is about 0.9 to55 mg/100 g, however safe for human consumption [18]. In plants, the glycoalkaloids serve as phytoanticipins, providing the plant with a preexisting chemical barrier against a broad range of pathogens [19,20]. Indeed, this glycoalkaloid acts via disruption of membranes, followed by the leakage of electrolytes and depolarization of the membrane potential [21,22]. However, it was suggested that the plants of *Lycopersiconesculentum* are not affected by its presence, possibly due the existence of sterol glycosides and acetylated sterol glycosides in their cell membranes [23]. In folk medicine, the leaves extract is used to treat mycosis. Any toxicity of this glycoalkaloid (solanine) by contact and inhalation was shown from up to now. The toxicity noted was by ingestion in opposite to many chemical fungicides such as Pyraclostrobin and methyl bromide used in agriculture which cause irritation and other toxicological effects by contact and by inhalation even at low concentrations [24]. The toxicity by ingestion of the solaninecontained in *Lycopersiconesculentum* leaves is observed at concentrations above 200 mg/kg of leaves.

Thus, this study was carried out to evaluate the effect of *Lycopersiconesculentum* leaves extract on fungi proliferation and mycotoxins production in order to contribute to the search for natural fungicides in alternative in synthetics fungicides which cause many toxicological effects on health and environment.

2. Material and Methods

2.1. Material

2.1.1. Biological Material

In this study, *Lycopersiconesculentum* Mill.leaveswere used. The culture medium used was the Czapeck Yeast Extract. Three *Aspergillus*strains (*Aspergillusflavus* K210fl, *Aspergillusnidulans* K213ni and *Aspergillusfumigatus* K301fu from the laboratory of Mycology of Pasteur Institute of Cocody-Abidjan (Ivory Coast) were also used.

2.2. Methods

2.2.1. Leaves Extract Preparation

Lycopersiconesculentum 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. 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 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. 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 and then filtrated separately onto 0.20µm cutoff membranes to eliminate residues which were not dissolved and eventual contaminants. These aqueous and ethyl acetate fractions were evaluated for their antifungal activities in order to use the one containing the antifungal compounds for the test of the inhibition of fungal growth and mycotoxins production.

2.2.2. 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. These suspensions of 10^6 spores/ml were used for the tests of fungal growth inhibition and mycotoxins production.

2.2.3. 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 CYA medium by inundation. A disc of 1cm of diameter was impregnated with 100µl of each fraction 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 fungalgrowth was observed was

identified as the disc impregnated with the fraction containing the antifungal compounds.

2.2.4. Monitoring of Growth

The fraction containing the antifungal compoundswas added to the medium to obtain mediums with different concentrations of 0.05%, 0.1%, 0.5% and 1%. 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. All the inoculated mediums were incubated at different temperatures as described above. The radial growth was determined by measuring the diameter of the colony each day according to the method of [25]. This experiment was carried out for 7 days.

2.2.5. Phenotypic Observations

The mediums incubated at appropriate temperatures were observed macroscopically and microscopically. The aspects of the colonies were recorded. The percentage of conidia germination was also recorded after 6, 12 and 24 hours of incubation.

2.2.6. Mycotoxins Analysis by Thin Layer Chromatography (TLC)

In order to extract the total mycotoxin produced, this experiment was carried out using liquid medium of Czapeck Yeast Extract. Into 2ml of this liquid medium without antifungal fraction and also with antifungal fraction, 10µl of the conidia suspension of 10^6 spores/ml of each *Aspergillusstrain* were inoculated. The antifungalextract contents in the liquid medium used were, 0.05%, 0.1%, 0.5% and 1%. The mediums inoculated were incubated at 30°C for *A. flavus* and 37°C for *A. fumigatus* and *A. nidulans* during 7 days. After these 7 days of incubation, 2ml of Chloroform were added to the culture into the tube and vortex for 10 seconds. The resulting mixture obtained was sat for 30min. with shaking every 5min. The mixture was centrifuged at 2000 rpm for 10min. The Chloroform layer (bottom layer) was transferred into glass vial for total evaporation in the Fume Hood. After evaporation, 100µl of Chloroform were added into the glass vial to recover the total mycotoxin extracted. For each sample of mycotoxin, 20µl were loaded onto the Thin Layer Chromatography (TLC) Silicate gel C18 plate containing a fluorescence indicator. Sterigmatocystin, gliotoxin and aflatoxin B1 standards were also loaded onto the TLC Silica gel C18 plate. For each mycotoxin standard, 5 µl were loaded onto this TLC Silicagel C18 plate. The spotted TLC plate was run in a TLC chamber including mobile phase for 40min.The mobile phase for aflatoxin B1 was Toluene/Ethyl acetate/Formic acid (100:80:20)while for gliotoxin and sterigmatocystin, it was Toluene/Ethyl acetate/acetic acid (160:20:20).

The TLC plates were then dried at room temperature for 3 min. For the visualization of aflatoxinB1 and sterigmatocistin, a solution of alumine chloride (20% AlCl13 7 H_2O in 95% ethanol) was sprayed onto the TLC plates. These plates were incubated at 80 °C for 5 min. and then observed under UV fluorescence.

2.2.7. Statistical Analysis

The statistical analysis of data was made 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 results showed that the antifungal compounds derived from *Lycopersiconesculentum* Mill. leaves are water-soluble compounds. Indeed, the discs around which any fungal growth was observed were those impregnated with the aqueous fraction.(Figure 1).

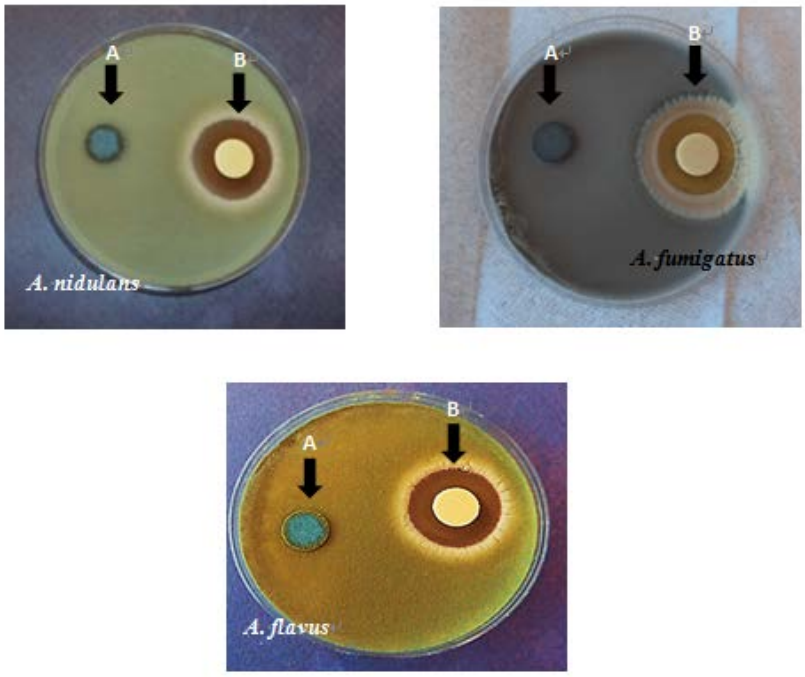

Figure 1. Inhibitory effect of (A): ethyl acetate fraction and (B): aqueous fraction of *Lycopersiconesculentum*leaves extract on fungi growth

It was noted that, with increases in the antifungal fraction content in the medium, there was less fungal growth (Figure 2). Indeed, the mean of radial growth which was 90 mm on the medium without antifungal fraction was decreased to reach the values of 55.67, 40, 20 and 0 mm for *A nidulans*, 70.67, 50, 24.33 and 0 mm for *A. fumigatus* and 85.33, 54.67, 24.33 and 0 mm for *A. flavus* after 7 days of incubation respectively on the medium at 0.05 %, 0.1 %, 0.5 % and 1 % of antifungal fraction. This reduction of growth started already on the medium at 0.05 %,of antifungal fraction (Figure 3).

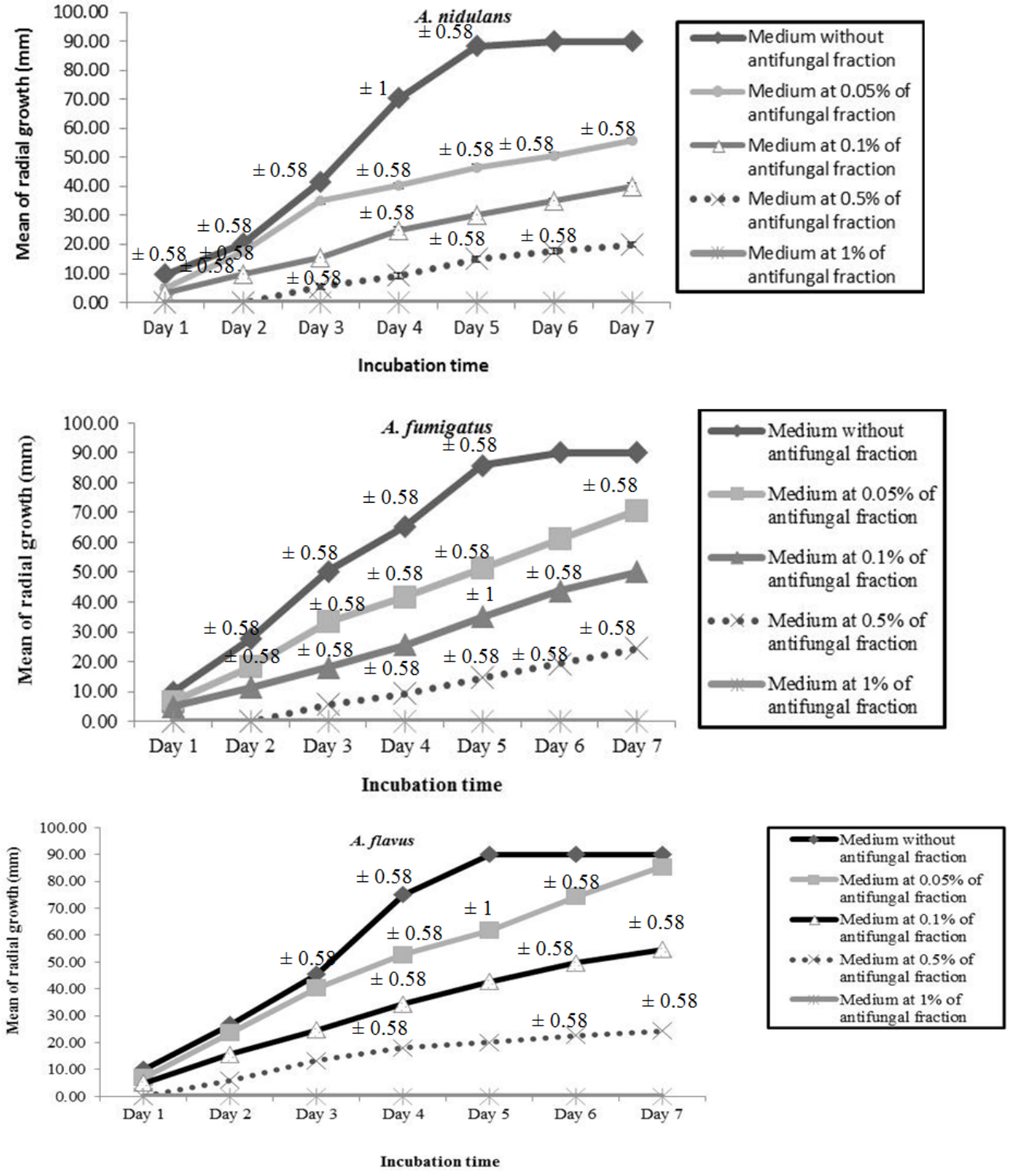

Figure 2. Inhibitory effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on the radial growth

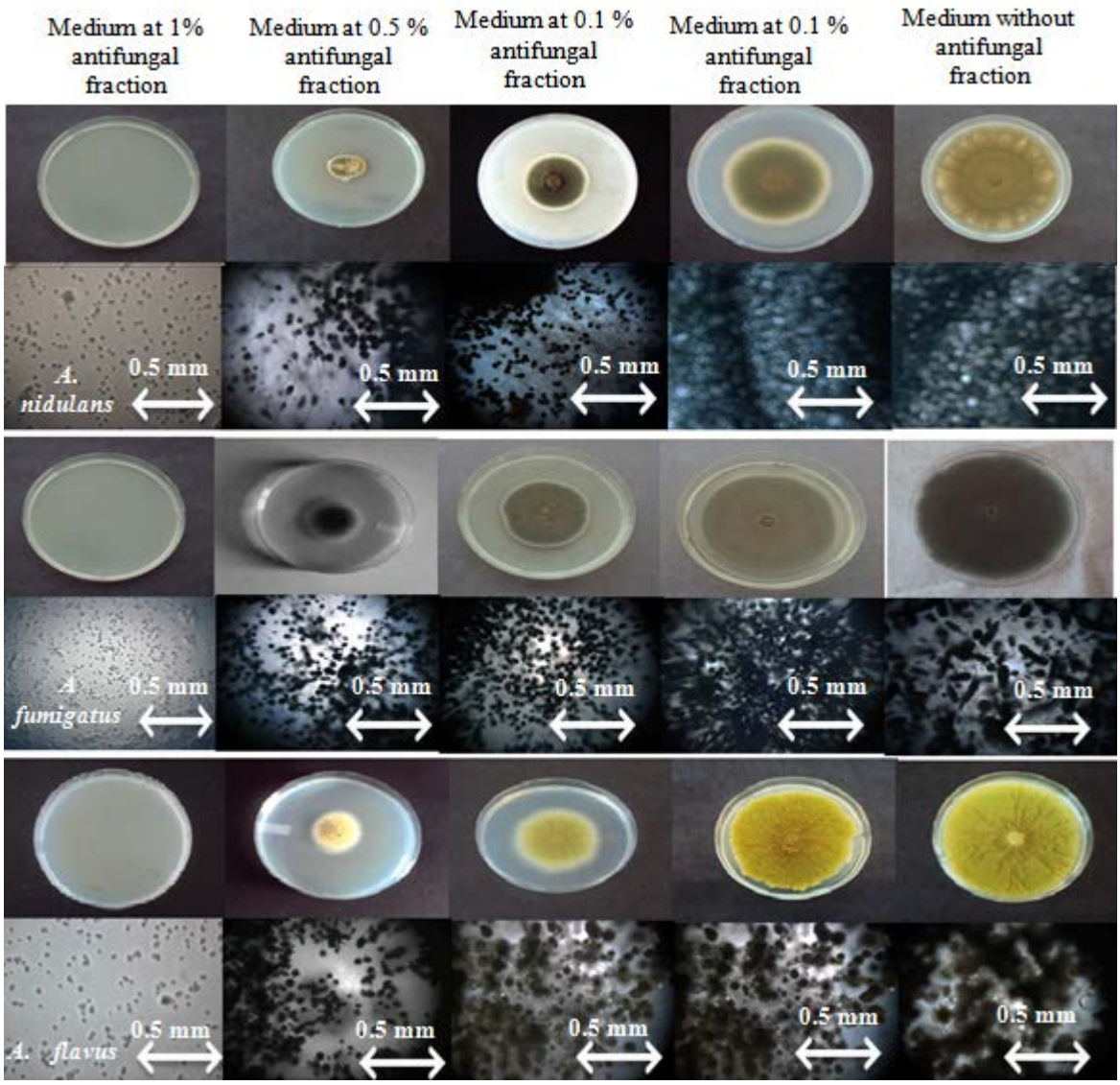

Figure 3. Effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on phenotypic aspects of *A. fumigatusA. nidulans*and *A. flavus* after 7 days of incubation

Table 1. Dose dependent effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on growth of *A. nidulansA. fumigatus*, and *A. flavus* after 7 days of incubation

Homogeneous Subsets Tukey HSD	**Radial growth (mm)**					
	Antifungal fraction content in medium	N	Subset for alpha=0.05			
			1	2	3	4	5
A.nidulans	Medium without antifungal fraction	3	90.00				
	Medium at 0.05 % of antifungal fraction	3		55.67			
	Medium at 0.1 % of antifungal fraction	3			40.00		
	Medium at 0.5 % of antifungal fraction	3				20.00	
	Medium at 0.5 % of antifungal fraction	3					0.00
	significance		1.000	1.000	1.000	1.000	1.000
A fumigatus	Medium without antifungal fraction	3	90.00				
	Medium at 0.05 % of antifungal fraction	3					
	Medium at 0.1 % of antifungal fraction	3			50.00		
	Medium at 0.5 % of antifungal fraction	3		70.67		24.33	
	Medium at 1 % of antifungal fraction	3					0.00
	significance		1.000	1.000	1.000	1.000	1.000
A flavus	Medium without antifungal fraction	3	90.00				
	Medium at 0.05 % of antifungal fraction	3	85.33				
	Medium at 0.1 % of antifungal fraction	3		54.33			
	Medium at 0.5 % of antifungal fraction	3			24.33		
	Medium at 13 % of antifungal fraction	3				0.00	
	significance		0.714	1.000	1.000	1.000	

Means of groups in homogeneous subsets are displayed.
Uses Harmonic Mean Sample size = 3.000

It is noted a dose-dependent inhibition of the growth with the increasing of the antifungal fraction content in the medium for the three strains of *Aspergillus*. However, no significance difference was noted between the radial growth on the medium without antifungal fraction and that observed on the medium at 0.05 % of antifungal fraction for *Aflavus*(Table 1).

The absence of growth noted on the medium at 1 % of the antifungal fraction is related to an absence of conidia germination for the three strains tested. Indeed, the absence of conidia germination for these three strainswas observed on the medium at 1 % of antifungal fractioneven after 24 hours of incubation, while at the other concentrations of antifungal fraction tested, the conidia germination was observed (Figure 4 A, B and C).

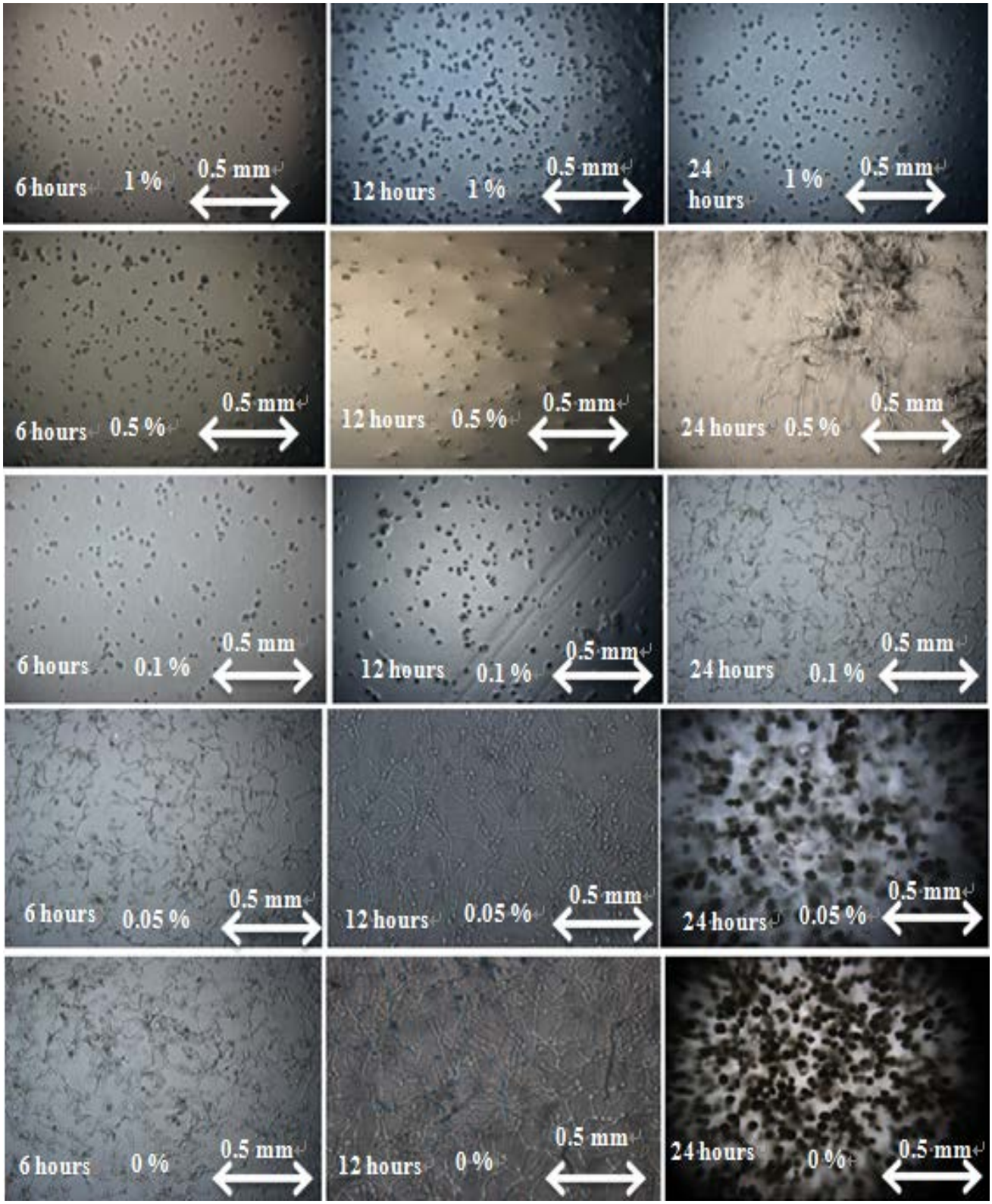

Figure 4 A. Effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on conidia germination of *A. nidulans*

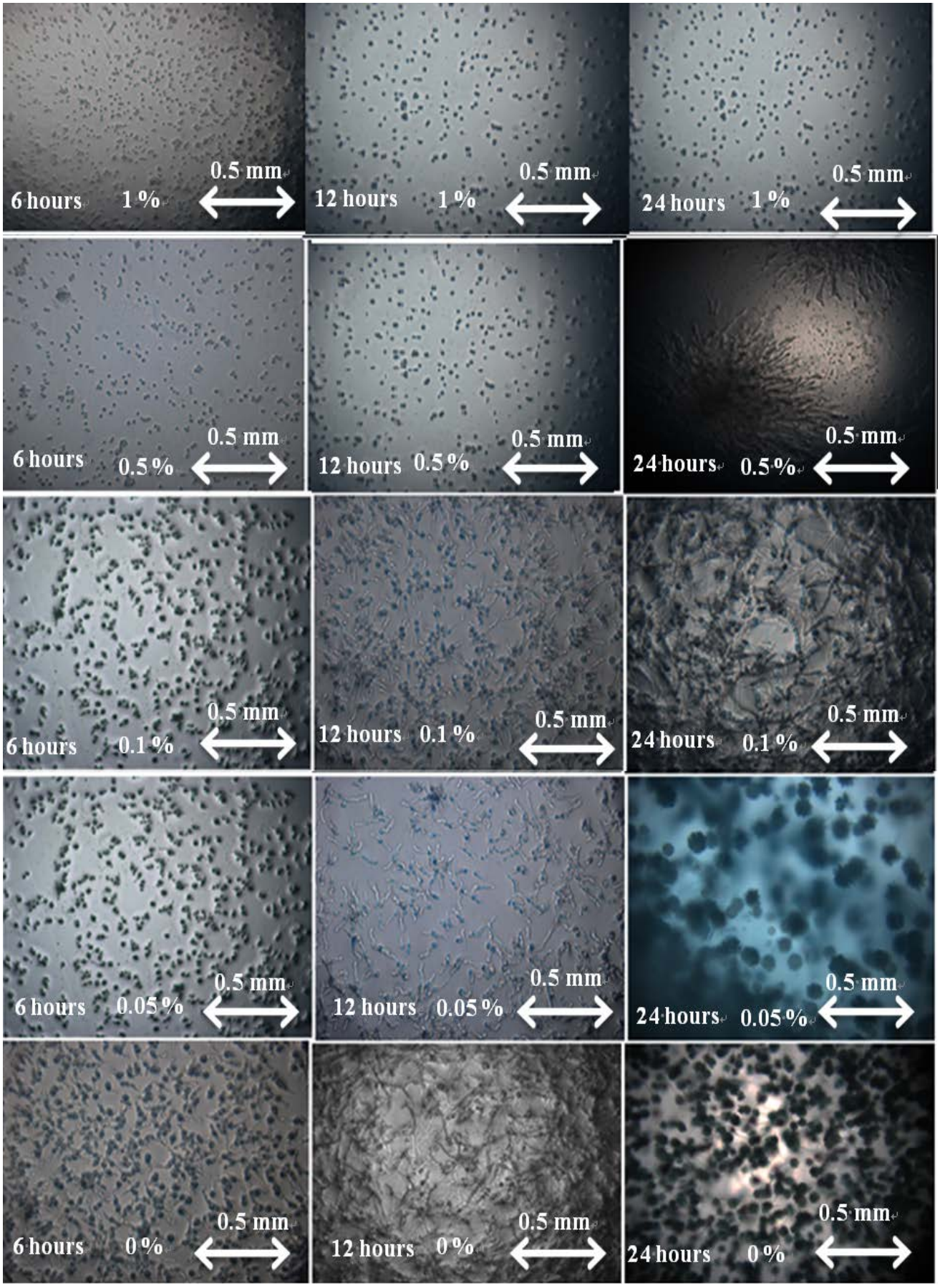

Figure 4 B. Effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on conidia germination of *A. fumigatus*

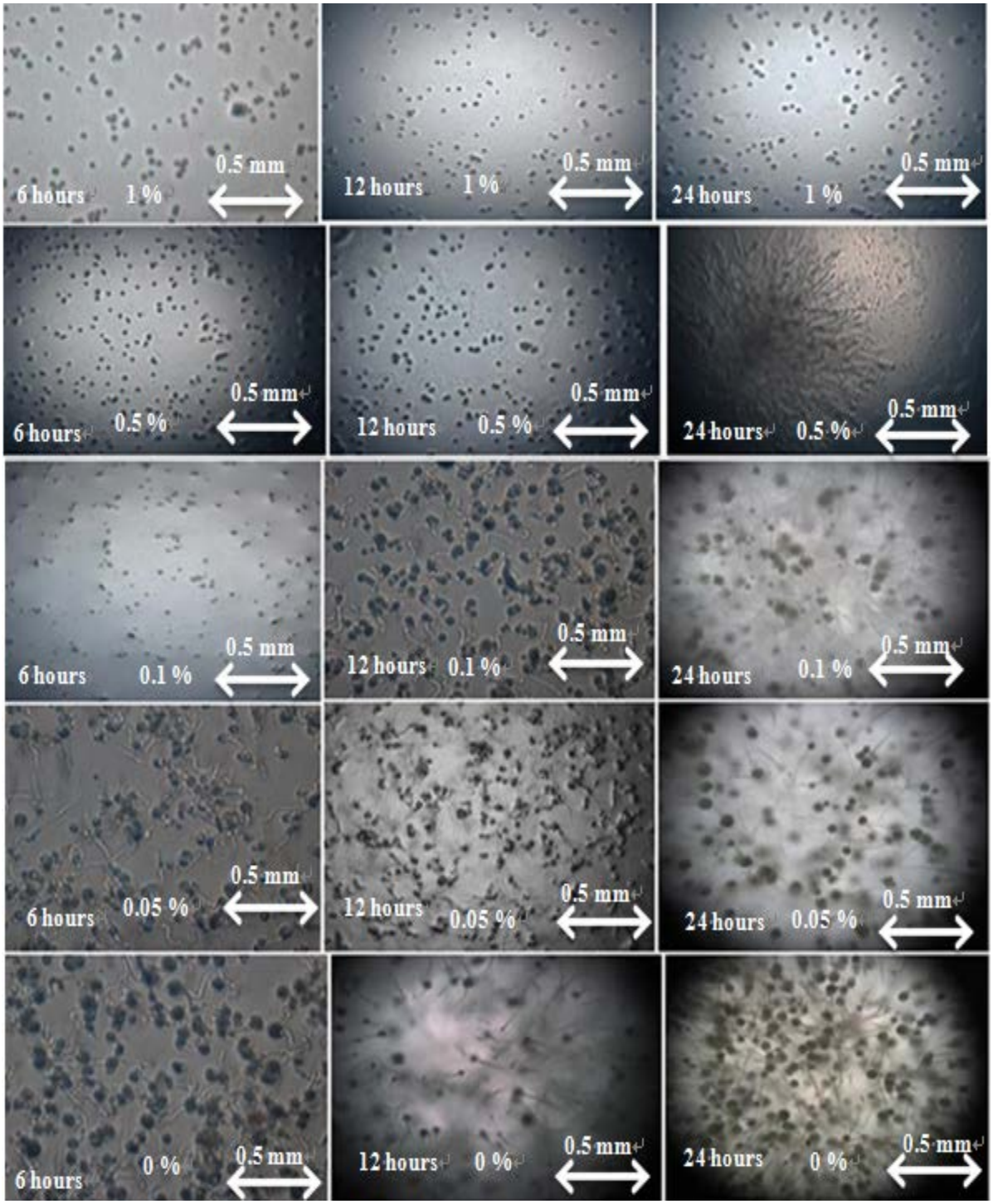

Figure 4 C. Effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on conidia germination of *A.flavus*

The evaluation of the conidia germination rate showed that the more the antifungal fraction in the medium increased, the less the conidia germinated. Indeed, after 24 hours of incubation, the percentages of conidia germinated were 100, 100, 100, 49 and 0 % for *A. nidulans*, 100. 100, 100, 42 and 0 % for *A. fumigatus* and 100, 100, 100, 55 and 0 % for *A. flavus* respectively on the medium without antifungal fraction, the medium at0.05 %, 0.1 %, 0.5 % and 1 % of antifungal fraction. However, from the medium without antifungal fraction to the medium at 0 .1 %, no significant difference was observed between the percentages of conidia germinated for the three strains tested. It was also noted that the rate of conidia germinated was already 100 % after 6 hours of incubation on the medium without antifungal fraction for the three strains tested (Figure 5).

The reduction of the growth of the strains when the antifungal fraction content in the medium increased, led also to the reduction of mycotoxins production. Indeed, the mycotoxins analysis by Thin Layer Chromatography showed any spots of sterigmatocistin and Gliotoxin in the medium at 0.5 % and 1 % of antifungal fraction respectively for *A. nidulans and A. fumigatus*, while for the other concentrations tested, spots of these mycotoxins were observed. For *A. flavus*, the absence of the spot of aflatoxin B1 was observed only in the medium at 1 % of antifungal fraction (Figure 6).

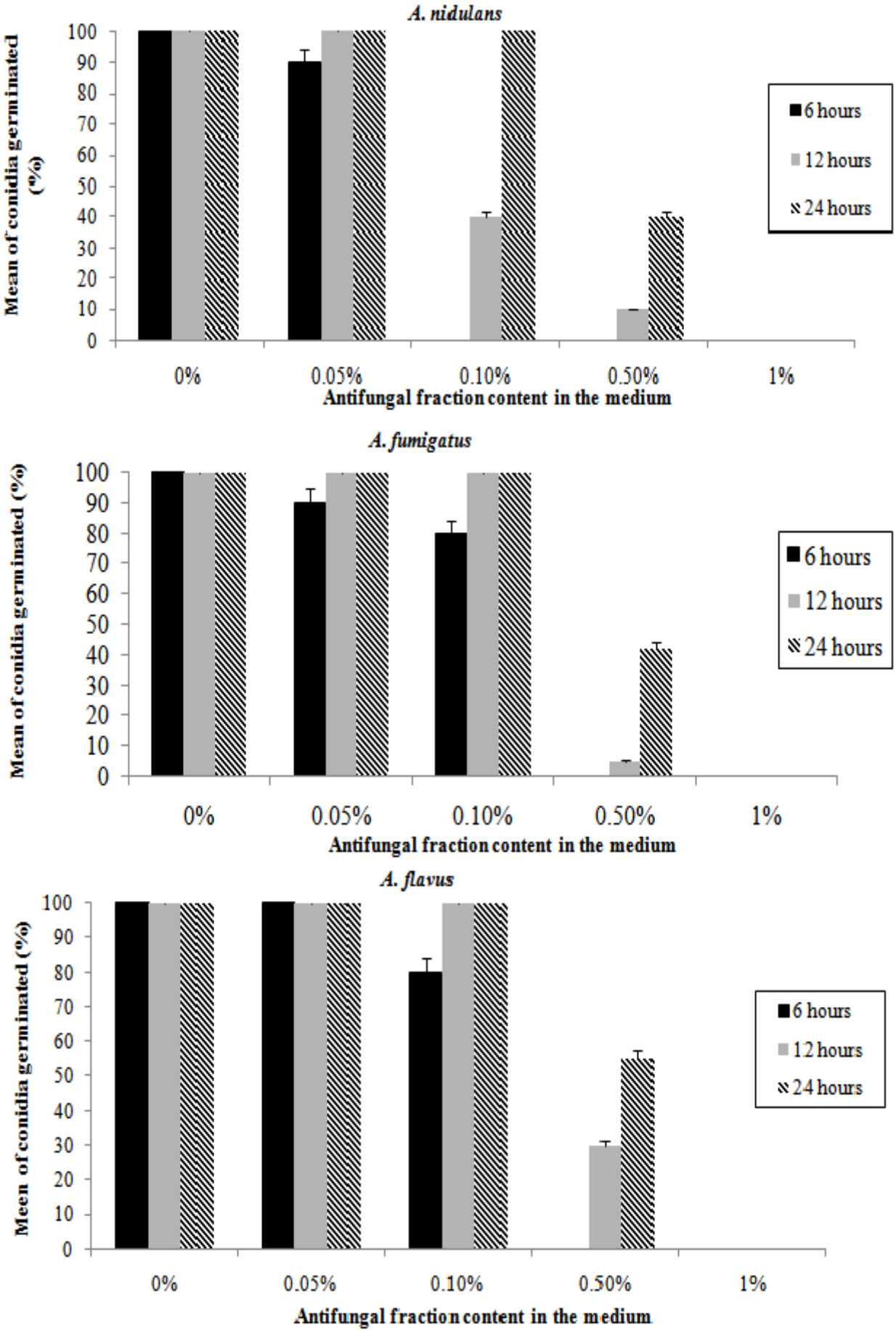

Figure 5. Effect of the antifungal fraction of *Lycopersiconesculentum*leaves extracton percentage of conidia germinated

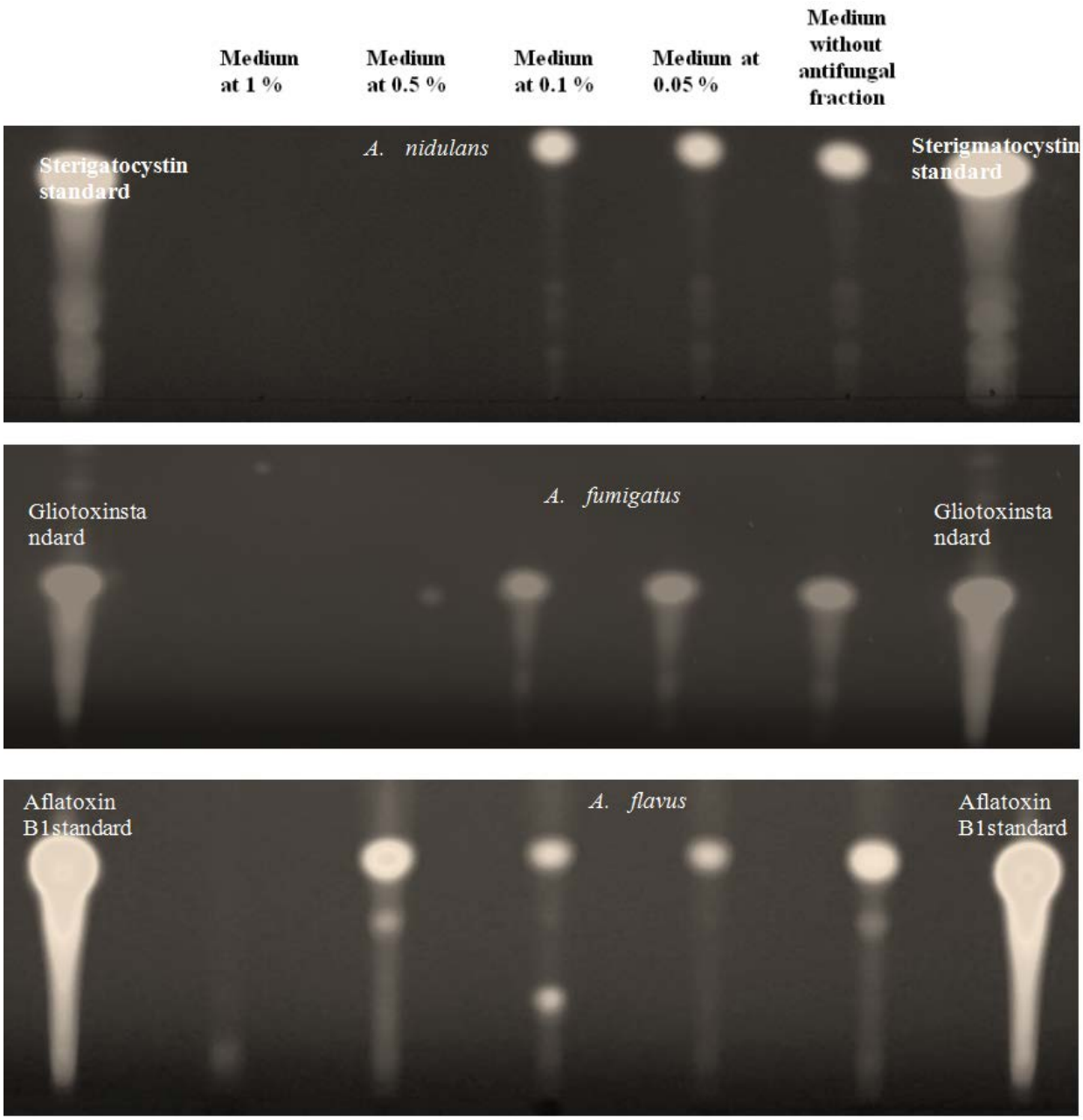

Figure 6. Effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on mycotoxins produced by *A.nidulans*, *A. fumigatus*and *A. flavus*

4. Discussion

In this study, the effect of the antifungal fraction of *Lycopersiconesculentum*leaves extract on fungi proliferation and their potential toxigenic was recorded. A significant reduction of the radial growth of the three strains tested (*A. nidulans*, *A. fumigatus* and *A. flavus*) was noted with the increasing of the antifungal fraction content in the medium. This antifungal fraction exhibited a significant inhibition on growth of the three *Aspergillus* strains with a dose-dependent manner. The highest reduction of the radial growth was observed on the medium containing 1 % of antifungal fraction. At this same concentration, an absence of conidia germination was also observed for the three strains. The absence of growth noted, could be explained by this inhibition of conidia germination. Previous studies showed also the antifungal activities of the glycoalkaloids. Indeed, they have shown that the glycoalkaloids inhibited conidia germination mainly of *Alternariabrassicicolia* [26,27,28]. In addition to molds, the inhibitory effect of the glycoalkaloids on yeasts has also been shown [29]. The inhibition of growth was already observed on the medium at 0.05 % of antifungal fraction indicating that the minimal inhibiting concentration may be at this value. As it has been shown previously by [14], .the most effective means to prevent contamination of food by mycotoxins is to avoid growth of mycotoxigenic fungi. Thus, the use of the antifungal compounds derived from *Lycopersiconesculentum* leaves could be a real mean to preserve food quality. Indeed, the results of the mycotoxins analysis showed an absence of spots of sterigmatocistinfor *A. nidulans* and gliotoxin for *A. fumigatus*in the medium at 0.5 % and 1 % of antifungal fraction. It is noted that although on the medium at 0.5 % of antifungal fraction, there was growth for these two strains, any spot of toxins was observed. Thus, this suggests that these antifungal compounds can inhibit mycotoxins production even if there is fungal growth. This shows also that fungal growth gives little indication about the occurrence of mycotoxins. These

results confirm those obtained by [30] who showed that fungal growth gives little indication about mycotoxins production. For *A. flavus*, the absence of the spot of aflatoxin B1 was observed only in the medium at 1 % of antifungal fraction. These results suggest that the antifungal fraction content at 1% may be the minimal inhibiting concentration for total inhibition of mycotoxins production by the three strains tested. At it has been shown previously, failure to prevent fungal invasion and toxin formation in the field will inevitably lead to an increased risk of adverse health effects and economic loss [7]. Thus, the ability of inhibiting mycotoxins production of the antifungal compounds derived from *Lycopersiconesculentum*leaves is a real hope.

5. Conclusion

We can conclude, regarding the obtained results, that the antifungal compounds of *Lycopersiconesculentum* leaves could be proposed as an effective and powerful antifungal agent against both fungi proliferation and mycotoxins production. The strains more sensitive were *A. nidulans* and *A. fumigatus* as any spot of mycotoxins was observed for these two strains at 0.5 % of antifungal fraction, while spot of aflatoxin B1 produced by the strain of *A. flavus* was observed at this concentration. The total inhibition of mycotoxins production was observed at only 1 % of antifungal fraction. These results suggest the use of the antifungal fraction of *Lycopersiconesculentum* leaves extract as a natural fungicide in alternative in chemical fungicides which cause environmental risks.

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References

[1] Silva C. F., Schwan R. F., Sousa-Diaz E. S. and Wheals A. E. Microbial diversity during maturation and natural processing of coffee cherries of Coffeaarabica in Brazil. International Journal of Food Microbiology 60: 251-260, 2000.

[2] Raju M. V. L. N. and Rama Rao S. V..Aflatoxins in poultry diet: natural ways for counteraction. Proc. Annual Conference. *Compound livestock feed manufacturers Association*, Mumbai, September 13 & 14, 2004.

[3] Akande, K. E., Abubakar, M. M., Adegbnola, T. A. and Bogoro, S. E.. Nutritional and health implications of mycotoxins in animal feeds: a review. Park.*J. Nutr*., 5(5):398-403, 2006.

[4] Word Health Organization. Water-related diseases: Malnutrition. 2001.

[5] Steyn P. S. Mycotoxins, general view, chemistry and structure. *Toxicol.Lett*., 82, 843-851, 1995.

[6] Binder, E. M., Tan, L. M., Chin, L. J., Handl J. & Richard J.Worldwide occurrence of mycotoxins in commodities feeds and feed ingredients. *Anim. Feed Sci. Technol.* 137:265-282, 2007.

[7] CAST (Council for Agricultural Science and Technology). Mycotoxins: Economic and Health Risks. Council for Agricultural Science and Technology *Task Force Report* No 116. Aimes, IA., 1989.

[8] Kuiper-Goodman T**.**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*, p. 3-31, 2004.

[9] Ciergle, A..Bioproduction of ochratoxin A and penicillic acid by members of *Aspergillusochraceus*group.*Can. J. of Microbiol*. 18: 631-636, 1975.

[10] Sharlin J. S., Howarth B., Thompson F. N. and Wyatt R. D. Decreased Reproductive Potential and Reduced Feed Consumption in Mature White Leghorn Males Fed Aflatoxin. *Poult. Sci*.,60: 2701-2708, 1981.

[11] Hafez A. M., Megalla S. E., Abdel-Fattah H. M. and Kamel Y. Y..Aflatoxin and Aflatoxicosis. II. Effects of Aflatoxin on Ovaries and Testicles in Mature Domestic Fowls.*Mycopathologia*, 77: 137-139, 1982.

[12] Jantrarotai W. and Lovell R. T..Pier A. C. 1992.Major biological consequences of Toxicity of Dietary Aflatoxin B1 to Channel Catfish*. J. Aquatic Anim. Health*, 2: 248-254, 1990.

[13] Choudhary A. K. and Kumari P. Management of mycotoxin contamination in pre-harvest and post-harvest crops: present status and future prospects. *Journal of Phytology*, 2(7): 37-52, 2010.

[14] Bullerman L. B. Incidence and control of mycotoxins producing molds in domestic and imported cheeses. *Annals of Nutr.and Food*, 31, 435-446, 1977.

[15] Directive 91/414/CEE du Conseil du 15 juillet 1991 concernant la mise sur le marché des produits phytopharmaceutiques. *Official Journal*, L 230 du 19.8.1991, p. 1. July, 1991.

[16] Rick, C. M..Tomato.In Hybridization of Crop Plants (ed. W. R. Fehr and H. H. Hadley.*American Society of Agronomy*, Madison, USA, pp. 669-680, 1980.

[17] Hui, Y.H., Roy Smith, David G. Spoerke, Foodborne Disease Handbook, vol. 3, Plant Toxicants, CRC; *2nd édition*, (ISBN 082470343X), p. 99, 2001.

[18] Friedman M. and Levin C. E. α-Tomatine content in tomato and tomato products determined by HPLC with pulsed amperometric detection, *Journal of agricultural and food chemistry*, vol. 43, no6, pp. 1507-1511 (1 p.), 1995.

[19] Sandrock R.W., VanettenH.D..Fungal sensitivity to and enzymatic degradation of the phytoanticipinα-tomatine.Phytopathology,88: 137-143, 1998.

[20] Hoagland R.E..Toxicity of tomatine and tomatidine on weeds, crops and phytopathogenic fungi.Allelopathy J.23: 425-436, 2009.

[21] Steel C.C., Drysdale R.B. Electrolyte leakage from plant and fungal tissues and disruption of liposome membranes by α-tomatine. Phytochemistry27: 1025-1030, 1988.

[22] Keukens E.A., de Vrije T., van den Boom C., de Waard P., Plasman H.H., Thiel F., Chupin V., Jongen W.M., de Kruijff B. Molecular basis of glycoalkaloid induced membrane disruption. Biochim.Biophys.Acta,1240: 216-228, 1995.

[23] Blankemeyer J.T., White J.B., Stringer B.K., Friedman M..Effect of α-tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin.Food Chem. Toxicol.35: 639-646, 1997.

[24] Iowa Department of Public Health. Acute Pesticide Poisoning Associated with Pyraclostrobin Fungicide --- Iowa, 2008.

[25] Pitt, J.I.. A laboratory guide to commun*Penicillium*species, Tingalpa :*Food Science*, 197 p. 1988.

[26] Fewell, A. M. and Roddick, J. G.. Interactive antifungal activity of the glycoalkaloidsα-solanine and α-chaconine. *Phytochemistry* 33: 323-328, 1993.

[27] Fewell, A. M., Roddick, J. G., and Weissenberg, M..Interactions between the glycoalkaloidssolasonine and s olamargine in relation to inhibition of fungal growth.*Phytochemistry* 37: 1007-1011 . 1994.

[28] Fewell, A. M. and Roddick, J. G. Potato glycoalkaloid impairment of fungal development. *Mycol. R e v*. 101: 597-603, 1997.

[29] Wang LD, Guo DA, Yuan L, He QH, Hu YQ, Tu PF, et al. Antifungal effect of three natural products on the genetic substance of Saccharomyces cerevisiaeGL7 and Protothecawickerhamii. *Yao XueXueBao*, 35(11): 860-863, 2000.

[30] Kouadio A. I., Agbo N. G., Lebrihi A., Mathieu F.and Dosso M. Effect of the frequency of the mixing of coffee cherries put out for dryingon the kinetics of drying and the relationship to ochratoxin A production. *Food Additives and Contaminants*,23(3): 295-304, March 2006.