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Effect of *Solanumindicum* L. Green Berries Extract on Proliferation and Survival of *Aspergillusnidulans*, *Aspergillusflavus*, and *Aspergillusfumigatus*

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Abstract The berries of *Solanumindicum* L. are known to be an appreciable source of antioxidants. In this study, the effect of the green berries' ethanolic extract on growth, RNA concentration representing the biomass and the percentage of reduced Alamar Blue indicating cell viability of *Aspergillusnidulans*, *Aspergillusfumigatus* and *Aspergillusflavus* was evaluated. It was noted a reduction of growth with the increasing of the extract content in the medium. Indeed, a radial growth of 90 mm was observed on the medium without extract for the three species after 7 days of incubation, while on the medium at 1 % of the berries extract, the radial growths were 0 mm for *A. nidulans*and *A. fumigatus* and 14.67 mm for *A. flavus*. In addition to the absence of growth of *Aspergillusnidulans* and *Aspergillusfumigatus* observed at 1 % of berries extract, an absence of their conidia germination was observed. It was noted also that the mean of RNA concentration observed in the medium without extract was about 285 times that observed in the medium at 1 % of berries extract for *A. nidulans*. For *A. fumigatus*and *A. flavus*, this mean of RNA concentrations observed in the medium without extract were respectively about 73 and 57 times that observed in the medium at 1 % of berries extract. The RNA bands of the three species tested observed after the electrophoresis seem to be degraded also in the medium at 1 % of berries extract. At this concentration, the percentages of surviving cells were less than 10 % for the three species tested, while that observed in the medium without the berries extract was 100 %.Thus, *Solanumindicum* green berries extract exhibit a real inhibitory effect against fungi with an ability of killing them.

Keywords: *Solanumindicum L., green berries, Aspergillus, biomass, cell viability*

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1. Introduction

The degradation of the quality of food and feed can occur in farms and also during postharvest treatments. Indeed, according to [1], before harvest and during postharvest treatments, crops and processed products are subjected to contamination by fungi. This development of fungi on food and feed leads to loss in nutritional quality. Indeed, according [2], energy, crude protein and crude fat contents of moldy maize may go down up to 5, 7 and 63% respectively. The team of [3] has 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 [4,5]. Many mycotoxins are stable under normal food processing conditions and can therefore be present not only in food and feed but also in processed products [6]. 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 [7]. 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 mycotoxins [8]. The economic losses are more important for developing countries which are exporters, due to stricter regulations imposed by importing countries.

Innovative technologies are urgently needed to reduce the risks of mycotoxin in food and feed. Current strategies to destroy mycotoxins in food include heating, treatment with ammonia, screening and radiation. Upon evaluation, these methods often are found too expensive, impractical for commercial application or destroy vital nutrients of the grain [9,10]. 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 [11]. The primary method of control is the use of chemical fungicides. However, they have become less favored by regulators due to the toxicological risks [12]. Also, some of these chemical fungicides do not kill the fungi. They simply inhibit growth for a period of days or weeks [13].

Furthermore, the general public demands a reduced use of chemical preservatives and additives in food and feed [14]. Therefore, the use of natural substances capable of inhibiting fungi development and killing them is of a great importance. Our preliminary investigations have shown that the berries of *Solanumindicum* L.,a wild plant consumed by rural populations in Ivory Coast seem not to be infected by microorganisms despite the ecological conditions which are favorable for their development. This plant species belongs to the genus *Solanum* and the family of *Solanaceae*. The berries of this plant 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 [15]. In addition to components mentioned above, these berries have been shown to contain polyphenols [16] and steroidal glycerides [17,18]. However, the use of this species has not limited to food. Indeed, *Solanumindicum* 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 beenclaimed in folk medicine to have an antihypertensive effect [19]. 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 some time at many dosages. From this background, we can move forward to explore the use of these berries against growth and cell viability of fungi for further contribution for the research for alternative in chemical additives and preservatives in food and feed and also in chemical fungicides.

2. Material and Methods

2.1. Material

2.1.1. Biological Material

In this study, green berries of *Solanumindicum* L. were used. These berries have been collected from rural zones of the central part of Ivory Coast where they are found in abundance. The medium used was the Minimal Medium plus Yeast Extract (MMYE). Three *Aspergillus* species tested capable of producing mycotoxins (*Aspergillusflavus*, *Aspergillusnidulans* and *Aspergillusfumigatus*) from the laboratory of Genetics of the Food Research Institute (University of Wisconsin Madison, USA) were also used. All the experiments in this study were carried out in this laboratory.

2.2. Methods

2.2.1. Medium Preparation

The Minimal Medium plus Yeats Extract (MMYE) used in this study was prepared by adding 10 g of Dextrose to 50 ml of 20X Salt solution. To the mixture obtained, 1 mL of 1000x trace elements and 1 g of yeast extract were added. Then, the deionized water was added to obtain a mixture of 1000 mL. The homogenate mixture obtained was sterilized in the autoclave at 121 °C during 20 min.

2.2.2. Berries Extract Preparation

The berries extract preparation was done according to the method of [20]. The resulting solution obtained after the green berries extraction was evaporated to dryness under Fume Hood. The residue obtained was dissolved into 15 mL of 100% ethanol and filtered onto 0.22µm cutoff membranes to eliminate residues which were not dissolved.

In order to make a partial purification and identify the fraction of the extract that contained the antifungal activity, the Thin Layer Chromatography (TLC) analysis was done. The migration solution in the chamber was composed of ethyl acetate, n-butanol and water with the proportions of 10:10:4. This antifungal fraction was identified by doing an inhibition test with discs impregnated with the different fractions removed from the TLC plate and dissolved into 100% ethanol. The antifungal fraction's Rf observed on the TLC plate was 0.25.

2.2.3. Preparation of the Tested Species

The preparation of the tested species was done according to the method of [21]. Once the spore's suspension at 10^6 pores/ml for each species was obtained, a quantity of 10µl of these suspensions was inoculated on solid medium for the monitoring of the growth. For the other experiments, 1 mL of this spore's suspension at 10^6 pores/mL was inoculated into liquid MMYE of 150 Ml for 18 hours 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 MMYE of 50 mL. Then, 2 mL of the ball suspension was put into different tubes aseptically. Into each tube, the ethanolic extract was added to obtain concentrations in extract of 0.01%, 0.1% and 1%. Medium without berries extract but containing 1% of ethanol 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 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 of reduced of Alamar Blue).

2.2.4. Monitoring of the Growth

The green berries extract was added to the medium to obtain mediums with different concentrations of 0.01%, 0.1% and 1%. A medium without extract but containing

1% of ethanol was used as a control medium. Each medium was put into a Petri dish and after solidification, 10 µl of the *Aspergillus* conidia suspension at 10^6 pores/mL were put aseptically in the center of this medium. The mediums inoculated 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 [22]. This experiment was carried out for 7 days.

2.2.5. Extraction and Determination of RNA Concentration

After 24 hours of incubation, the RNA was extracted. For this RNA extraction, the method of [23] was used. 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.

2.2.6. Bioassay Analysis

The experiment was conducted over a span of 5 days. After each 24 hours of incubation, 700 µl of liquid MMYE 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 hours. MMYE 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 the apparatus Bio-Teck ELISA.

2.2.7. 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 results (Figure 1) showed that with increases inberries extract content in the medium,there was less fungal growth.

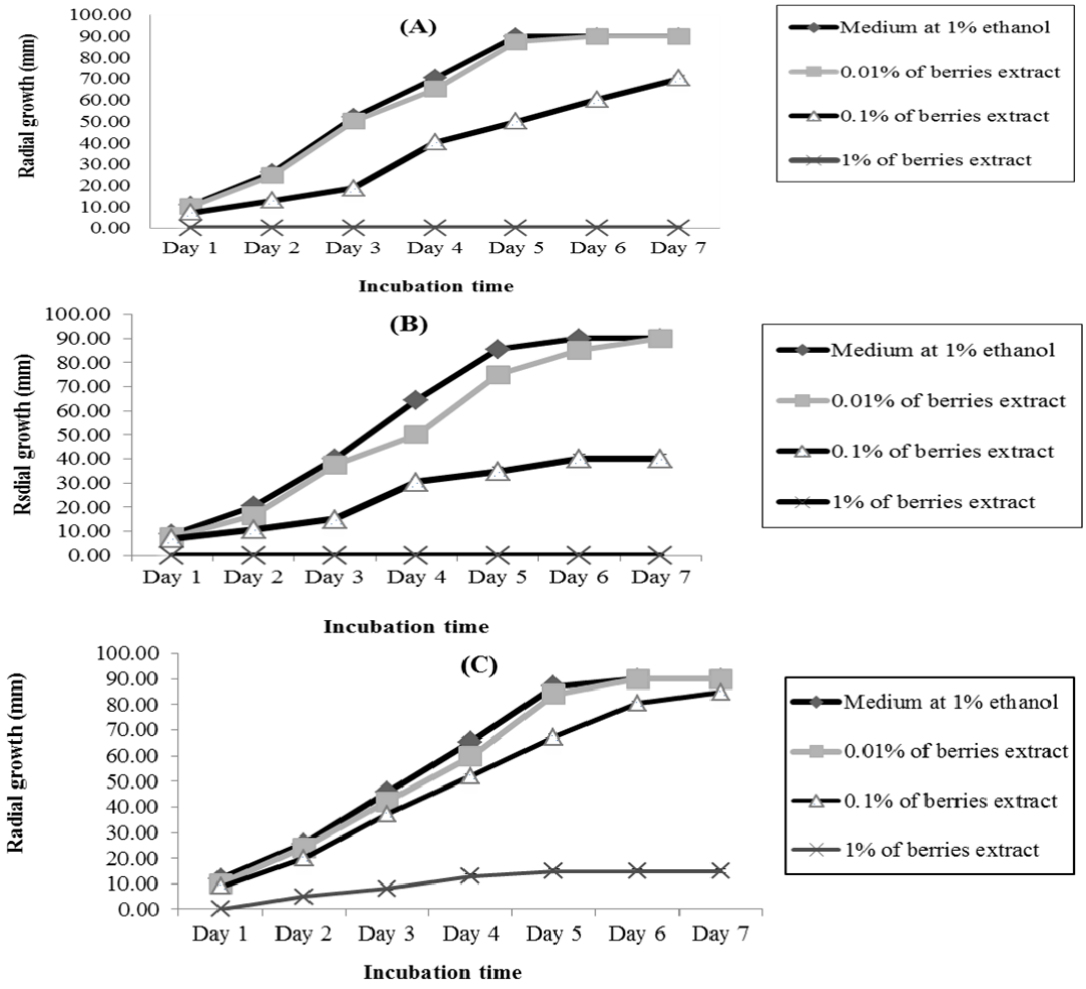

Figure 1. Effect of *Solanumindicum* L. green berries extract on growth of (A): *A. fumigatus*, (B): *A. nidulans* and (C): *A. flavus*

Indeed, the mean of radial growth which was 90 mm on the medium without extract was decreased to reach the values of 0 mm for *A. nidulans* and *A. fumigatus* and 14.67 mm for *A. flavus*after 7 days of incubation on the medium containing 1 % of berries extract. A dose-dependent inhibition of the growth was observed with the increasing of the berries extract content in the medium for the three species. However, from the medium without extract to the medium at 0.01 % of berries extract, no significance difference between the radial growths was observed (Table 1).

Table 1. Dose-dependent effect of *Solanumindicum*L. green berries extract on growth of *A. fumigatus*, *A. nidulans* and *A. flavus* after 7 days of incubation

Homogeneous Subsets Radial growth (mm) Tukey HSD					
	Extract content in medium	N	Subset for alpha=0.05		
			1	2	3
A.nidulans	Medium at 1 % of berries extract	3	0.00		
	Medium at 0.1 % of berries extract	3		40.33 ± 0.58	
	Medium at 0.01 % of berries extract	3			90.00
	Control (Medium at 1% ethanol)	3			90.00
	Sig		1.000	1.000	0.81
A fumigatus	Medium at 1 % of berries extract	3	0.00		
	Medium at 0.1 % of berries extract	3		70.33 ± 0.58	
	Medium at 0.01 % of berries extract	3			90.00
	Control (Medium at 1% ethanol)	3			90.00
	Sig		1.000	1.000	0.81
A flavus	Medium at 1 % of berries extract	3	14.67 ± 0.58		
	Medium at 0.1 % of berries extract	3		90.00	
	Medium at 0.01 % of berries extract	3		90.00	
	Control (Medium at 1% ethanol)	3		85.00	
	Sig		1.000	.79	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmoric Mean Sample Size = 3.000.

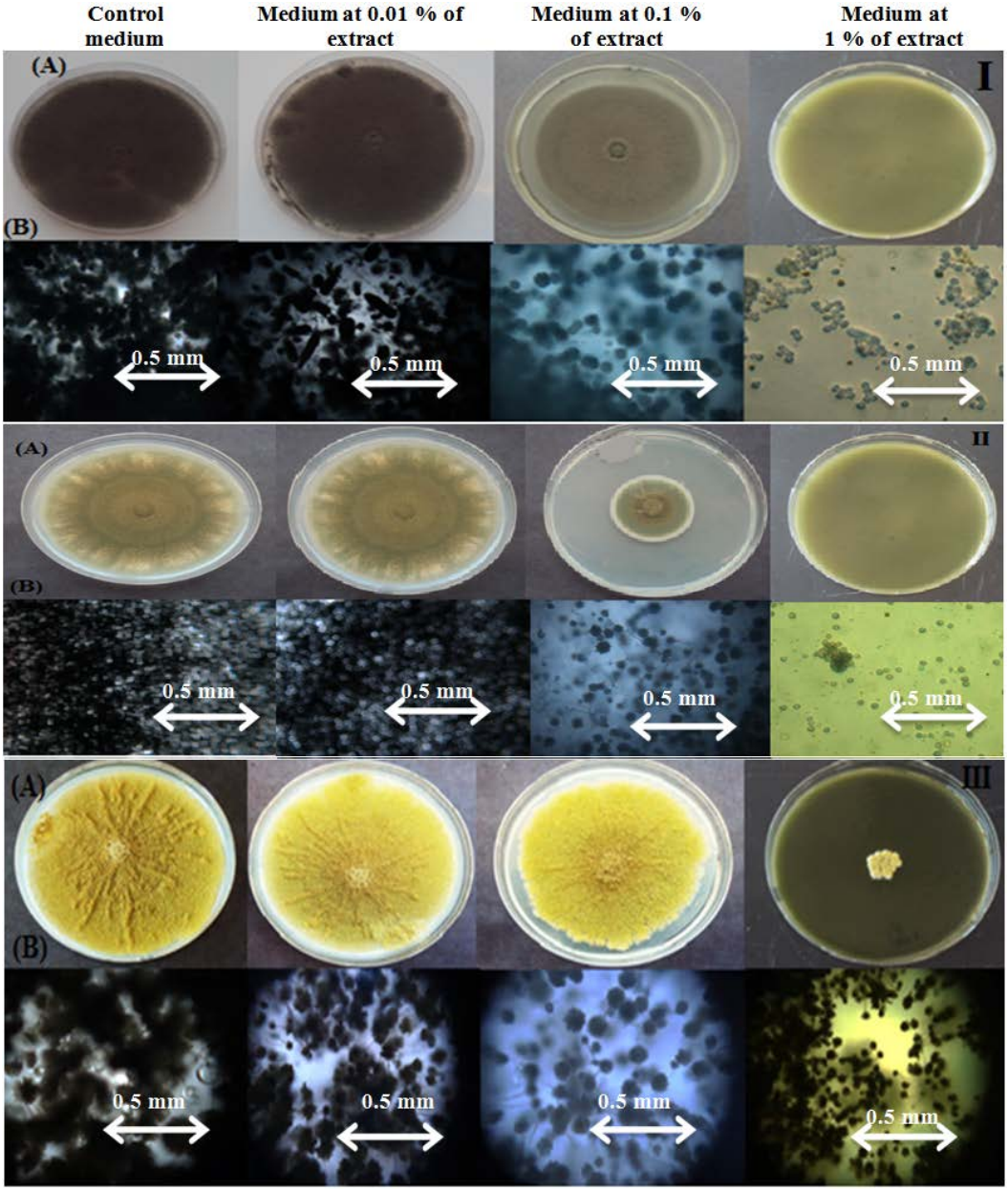

Figure 2. Effect of *Solanumindicum* green berries on macroscopic (A) and microscopic (B) aspects of *A. fumigatus* (I), *A. nidulans* (II) and *A. flavus* (III) after 7 days of incubation

The absence of growth observed on the medium at 1 % of berries extract for *A. nidulans* and *A. fumigatus* was related to an absence of conidia germination (Figure 2).

A decrease of the RNA concentration representing the biomass was also observed when the berries extract content in the medium increased. Indeed, the mean of RNA concentrations(Figure 3) which were 5400.23, 6087.17 and 5520.53l µg/ml in the control medium respectively for *A. nidulans*, *A. fumigatus* and *A. flavus*decreased to reach respectively, the values of 18.9,82.6 and 95.73 µg/ml in the medium at 1% of berries extract.

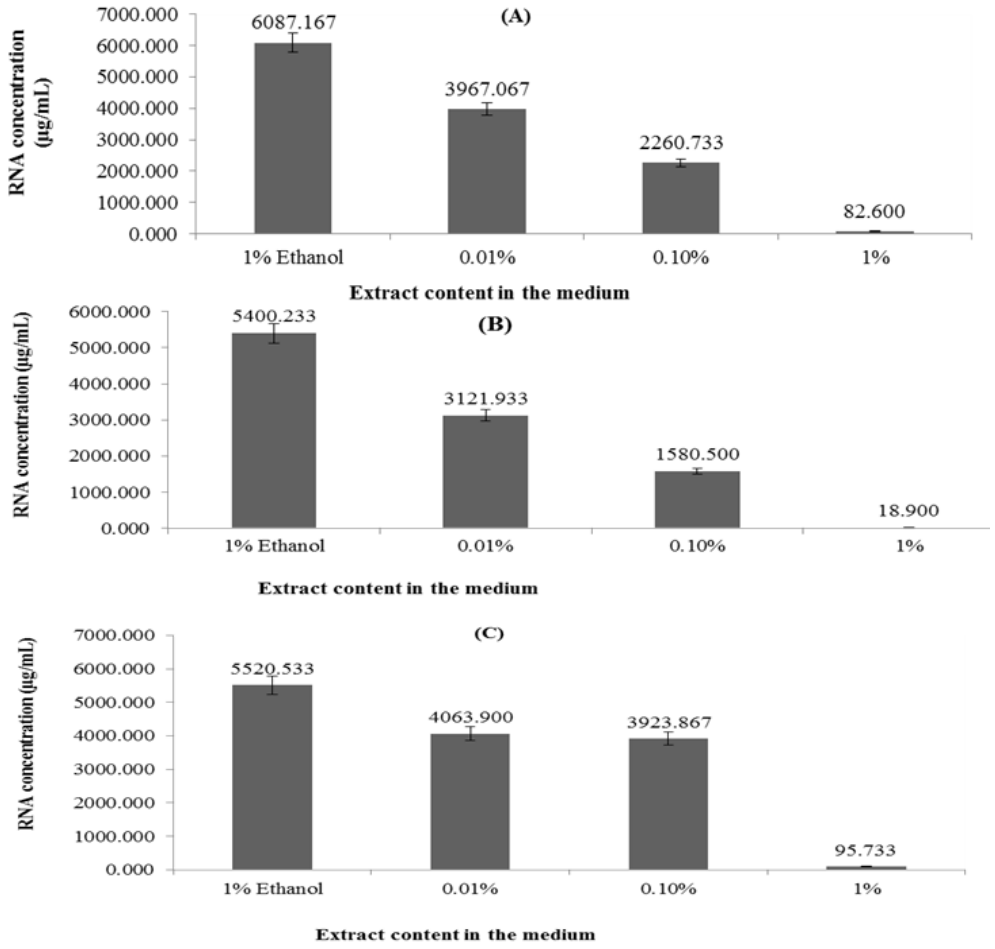

Figure 3. Effect of *Solanumindicum* L. green berries extract on RNA concentration of **(A)**: *A. fumigatus*,**(B):***A. nidulans*,and**(C):** *A. flavus*

Table 2. Dose-dependent effect of *Solanumindicum* L. green berries extract on biomass of *A. fumigatus, A. nidulans* and *A. flavus*

Post Hoc Tests

Multiple Comparisons

Dependent Variable: RNA concentration (µg/ml)
Tukey HSD

	(I) Extract Content in medium		Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval	
A fumigatus	Control (Medium contained 1% ethanol) **VS**	Medium at 0.01% of berries extract	2153.4333*	153.2256	.000	1662,751	2644.116
		Medium at 0.1% of berries extract	4826.4333*	153.2256	.000	4335.751	5317.116
		Medium at 1% of berries extract	6004.5667*	153.2256	.000	5513.884	6495.249
	Medium at 0.01% of berries extract **VS**	Medium at 0.1% of berries extract	2673.0000*	153.2256	.000	2182.318	3163.682
		Medium at 1% of berries extract	3851.1333*	153.2256	.000	3360.451	4341.816
	Medium at 0.1% of berries extract **VS**	Medium at 1% of berries extract	1178.1333*	153.2256	.000	687.451	1668.816
A.nidulans	Control (Medium contained 1% ethanol) **VS**	Medium at 0.01% of berries extract	2278.3000*	66.6393	.000	2064.898	2491.702
		Medium at 0.1% of berries extract	3819.7333*	66.6393	.000	3606.331	4033.136
		Medium at 1% of berries extract	5381.3333*	66.6393	.000	5167.931	5594.736
	Medium at 0.01% of berries extract **VS**	Medium at 0.1% of berries extract	1541.4333*	66.6393	.000	1328.031	1754.836
		Medium at 1% of berries extract	3103.0333*	66.6393	.000	2889.631	3316.436
	Medium at 0.1% of berries extract **VS**	Medium at 1% of berries extract	1561.6333*	66.6393	.000	1348.198	
A flavus	Control (Medium contained 1% ethanol) **VS**	Medium at 0.01% of berries extract	1456.6333*	98.9637	.000	1139.717	1773.550
		Medium at 0.1% of berries extract	3596.6667*	98.9637	.000	3279.750	3913.583
		Medium at 1% of berries extract	5424.8000*	98.9637	.000	5107.883	5741.717
	Medium at 0.01% of berries extract **VS**	Medium at 0.1% of berries extract	2140.0333*	98.9637	.000	1823.117	1456.950
		Medium at 1% of berries extract	3968.1667*	98.9637	.000	3651.250	4285.083
	Medium at 0.1% of berries extract **VS**	Medium at 1% of berries extract	1828.1333*	98.9637	.000	1511.217	2145.050

* The mean difference significant at the 0.05 level.

It was noted that inhibition of fungiproliferation was influenced significantly ($P < 0.05$; Table 2) by the extract content in medium.

The result of the electrophoresis done showed also an absence of RNA bands in the medium at 1 % of green berries extract for the three species tested (Figure 4).

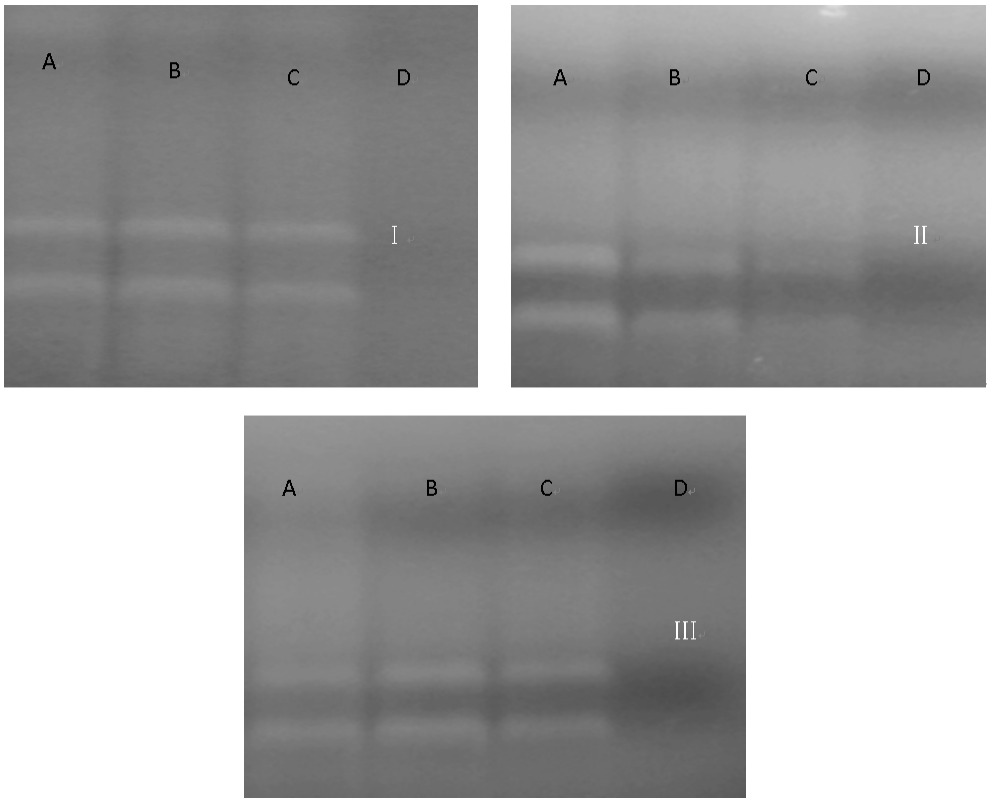

Fig. 4: Effect of *Solanumindicum*L. green berries on RNA bands of (I): *A. fumigatus*, (II): *A. nidulans* and (III): *A. flavus* (**A**:medium without extract; **B**: medium at 0.01 %; **C**: medium at 0.1 % and **D**: medium at1 % of berries extract)

Table 3. Variation of the mean of percentage in reduction of Alamar Blue in relation to *Solanumindicum* L. green berries extract content in the medium during 5 days of incubation of *A. fumigatus*, *A. nidulans* and *A. flavus*

	Percentage in reduction of Alamar blue								
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
A fumigatus	Control (Medium at 1% ethanol)	3	100.0000	0.0000	0.0000	100.0000	100.0000	100.0000	100.0000
	Medium at 0.01% of berries extract	3	29.224-71.1720	0.3637-0.2644	0.21-0.1527	78.3204-70.5152	80.1272-71.8288	78.8643-70.8681	79.5915-71.3495
	Medium at 0.1% of berries extract	3	77.6605-59.8882	0.1479-0.1027	0.08541-0.0593	77.2930-59.6332	78.0280-60.1433	77.5331-59.8011	77.8228-60.0014
	Medium at 1% of berries extract	3	13.2889-5.0522	2.0307-0.1993	1.1725-0.1151	8.2442-4.5572	18.3335-5.5473	11.2716-4.8385	15.3328-5.2330
	Total	12	67.5433-59.0281	33.9998-53.9560	9.8149-10.3796	45.9408-36.1828	89.1457-81.8734	11.2716-4.8385	100.0000
A.nidulans	Control (Medium at 1% ethanol)	3	100.0000	0.0000	0.0000	100.0000	100.0000	100.0000	100.00000000
	Medium at 0.01% of berries extract	3	81.9230-74.8843	0.8739-0.0597	0.5045-0.0345	79.7523-74.7360	84.0938-75.0326	81.2184-74.8174	82.9009-74.9321
	Medium at 0.1% of berries extract	3	65.9230-61.1552	0.2132-0.3112	0.1231-0.1797	64.4892-60.7787	65.5482-62.3251	64.7763-61.1934	65.1768-61.7527
	Medium at 1% of berries extract	3	6.2017-3.5805	0.1208-0.1604	0.0698-0.0926	5.9016-3.1820	6.5019-3.9789	6.0888-3.4705	6.3291-3.7645
	Total	12	63.2859-60.0042	36.7699-39.9540	10.6145-10.6677	39.9234-36.5247	86.634-83.4836	6.0888-3.4705	100.0000
A flavus	Control (Medium at 1% ethanol)	3	100.0000	0.0000	0.0000	100.0000	100.0000	100.0000	100.00000000
	Medium at 0.01% of berries extract	3	91.0932-87.2129	0.1683-0.8667	0.0972-0.5004	90.6749-85.0599	91.5114-89.3659	90.9062-86.2634	91.2328-87.9616
	Medium at 0.1% of berries extract	3	80.6371-76.8352	0.1645-0.4451	0.0950-0.2570	80.2285-75.7294	81.0457-77.9410	80.4851-76.4598	80.8117-77.3270
	Medium at 1% of berries extract	3	10.7087-6.1142	0.3945-0.07184	0.2277-0.0415	9.7289-5.9357	11.6887-6.2927	10.4335-6.0640	11.1607-6.1965
	Total	12	70.6097-67.5406	36.8245-38.0221	10.6303-10.9760	47.2125-43.3824	94.0069-91.6987	10.4335-6.0640	100.00000000

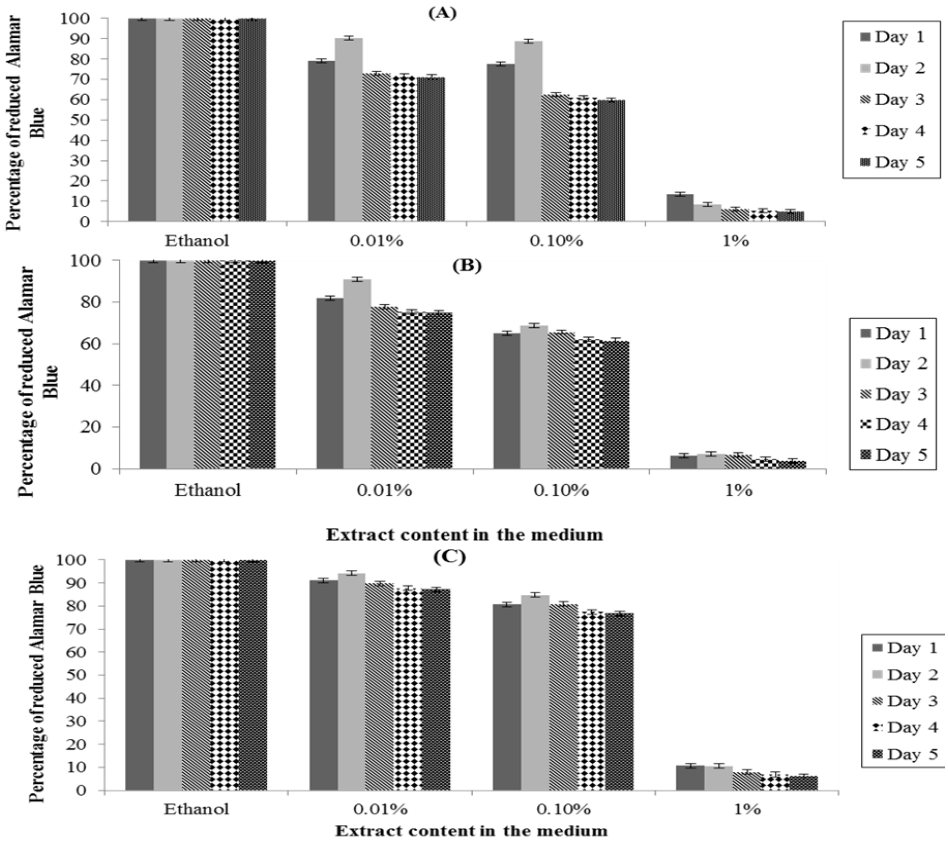

Fig. 5: Effect of *Solanumindicum*L. green berries extract on percentage in reduction of Alamar Blue of **(A)**: *A. fumigatus***(B):***A. nidulans*and**(C):** *A. flavus*

Table 4: Dose-dependent effect of *Solanumindicum* L. green berries extract on cell viability during 5 days of incubation of *A. fumigatus, A. nidulans*and *A. flavus*

	Post Hoe Tests **Multiple Comparisons** Dependent Variable: Percentage in reduction of Alamar blue Tukey HSD						
	(I) Extract Content in medium		Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
A fumigatus	Control (Medium contained 1% ethanol) **VS**	Medium at 0.01% of berries extract	20.7762*-28.8280*	0.8444-0.1415	.000	18.0722-28.3748	23.4803-29.2812
		Medium at 0.1% of berries extract	22.3395*-40.1117*	0.8444-0.1415	.000	19.6354-39.6586	25.0435-40.5649
		Medium at 1% of berries extract	86.7111*-94.9478*	0.8444-0.1415	.000	84.0071-94.4946	89.4152-95.4010
	Medium at 0.01% of berries extract **VS**	Medium at 0.1% of berries extract	1.5633-11.2837*	0.8444-0.1415	0.319-0.000	-1.1408-10.8305	4.2673-11.7369
		Medium at 1% of berries extract	65.9349*-66.1197*	0.8444-0.1415	.000	63.2308-65.6666	68.6390-66.5729
	Medium at 0.1% of berries extract **VS**	Medium at 1% of berries extract	64.3716*-54.8360*	0.8444-0.1415	.000	61.6676-54.3828	67.0757-55.2892
A.nidulans	Control (Medium contained 1% ethanol) **VS**	Medium at 0.01% of berries extract	18.0769*-25.1157*	0.3705-0.1450	.000	16.8905-24.6513	19.2634-25.5800
		Medium at 0.1% of berries extract	34.9813*-38.4481*	0.3705-0.1450	.000	33.8905-24.6513	36.1678-38.9124
		Medium at 1% of berries extract	93.79825*-96.3324*	0.3705-0.1450	.000	92.6117-95.9551	94.9847-96.8838
	Medium at 0.01% of berries extract **VS**	Medium at 0.1% of berries extract	16.9043*-13.3324*	0.3705-0.1450	.000	15.7178-12.8681	18.0908-13.7968
		Medium at 1% of berries extract	75.7213*-71.3038*	0.3705-0.1450	.000	74.5348-70.8395	76.9078-71.7682
	Medium at 0.1% of berries extract **VS**	Medium at 1% of berries extract	58.8170*-57.9714*	0.3705-0.1450	.000	57.6305-57.50706	60.0034-58.4358
A flavus	Control (Medium contained 1% ethanol) **VS**	Medium at 0.01% of berries extract	8.9068*-12.7871*	0.18753-0.3988	.000	8.3063-11.5099	9.5074-14.0644
		Medium at 0.1% of berries extract	19.3629*-23.1648*	0.18753-0.3988	.000	18.7624-21.8876	19.9634-24.4421
		Medium at 1% of berries extract	89.2912*-93.8858*	0.18753-0.3988	.000	88.6907-92.6085	89.8918-95.1630
	Medium at 0.01% of berries extract **VS**	Medium at 0.1% of berries extract	10.4561*-10.3777*	0.18753-0.3988	.000	9.8555-9.1004	11.0566-11.6550
		Medium at 1% of berries extract	80.3844*-81.0987*	0.18753-0.3988	.000	79.7839-79.8214	80.9850-82.3760
	Medium at 0.1% of berries extract **VS**	Medium at 1% of berries extract	69.9283*-70.7209*	0.18753-0.3988	.000	69.3278-69.4437	70.5289-71.9982

* The mean difference significant at the 0.05 level.

In addition to this reduction of the biomass related to the increasing of the extract content in the medium, a decreasing of the percentage in reduction of the Alamar Blue indicating the decreasing of surviving cells was noted (Figure 5).

This percentage in reduction of Alamar Blue which was 100% in the control medium for the three strains tested was decreased to reach the values of 6.11, 10.708 and 13.29% in the medium at 1% of berries extract after one day of incubation respectively for *A. nidulans*, *A. fumigatus* and *A. flavus*. From the first day to the fifth day of incubation, these percentages in reduction of Alamar Blue decreased also to reach the values of 3.58, 5.05and 6.11%in the medium at 1% of berries extract respectively for *A. nidulans*, *A. fumigatus* and *A. flavus* (Table 3).

Table 5: Effect of incubation time on the percentage in reduction of Alamar Blue for *A. fumigatus A. nidulans and A. flavus* grown in the medium at 0.01%, 0.1% and 1% of *Solanumindicum* L. green berries extract

	Homogeneous Tukey HSD		**Percentage in reduction of Alamar Blue**			
	Extract content in medium	N	Subste for alpha = 0.05			
			1	2	3	4
A fumigatus	Medium at 0.01% at dye 5	3	71.1720 ± 0.3637			
	Medium at 0.01% at dye 4	3	71.6979 ± 0.4326			
	Medium at 0.01% at dye 3	3		72.98009 ± 0.36589		
	Medium at 0.01% at dye 2	3			79.2238 ± 0.3637	
	Medium at 0.01% at dye 1	3				90.4655 ± 0.41527
	Significance		.461	1.000	1.000	1.000
	Medium at 0.1% at dye 5	3	59.8882 ± 0.1027			
	Medium at 0.1% at dye 4	3	60.9073 ± 0.721			
	Medium at 0.1% at dye 3	3		62.51809 ± 1.2407		
	Medium at 0.1% at dye 2	3			77.6605 ± 0.1479	
	Medium at 0.1% at dye 1	3				88.6804 ± 0.1689
	Significance		.254	1.000	1.000	1.000
	Medium at 1% at dye 5	3	5.0522 ± 0.1993			
	Medium at 1% at dye 4	3	5.3809 ± 0.2149			
	Medium at 1% at dye 3	3	6.1699 ± 0.2149	6.16989 ± 0.2149		
	Medium at 1% at dye 2	3		8.2640 ± 0.2373		
	Medium at 1% at dye 1	3			13.2889 ± 2.0307	
	Significance		.599	.113	1.000	
A.nidulans	Medium at 0.01% at dye 5	3	74.884 ± 0.0597			
	Medium at 0.01% at dye 4	3	75.1903 ± 0.2348			
	Medium at 0.01% at dye 3	3		77.6561 ± 0.0632		
	Medium at 0.01% at dye 2	3			81.9230 ± 0.8738	
	Medium at 0.01% at dye 1	3				91.0496 ± 0.5843
	Significance		.932	1.000	1.000	1.000
	Medium at 0.1% at dye 5	3	61.5519 ± 0.3112			
	Medium at 0.1% at dye 4	3	62.2020 ± 0.5139	62.2020 ± 0.5139		
	Medium at 0.1% at dye 3	3		65.0187 ± 0.2131		
	Medium at 0.1% at dye 2	3		65.6204 ± 2.5147	65.6204 ± 2.5147	
	Medium at 0.1% at dye 1	3			68.8027 ± 1.1941	
	Significance		.968	.051	.072	
	Medium at 1% at dye 5	3	3.5805 ± 0.16039			
	Medium at 1% at dye 4	3	4.6130 ± 0.6286			
	Medium at 1% at dye 3	3		6.2017 ± 0.1208		
	Medium at 1% at dye 2	3		6.5558 ± 0.5688		
	Medium at 1% at dye 1	3		6.9222 ± 0.1463		
	Significance		.57	.243		
A flavus	Medium at 0.01% at dye 5	3	87.2129 ± 0.8667			
	Medium at 0.01% at dye 4	3	87.7910 ± 0.4952			
	Medium at 0.01% at dye 3	3		89.7822 ± 0.3062		
	Medium at 0.01% at dye 2	3		91.0931 ± 0.1683		
	Medium at 0.01% at dye 1	3			94.2234 ± 0.3512	
	Significance		.629	.056	1.000	
	Medium at 0.1% at dye 5	3	76.8352 ± 0.4451			
	Medium at 0.1% at dye 4	3	77.3811 ± 0.0493			
	Medium at 0.1% at dye 3	3		80.6371 ± 0.1645		
	Medium at 0.1% at dye 2	3		80.9461 ± 0.2780		
	Medium at 0.1% at dye 1	3			84.7607 ± 1.1268	
	Significance		.756	.958	1.000	
	Medium at 1% at dye 5	3	6.1142 ± 0.0718			
	Medium at 1% at dye 4	3	6.8850 ± 0.3074			
	Medium at 1% at dye 3	3		8.0412 ± 0.5885		
	Medium at 1% at dye 2	3			10.5347 ± 0.2466	
	Medium at 1% at dye 1	3			10.7087	
	Significance		.145	1.000	.974	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3000.

It was noted that the reduction of cell viability was influenced significantly by the extract content in medium ((P<0.05; Table 4).This percentage of reduced Alamar Blue indicating cell viability was also influenced

significantly by the incubation time (P<0.05; Table 5). However, from the fourth to the fifth day of incubation (Table 5), no significance difference was observed between these percentages of reduced Alamar Blue whatever the *Aspergillus* species tested (P>0.05).

4. Discussion

In this study, the effect of the ethanolic extract of *Solanumindicum* L. green berries on fungal growth and their cell viability was recorded. A significant reduction of the radial growth and the biomass of the three tested species(*A. flavus*, *A. fumigatus* and *A. nidulans*) was noted with the increasing of the extract content in the medium. The highest reductions of the radial growth and the RNA concentration representing the biomass were observed in the medium containing 1% of berries extract. At this concentration of 1 % of berries extract, an absence of growth and conidia germination was observed for *A. fumigatus* and *A. nidulans*. This absence of growth noted for these two species, could be explained by this inhibition of conidia germination. In addition, in this medium at 1 % of extract, the RNA extracted seems to be damaged as no band was observed on the Formaldehyde Agarose Gel Electrophoresis for the three species tested. This shows the effective inhibitory effect of the green berries extract on the proliferation of the three species tested. However, the more sensitive species to the green berries extract was *A. nidulans.* Indeed, the mean of RNA concentration observed in the medium without extract was about 285 times that observed in the medium at 1% of berries extract for this species, while, for *A. fumigatus*and *A. flavus*, the mean of RNA concentrations observed in the medium without extract were respectively about 73 and 57 times that observed in the medium at 1% of berries extract.In opposite to these results, the experiments carried out [24] on *Solanumindicum* L. leaves didn't show any antifungal activity whatever the extraction solvent used. Similar results were obtained [25]. Thus, the antifungal activity in this wild plant seems to be located in its berries.

The research of antifungal activity carried out on the leaves of *Solanumnigrum* L., another wild plant from the family of *Solanaceae*, showed a poor antifungal activity [26]. This reduction of the biomass when the extract content was increased in the medium could be explained by the death of the fungal cells. Indeed, the more the extract 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 berries extract which was the highest concentration of berries extract tested in this study. Indeed, from the first to the fifth day of incubation, the lowest percentages of reduced Alamar Blue were 6.92 to 3.58%, 13.28 to 5.05% and 10.71 to 6.11% respectively for *A. nidulans, A. fumigatus* and *A. flavus* in this medium at 1 % of extract.

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 0.01% of berries extract. This indicates that the minimum killing concentration could beat this value.

5. Conclusion

This investigation revealed that *Solanumindicum* L. Green berries possess significant antifungal activities due to their inhibitory effect on fungi proliferation and their capacity of killing them. It highlights the discovery of natural substances for the research for alternative in chemical fungicides that inhibit fungal growth without killing them. For many years now, these berries were 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, *Solanumindicum* L. green berries extract can be used. This inhibitory effect of these berries could be due to their richness in polyphenols. Further research must be carried out on the polyphenols of these berries in order to confirm this fact.

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