

Effect of Fluorescent and UV Light on Mycotoxin Production under Different Relative Humidities in Wheat Grains

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Abstract

UV and fluorescent light were investigated for their ability to detoxify mycotoxins or inhibit the production of mycotoxins in wheat grains to avoid their hazardous effect. Four fungal isolates known to be highly toxin producers, i.e., *Aspergillus parasiticus*, *Fusarium verticillioides*, *Scopulariopsis fusca* and *Verticillium lecanii* were grown on wheat grains. The inoculated wheat grains were exposed to fluorescent light, short and long UV and stored for three weeks under different relative humidities (50-80%) at room temperature. Some mycotoxins were completely eliminated while others varied in their concentrations depending on relative humidity and period of illumination. Also isolates varied in their sensitivity to illumination at any exposure period.

Key words: Fungi; Mycotoxins; Light; Relative humidity; Wheat grains.

Introduction

Few studies have dealt with the effects of light on mycotoxin production. The use of any applicable treatment conditions should not cause undesirable alterations to the nutritional and organoleptic qualities of foods (Samarajeewa *et al.*, 1990).

Aflatoxin B₁ absorbs ultraviolet (UV) radiation at 222, 265 and 362 nm with the greatest absorption at 362 nm. Irradiation at 362 nm activates AFB₁ and increases its susceptibility to degradation. AFB₁ is reported to be highly sensitive to UV radiation at a pH of less than 3 or greater than 10 (Lillard and Lantin, 1970).

Bennett *et al.* (1981) examined the effects of continuous light and continuous darkness on the production of aflatoxin and three polyhydroxyanthraquinones (averufin, versicolorin A and C) by *Aspergillus parasiticus*. No growth was observed at 15°C in the light. No aflatoxin or anthraquinones were produced in the light or dark at 35 and 40°C, although growth was good at these temperatures.

Loss of the toxin and development of reaction products in aqueous solutions were monitored during the treatment with 365 nm, low energy UV irradiation (Yousef and Marth, 1987). Aziz

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and Smyk (2002) found that near-UV radiation and nitrosamines had a mutagenic effect on the induction of aflatoxins and ochratoxin A synthesis by nontoxigenic moulds. The aim of the present work is to study the effect of UV and fluorescent light on mycotoxin production by different fungal isolates.

Materials and Methods

Preparation of mycotoxin standard solutions

Aflatoxin B₁ and ochratoxin A and B were brought from Fluka AG, Buchs SG, Switzerland. Aflatoxin B₂, G₁ and G₂, zearalenone, sterigmatocystin, nivalenol, deoxynivalenol and T-2 toxin were brought from Sigma Chemical Co., St. Louis, USA.

For aflatoxins, 1 mg was dissolved in methanol and diluted to the desired concentration.

For ochratoxins, original solution (ca 40 µg/ml) was prepared in glacial acetic acid-benzene (1+99).

For sterigmatocystin and zearalenone, original solution (ca 20 µg/ml) was prepared in benzene (Stoloff *et al.*, 1971).

For deoxynivalenol (DON), nivalenol and T-2 toxin, original solution (ca 20 µg/ml) was prepared in methanol.

Moisture content of wheat grains

A known weight of wheat grains was heated at 130° C up to a constant weight. The moisture content was calculated on dry weight basis according to the method of Helrich (1990). The initial moisture content was found to be 13%.

Mycotoxin extraction from wheat grains

a- Extraction of aflatoxins, ochratoxins, sterigmatocystin and zearalenone. The extraction method of Stoloff *et al.* (1971) was used. 25 g of wheat grains were transferred to a 250 ml glass-stoppered Erlenmeyer flask and 90 ml CH₃CN and 10 ml (4 %) KCl solution were added. After shaking for 10 min. the mixture was filtered and 50 ml isooctane was added to 100 ml filtrate. This was repeated three times. 25 ml water was added to the CH₃CN extract followed by 50 ml CHCl₃ and the mixture was shaken. The CHCl₃ extract was separated three times with 10 ml portions of CHCl₃. The CHCl₃ extract was evaporated and dried under vacuum. 5 ml Benzene- CH₃CN (98+2) was added to make an extract in solution for TLC and subsequent analysis..

b- Extraction of nivalenol, deoxynivalenol and T-2 toxin. The extraction method of Luo *et al.* (1990) was used. 25 g of finely ground wheat grains were extracted with 100 ml of acetonitrile-water (3:1). After shaking for 30 min., the filtrate was defatted with n-hexane, followed by concentration to dryness. The residue was dissolved in 5 ml methanol.

Determination of mycotoxins

HPLC assay. The HPLC system used included a Shimadzu model LC-9A liquid chromatograph with a Shimadzu SPD-MGA photodiode array UV-via detector (Shimadzu corporation). A reverse phase Ultrasphere ODS C18 column was used (25cm X 4.6 mm, 5 µm particle size) with a 4.5 cm X 4.6 mm guard column of identical composition for DON, zearalenone and T-2 toxin. The injection volume was 20 µl.

For DON, a gradient separation was used. The mobile phase was initially 15 % methanol in water, and was increased to 22 % over a 15 min. period in a linear fashion. The flow rate was 1.2 ml min.⁻¹ Detection was at 220 nm. For zearalenone, a gradient separation was also used.

The mobile phase was 70 % methanol in water, which was maintained for 15 min. and then the methanol level was increased to 100 % over 1 min. period in a linear gradient. The flow rate was 1.2 ml min⁻¹ and detection was at 236 nm. An isocratic separation was used for T-2 toxin and was detected at 200 nm. (Stratton *et al.*, 1993).

For aflatoxins and ochratoxins, Apex ODS C18 column was used (25cm X 4.6 mm, 5 µm particle size). A gradient separation was used. The mobile phase was acetonitrile : water : acetic acid glacial (45 : 55 : 2). The flow rate was 1 ml min⁻¹. Detection was at 254 and 365 nm (Engstrom *et al.*, 1977).

For sterigmatocystin, a cyano column and gradient separation were used. The mobile phase was n-hexane : chloroform : acetic acid glacial (65 : 35 : 1). The flow rate was 2 ml min⁻¹. Detection was at 254 nm (Kingston and Chen, 1976).

For nivalenol, column 5 C28 and gradient separation were used. The mobile phase was water: tetrahydrofuran (76 : 24). The flow rate was 0.5 ul/min. Detection was at 224 nm. (Lanin and Nikitin, 1991)

Mycotoxin detection limits were determined by dilution, *i.e.* a standard was prepared using mobile phase solvent and serially diluted to the point where there was no detectable response. Detection limits were defined here as the analytical limit of detection for the equipment and operating parameters used.

Effect of Light on mycotoxins production under different relative humidities

The wheat grains were inoculated with four fungal isolates that proved to be highly toxin producer (Atalla *et al.*, 2003), *i.e.* *A. parasiticus*, *Fusarium verticillioides*, *Scopulariopsis fusca* and *Verticillium lecanii* at a concentration of 1 ml spore suspension/100 g. The inoculated wheat grains were irradiated with short and long UV. (with UV lamp Model UVSL-25, 220 volt, 0.12 Amps, Ultra Violet products, Inc. San Gabriel, California, USA) and fluorescent tube (30 watt). The grains were irradiated at a distance of 5 cm from the UV lamp for 30, 60 and 120 min respectively while at a distance of 25 cm from the fluorescent tube for the same periods. The irradiated wheat grains were stored in different relative humidities (50, 74 and 80 %) at room temperature as mentioned by Rambo *et al.* (1975), Atalla (1978) and Atalla *et al.* (1999). Mycotoxins were assayed after 3 weeks using HPLC. Data were analyzed statistically according to the simple factorial method (LSD).

Results and Discussion

The use of any applicable treatment conditions should not cause undesirable alterations to the nutritional and organoleptic qualities of foods (Samarajeewa *et al.*, 1990).

Few studies, however, have dealt with the effects of light on mycotoxin production. In the present study, wheat grains were inoculated with mycotoxin producing fungi exposed to two different illumination sources varying in wave length for different periods of time and stored at different relative humidities.

The mycotoxin producing fungi were found to react in a varying way regarding the production of mycotoxins as seen in Tables 1-12. Aflatoxins, ochratoxins, sterigmatocystin and zearalenone production were eliminated on exposure to UV short, long wave or fluorescent light for 30 min. at different relative humidities specially with *A. parasiticus* and in some cases completely inhibited after 60 min exposure to light as in case of *Aspergillus parasiticus*. *Fusarium verticillioides* became unable to produce T-2 toxin at 30 min. exposure to any source of light.

As for wheat grains inoculated with *Scopulariopsis fusca*, treatment with UV short wave at different relative humidities for different duration periods resulted in reduction in mycotoxins

concentration. On exposure to UV short wave for 30 and 60 min at relative humidity 50 and 74%, aflatoxin G₁ and ochratoxin B were completely eliminated. While most other mycotoxins were reduced at different levels, e.g. zearalenone was reduced after 30 min by 87 %. Longevity of exposure time led to a decrease in mycotoxin production e.g aflatoxin G₂ showed its lowest concentration after 60 min. (3.06 mg/kg) while DON and T-2 were lower after 120 min. (0.04 and 0.48 mg/kg). On treatment of wheat grains with fluorescent light at relative humidities (50, 74 and 80%) for different durations, mycotoxins production were greatly affected. Aflatoxin G₁, ochratoxin B, and T-2 toxin were absent on treatment for 30 min. while on using UV long wave for different periods of time at different relative humidities 50, 74 and 80 %. Aflatoxin G₁ and ochratoxin B were completely absent after treatment for 30 and 60 min. Aflatoxin B₂ was decreased on treatment for 120 min. (2, 1.05 and 0.79 mg/kg) at different relative humidities. DON was decreased on treatment for 30 min. (0.02, 0.02 and 0.01 mg/kg) at different relative humidities. Both aflatoxin B₁ and ochratoxin B were absent under all circumstances in this treatment.

Regarding wheat grains inoculated with *Verticillium lecanii*, none of aflatoxins B₁, B₂, ochratoxin B and ZEA were detected after 30 min. exposure to UV light, while sterigmatocystin and ZEA were omitted after 60 min. exposure. When the grains were exposed to fluorescent light, both aflatoxin B₁ and ochratoxin B were eliminated after 30 min. Other toxins as DON, T-2 toxin and NIV reduced greatly by exposure of grains at prolonged periods of time (120 min.) to UV or fluorescent light. The difference is highly significant.

Presence or absence of toxins after wheat grains were exposed to fluorescent light or irradiated under UV short or long wave may be attributed to chemical changes in the toxins which alters the known formula of the toxin. Such results are in accordance with those of many previous investigators. Bennett *et al.* (1981) found that aflatoxins, versicolorins and averufin arise biosynthetically by a polyketide pathway. The earliest precursors were acetyl and malonyl units. Both averufin and versicolorin A are experimentally identified precursors of aflatoxin B₁. Therefore, the coordinate mediated increase or decrease of aflatoxins probably reflects a light response that affects the whole polyketide pathway.

Carlile (1971) has hypothesized that an early step may show increased or decreased activity in one or more of the electron transfer pathways because respiratory enzymes commonly have light absorbing prosthetic groups. Thus, the small, light sensitive changes in levels of polyketide compounds are most likely a secondary effect of changes in primary metabolism. These changes alter or redirect the precursor pool of acetyl and malonyl coenzyme A units.

Irradiation at 362 nm activates aflatoxin B₁ and increases its susceptibility to degradation. Aflatoxin B₁ is reported to be highly sensitive to UV radiation affecting the structure of the terminal furan ring and thus eliminating the active binding site. Oxygen appears to enhance UV-mediated free radical degradation of aflatoxin crystal (Lillard and Lantin, 1970). The production of 12 new aflatoxin degradation compounds after the UV irradiation of aflatoxin B₁ suggests that a series of reactions occurs during aflatoxin B₁ breakdown. Kinetic studies on the degradation of aflatoxins indicate a first order reaction with possible photodimerization of the coumarin molecule (Aibara and Yamagishi, 1968).

On the other hand Aziz and Moussa (1997) reported that only a low percentage of aflatoxin degradation had been observed after the exposure of foods to light from fluorescent or incandescent bulbs. The exposure to fluorescent light increased mycelial dry weight and mycotoxin production.

In the present investigation, some toxins were found to increase after exposure to UV or fluorescent light while others decreased, also isolates varied in their sensitivity to illumination at any exposure time under the different relative humidities. These observations might be

attributed to the effect of light as mutagenic agent, which was reflected on the toxin production ability of the isolate.

A. parasiticus became a highly toxin producer when exposed to UV or fluorescent light especially for DON, NIV and T-2 toxin under the effect of UV short wave. *F. verticillioides* was highly sensitive to illumination, except when exposed to UV long wave. Both aflatoxin G₂, ochratoxin A and NIV were produced in large quantities. The different sources of light used altered toxin production ability of *Scopulariopsis fusca* against aflatoxins G₂, B₂, ochratoxin A, NIV, ZEA and T-2 toxin. The isolate changed to highly toxic organism. UV short wave and fluorescent light enhanced *Verticillium lecanii* towards T-2 toxin and NIV production as well as UV long wave effects towards aflatoxin B₂ and G₂ at the different relative humidities. These observations coincide with Aziz *et al.* (1997) results on the effect of gamma irradiation on the natural occurrence of *Fusarium* mycotoxins.

Table 1. Effect of UV short wave (254 nm) on mycotoxin production by *Aspergillus parasiticus* under different relative humidities.

Light source	R.H. %	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxin		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
UV short wave (254 nm)	50	30	0	0	0	0	0	0.05	0	0	0.36	0.60	9.78
		60	0	0	0	0	0	0	0	0	11.8	0.09	4.97
		120	0	0	0	0	0.58	2.58	4.18	0	0.16	0.09	0.17
		Control	0.10	0.43	0.19	1.02	0.01	0.08	0	0.01	0.12	0.08	0
	74	30	0	0	0	0	0	0.02	0	0	0	0.10	0.34
		60	0	0	0	0	0	0	0	0	1.18	0.02	5.71
		120	0.14	0	0	0	0.73	0	2.45	0	3.08	0.40	6.35
		Control	0.06	0.06	0.11	0.33	0.27	0.03	0.08	0.01	0.06	0.12	29.2
	80	30	0	0	0	0	0	0.06	0	0	0	0.48	0
		60	0	0	0	0	0	0	0	0	9.45	0.01	8.60
		120	0.20	0	0.71	4.11	0.67	2.07	0.95	0	0.10	0.20	6.42
		Control	0.09	0	0.13	0	0.02	0.10	0.05	0.02	0	2.77	20.2
L.S.D.	1%		0.03	0.04	0.07	0.37	0.10	0.29	0.43	0.00	1.33	0.10	3.41
	5%		0.02	0.03	0.04	0.25	0.07	0.20	0.29	0.00	0.92	0.07	2.35

Table 2. Effect of Fluorescent light on mycotoxin production by *Aspergillus parasiticus* under different relative humidities.

Light source	R.H. %	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxi		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
Fluorescent	50	30	0	0	0	0	0	0	0	0	2.12	0.70	9.11
		60	0	0.06	0.15	0.64	0.43	0	0.04	0	3.44	0.08	0.63
		120	0	0	0	0	0.61	0	2.59	0	1.04	0.07	0
		Control	0.10	0.43	0.19	1.02	0.01	0.08	0	0.01	0.12	0.08	0
	74	30	0	0	0	0	0	0	0	0	0.06	0.34	0
		60	0	0.05	0	2.80	4.11	0	0.03	0	0.65	0.07	0.26
		120	0	0	0	0	0.67	0	2.83	0	0.40	0.10	0.14
		Control	0.06	0.06	0.11	0.33	0.27	0.03	0.08	0.01	0.06	0.12	29.2
	80	30	0	0	0	0	0	0	0	0	0	0.52	18.2
		60	0	0.06	0.47	5.75	0.60	0.19	0.01	0	4.98	0.06	0.47
		120	0.04	0	0.33	0	0.58	2.52	2.73	0	0.90	0.43	0
		Control	0.09	0	0.13	0	0.02	0.10	0.05	0.02	0	2.77	20.2
L.S.D.	1%		0.01	0.04	0.06	0.56	0.37	0.22	0.41	0.00	0.57	0.26	3.53
	5%		0.01	0.03	0.04	0.39	0.26	0.15	0.28	0.00	0.39	0.18	2.43

Table 3. Effect of UV long wave (362 nm) on mycotoxin production by *Aspergillus parasiticus* under different relative humidities.

Light source	R.H . %	Duration time min.	Mycotoxins (mg/kg)											
			Aflatoxin				Ochratoxi n		Ster	ZE A	NIV	DON	T-2	
			B ₁	B ₂	G ₁	G ₂	A	B						
UV long wave (362 nm)	50	30	0	0	0	0	0	0	0	0	0	0.07	0.82	0
		60	0	0.06	1.25	0.45	0.41	1.83	0.22	0	3.44	0.48	0	
		120	0.22	0	0.67	4.37	0.26	0.76	1.82	0	4.36	0.56	0	
		Control	0.10	0.43	0.19	1.02	0.01	0.08	0	0.01	0.12	0.08	0	
	74	30	0	0	0	0	0.08	0	0	0	0.60	0.62	0	
		60	0	0.07	1.15	3.02	2.57	1.20	0.25	0	7.28	0.15	0.58	
		120	0.09	0	0.30	12.2	0.01	0	1.34	0	0.53	0.18	0	
		Control	0.06	0.06	0.11	0.33	0.27	0.03	0.08	0.01	0.06	0.12	29.2	
	80	30	0	0	0	0	0.04	0	0	0	0.46	0.83	0	
		60	0	0.10	2.90	0.84	0	0.84	0.65	0	0.38	0.40	0.39	
		120	0	0	0.71	0	0.33	1.90	1.25	0	0.81	0.21	0.41	
		Control	0.09	0	0.13	0	0.02	0.10	0.05	0.02	0	2.77	20.2	
L.S.D.	1%		0.02	0.04	0.04	1.16	0.23	0.27	0.23	0.00	0.80	0.28	3.07	
	5%		0.02	0.03	0.03	0.80	0.16	0.18	0.16	0.00	0.55	0.19	2.10	

Table 4. Effect of UV short wave (254 nm) on mycotoxins production by *Fusarium verticillioides* under different relative humidities.

Light source	R.H. %	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxin		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
UV short wave (254 nm)	50	30	0.09	0.06	0	0.76	0	0	0	0	0.46	0.16	0
		60	0.14	0	0	0	0	0	0	0	4.07	0.05	0
		120	0	0	0	0	0	0	0	0	0	0	0
		Control	0.14	0.06	0.25	0.82	0.07	0.12	0	0.01	0.07	1.69	6.9
	74	30	0.05	0	0	0.57	0	0	0	0.38	0.49	0.05	0
		60	0.10	0.07	0.32	0.68	0	0	0.33	0	2.65	0.08	0
		120	0	0	0	0	0	0	0	0	0	0	0
		Control	0.30	0.12	0.12	0.86	0.07	0	0.01	0	0.15	0.08	4.49
	80	30	0	0.06	0	4.43	0	0	0	0	0.11	0	0
		60	0.40	0.03	0.32	2.00	0	0	0	0	0	0	0
		120	0.16	0	0	0.63	0	0	0	0	0	0	0
		Control	0.10	1.86	1.14	0.86	0.08	0.09	0.03	0	0.07	0.55	12.9
L.S.D.	1%		0.05	0.16	0.11	0.45	0.01	0.01	0.03	0.03	0.42	0.15	1.32
	5%		0.04	0.11	0.07	0.31	0.01	0.01	0.02	0.02	0.29	0.11	0.91

Table 5. Effect of fluorescent light on mycotoxins production by *Fusarium verticillioides* under different relative humidities.

Light source	R.H. %	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxi		Ster	ZE A	NI V	DO N	T-2
			B ₁	B ₂	G ₁	G ₂	n						
Fluorescent	50	30	0.0 6	0.0 7	0	0.6 2	0.7 5	0	0	0	0	0.08	0
		60	0.8 0	0	0.2 4	1.0 1	0	0	0.8 6	0	0	0	0
		120	0.1 8	0.0 1	0	0.2 5	0	0	0	0	0	0	0
		Control	0.1 4	0.0 6	0.2 5	0.8 2	0.0 7	0.1 2	0	0.0 1	0.0 7	1.69	6.9
	74	30	0	0.0 8	0	0.4 1	1.0 5	0	0	0	0	0.04	0
		60	0	0	0.1 4	0.9 6	0	0	1.3 5	0.2 4	2.0 7	0	0
		120	0.1 3	0.0 1	0	1.0 6	0.6 6	0	0	0	1.6 4	0	0
		Control	0.3 0	0.1 2	0.1 2	0.8 6	0.0 7	0	0.0 1	0	0.1 5	0.08	4.4 9
	80	30	0.1 6	0.2 2	0	0.3 1	0.1 9	0	0	0.0 5	0.0 8	0.09	0
		60	0.0 5	0.2 2	0.1 6	1.3 1	0	0	1.1 5	0	3.4 2	0.10	0
		120	0	0	0	5.9 2	0	0	0	0	0.8 1	0	0
		Control	0.1 0	1.8 6	1.1 4	0.8 6	0.0 8	0.0 9	0.0 3	0	0.0 7	0.55	12. 9
L.S.D.	1%	0.0 8	0.1 6	0.1 1	0.5 6	0.1 3	0.0 1	0.1 7	0.0 2	0.3 8	0.15	1.3 2	
	5%	0.0 6	0.1 1	0.0 7	0.3 9	0.0 9	0.0 1	0.1 2	0.0 2	0.2 6	0.11	0.9 1	

Table 6. Effect of UV long wave (362 nm) on mycotoxins production by *Fusarium verticillioides* under different relative humidities.

Light source	R.H .%	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxi n		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
UV long wave (362 nm)	50	30	0.08	0.11	0	1.55	1.14	0	0	0	0	0.01	0
		60	0.38	0.02	1.49	0	1.07	0	0.80	0	2.66	0.13	0
		120	0.11	0	0	6.90	0.96	0	0	0	2.39	0	0
		Control	0.14	0.06	0.25	0.82	0.07	0.12	0	0.01	0.07	1.69	6.9
	74	30	0.03	0.04	0	0.75	0.44	0	0	0	0	0.01	0.17
		60	0.10	0.02	0.07	1.27	0	0	0	0	0	0	0
		120	0.08	0	0	4.97	1.02	0	0	0	2.54	0	0
		Control	0.30	0.12	0.12	0.86	0.07	0	0.01	0	0.15	0.08	4.49
	80	30	0.13	0	0	0.82	0.60	0	0	0	0	0.01	0.06
		60	0.04	0.05	0.16	2.05	0	0	0	0	0	0	0
		120	0	0	0	0	0	0	0	0	0.43	0.10	0
		Control	0.10	1.86	1.14	0.86	0.08	0.09	0.03	0	0.07	0.55	12.9
	L.S.D.	1%		0.05	0.16	0.6	0.79	0.19	0.01	0.07	0.00	0.38	0.15
5%			0.03	0.11	0.11	0.54	0.13	0.01	0.05	0.00	0.26	0.11	0.91

Table 7. Effect of UV short wave (254 nm) on mycotoxins production by *Scopulariopsis fusca* under different relative humidities.

Light source	R.H. %	Duration time (min)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxin		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
UV short wave (254 nm)	50	30	0	1.65	0	9.96	6.64	0	0	0.79	5.75	0.10	67.3
		60	0	0	2.66	3.06	0	0	0	1.12	0	0.06	1.25
		120	0	1.83	0	7.69	0	0	31.2	0.99	0	0.04	0.48
		Control	0	6.20	0.28	0.28	0.01	0.61	0.02	6.18	0.92	0.04	0
	74	30	0	2.04	0	11.6	5.91	0	29.8	1.69	5.96	0.02	55.4
		60	0	4.11	3.56	16.4	5.87	0	0	2.83	0	0.19	2.64
		120	0	1.40	0	0	0	0	22.2	0.68	0	0.23	0
		Control	0	7.63	0.35	0.21	0.01	0.17	0.07	2.50	0.06	0.06	0
	80	30	0	3.59	0	16.8	5.97	0	28.5	4.81	4.22	0.02	34.2
		60	0	1.21	0	5.42	5.59	0	20.1	0.85	0	0.24	3.21
		120	0	1.91	0	15.0	16.9	0	14.4	1.24	0	0.07	0.04
		Control	0	6.10	0.18	0.2	0.01	1.4	0.02	4.1	0.08	14.3	0
L.S.D.	1%		0	1.37	0.47	5.13	2.31	0.13	3.63	0.87	1.39	1.23	3.28
	5%		0	0.94	0.33	3.52	1.59	0.09	2.49	0.60	0.96	0.85	2.25

Table 8. Effect of fluorescent light on mycotoxins production by *Scopulariopsis fusca* under different relative humidities.

Light source	R.H. %	Duration time (min)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxin		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
Fluorescent	50	30	0	2.51	0	10.6	7.02	0	26.9	1.52	5.48	0.01	0
		60	0	3.38	0.08	5.07	4.77	0	0.05	0	0	0.04	2.60
		120	0	1.50	0	1.36	0	0	2.27	0.12	0.04	0.01	0.05
		Control	0	6.2	0.28	0.28	0.01	0.61	0.02	6.18	0.92	0.04	0
	74	30	0	1.82	0	19.1	3.26	0	1.25	1.63	8.84	0	15.1
		60	0	0	0	0	0	0	0	0	0	0	0.06
		120	0	0.78	4.12	10.1	0	0	4.61	0	0	0.05	0
		Control	0	7.63	0.35	0.21	0.01	0.17	0.07	2.5	0.06	0.06	0
	80	30	0	1.70	0	16.9	14.5	0	0	3.15	6.41	0.22	31.0
		60	0	2.84	0.07	22.0	3.04	0	1.38	0	0	0.04	0
		120	0	0.75	0	0.92	0	0	0.11	0	0.22	0.07	0.09
		Control	0	6.10	0.18	0.2	0.01	1.4	0.02	4.1	0.08	14.3	0
L.S.D.	1%		0	1.12	0.36	3.20	1.50	0.13	2.37	0.75	1.06	1.23	2.98
	5%		0	0.77	0.25	2.20	1.03	0.09	1.63	0.52	0.73	0.85	2.05

Table 9. Effect of UV long wave (362 nm) on mycotoxins production by *Scopulariopsis fusca* under different relative humidities.

Light source	R.H. %	Duration time (min)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxin		Ster	ZEA	NI V	DO N	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
UV long wave (362 nm)	50	30	0	3.41	0	26.1	11.3	0	0	3.52	6.9	0.0	16.6
		60	0	4.35	0	22.3	9.74	0	0	0.60	0	0.0	0.0
		120	0	2.00	0.77	7.57	14.7	0	17.5	0.31	0	0.0	0.0
		Control	0	6.20	0.28	0.28	0.01	0.61	0.0	6.18	0.9	0.0	0
	74	30	0	2.33	0	18.5	2.25	0	0.1	3.88	13.4	0.0	0.1
		60	0	3.81	0	10.8	8.11	0	0	1.65	0	0.1	0.0
		120	0	1.05	0.11	17	0	0	28.4	1.37	0	0.0	34.2
		Control	0	7.63	0.35	0.21	0.01	0.17	0.0	2.50	0.0	0.0	0
	80	30	0	3.62	0	17.6	10.6	0	0.0	1.59	5.7	0.0	0
		60	0	6.92	0	31.3	9.86	0	0	2.14	0.2	0.0	0.5
		120	0	0.79	5.42	15.9	0	0	25.5	0	0	0.0	0.0
		Control	0	6.10	0.18	0.20	0.01	1.4	0.0	4.1	0.0	14.8	0
L.S.D.	1%		0	1.37	0.47	5.12	2.31	0.13	3.6	0.87	1.3	1.2	3.2
	5%		0	0.94	0.33	3.52	1.59	0.09	2.4	0.60	0.9	0.8	2.2

Table 10. Effect of UV short wave (254 nm) on mycotoxins production by *Verticillium lecanii* under different relative humidities.

Light source	R.H .%	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxi		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
UV short wave (254 nm)	50	30	0	0	0.08	0.67	0.06	0	0.69	0	0.05	0.73	0.44
		60	0.10	0.02	0.40	0.89	0.77	0	0	0.02	0.43	0.43	10.1
		120	0	0	0	0.59	0.40	0.02	0.12	0.05	0.33	0.01	1.61
		Control	0	0.03	0.68	1.00	0.80	0.12	0.60	0.02	0.71	0.8	0.9
	74	30	0	0	0.59	0.46	0.06	0	0.90	0	0.10	0	0
		60	0.09	0.06	0.65	0	0.45	0	0.06	0.01	0.86	0.20	7.05
		120	0	0.04	0	0.18	0.17	0	0.06	0.02	1.71	0.01	0.80
		Control	0.06	0.04	0.9	1.43	1.7	0.06	0.90	0.02	0.04	1.2	1.01
	80	30	0	0.03	0.24	0	0.09	0	0.52	0.01	0.52	0.19	1.51
		60	0.06	0.04	0.51	1.75	0.10	0	0.04	0	0.16	0.02	1.90
		120	0	0.05	0	0	0.19	0	0.12	0.01	0.13	0.18	2.01
		Control	0.11	0.05	0.89	6.12	0.80	0.11	1.02	0.02	0.21	1.17	1.37
L.S.D.	1%		0.02	0.02	0.16	0.58	0.20	0.02	0.17	0.01	0.19	0.18	1.12
	5%		0.01	0.01	0.11	0.40	0.14	0.01	0.12	0.00	0.13	0.12	0.77

Table 11. Effect of fluorescent light on mycotoxins production by *Verticillium lecanii* under different relative humidities.

Light source	R.H. %	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxi n		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	A	B					
Fluorescent	50	30	0	0	0.11	0	0.32	0	0.37	0.01	0.07	0.02	0.78
		60	0.08	0.02	0.54	0.18	0.21	0	0.12	0.03	0.30	0.01	0.38
		120	0	0.06	0	0	0.14	0.14	0.08	0.01	0.75	0.01	0.78
		Control	0	0.03	0.68	1.00	0.80	0.12	0.60	0.02	0.71	0.8	0.9
	74	30	0	0.01	0.10	1.94	0.55	0	0.40	0.01	0.05	0	1.26
		60	0.04	0.12	0.33	2.22	0.74	0	0.08	0.02	0.60	0.02	6.98
		120	0	0.04	0	0.21	0	0	0	0.01	0	0	3.46
		Control	0.06	0.04	0.9	1.43	1.7	0.06	0.90	0.02	0.04	1.2	1.01
	80	30	0	0.04	0.64	0.72	0	0	0.61	0.01	0.31	0	0
		60	0.14	0.08	0.27	0.40	0	0	0.03	0.01	0.05	0	1.26
		120	0	0.03	0	0.30	0	0.06	0.08	0	0.28	0.02	0.31
		Control	0.11	0.05	0.89	6.12	0.80	0.11	1.02	0.02	0.21	1.17	1.37
L.S.D.	1%		0.02	0.02	0.15	0.61	0.20	0.02	0.15	0.01	0.60	0.16	0.72
	5%		0.01	0.01	0.10	0.42	0.14	0.01	0.10	0.01	0.41	0.11	0.49

Table 12. Effect of UV long wave (362 nm) on mycotoxins production by *Verticillium lecanii* under different relative humidities.

Light source	R.H. %	Duration time (min.)	Mycotoxins (mg/kg)										
			Aflatoxin				Ochratoxi		Ster	ZE A	NIV	DON	T-2
			B ₁	B ₂	G ₁	G ₂	n	A					
UV long wave (362 nm)	50	30	0	0.03	0	1.04	0.40	0	0.05	0.02	0.38	1.01	0.95
		60	0.06	0.06	0.15	0.63	0.56	0	0.05	0.02	0.28	0.02	1.76
		120	0	0.08	0	0.29	0	0.03	0.04	0.01	0.25	0	1.04
		Control	0	0.03	0.68	1.00	0.80	0.12	0.60	0.02	0.71	0.8	0.9
	74	30	0	0.03	0	1.96	0.34	0	0.67	0.01	0.34	1.57	0
		60	0.14	0.06	0.33	0.49	0.77	0	0.05	0.03	0.68	0.07	1.13
		120	0	0.05	0	0.42	0.01	0	0.05	0.02	0.15	0	0
		Control	0.06	0.04	0.9	1.43	1.7	0.06	0.90	0.02	0.04	1.2	1.01
	80	30	0	0.05	0	1.11	0	0	0.19	0.01	0.90	2.67	0
		60	0.05	0.07	0	0.21	0	0	0	0.01	0.50	0.01	6.56
		120	0	0	0	4.40	0	0	0	0.01	0.16	0.03	0.11
		Control	0.11	0.05	0.89	6.12	0.80	0.11	1.02	0.02	0.21	1.17	1.37
L.S.D.	1%		0.02	0.02	0.13	0.71	0.20	0.02	0.14	0.02	0.85	0.32	0.32
	5%		0.01	0.01	0.09	0.49	0.14	0.01	0.10	0.01	0.59	0.22	0.22

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