ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Impact of Mg^{2+}, Fe^{2+} and Co^{2+} Metal Ions on Growth and Ochratoxin A Production by Strains of *Aspergillus westerdijkiae* and *Aspergillus carbonarius.*

Yousef Sultan[a]*, Esther S Baxter[b], Giuseppe Salis[b], and Naresh Magan[b].

[a]Department of Food Toxins and Contaminants, National Research Centre, Dokki, Cairo 12622, Egypt.
[b]Applied Mycology Group, AgriFood Institute, Cranfield University, Cranfield, Bedford MK43 0AK, United Kingdom.

ABSTRACT

The efficacy of metal ions (150, 500, 1000 µM; Mg^{2+}, Fe^{2+} and Co^{2+}) on growth of 2 strains each *of Aspergillus westerdijkiae* and *Aspergillus carbonarius* and chratoxin A (OTA) production was studied on a conducive yeast extract sucrose agar medium (YES) at different water activity levels (a_w, 0.92, 0.97). A_w was the main factor affecting growth of the tested strains. In general, the growth of strains of *A. carbonarius* was faster than those of *A. westerdijkiae.* At 0.97 a_w the metal ion Co^{2+} inhibited growth of the tested strains, with the exception of a type strain of *A. westerdijkiae.* Addition of the metal ions had contrasting results on OTA production. Addition of metal ions was the main factor affecting OTA production. Mg^{2+}, notably at 1000 µM, promoted OTA production by the tested strains at both a_w levels. The metal ions Fe^{2+} and Co^{2+} had either no effect or inhibited OTA production at both a_w levels with exception of Co^{2+} at 1000 µM which significantly increased OTA production by the type strains of *A. westerdijkiae* at 0.92a_w.

Keywords: Metal ions, *Aspergillus westerdijkiae*, *Aspergillus carbonarius* and ochratoxin A

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Metals are an important part of all ecosystems. Some microelements are essential for the normal functions of organisms. Important processes are affected by the presence or absence of these essential metal ions, as they may be responsible for enzymatic reactions (Zn, Co, Ni, Mn, Fe, Cr, Al), glycolysis (Mn, Zn), nucleotide synthesis (Mg, Fe), erythropoesis (Fe, Cu), organic acid transformation (Fe, Zn, Ni, Mn), nitrogen exchange (Fe, Mo, Cu, Mn, V, Co) and photosynthesis (Fe, Ti, Mg, Mn) (Protasowicki, 2005). In fungi, the production of various antibiotics, hormones, and mycotoxins are significantly influenced by the presence of metals and other micronutrients in the growth media (Weinberg,1970).

Ochratoxin A (OTA) is an important mycotoxin which is a toxic secondary metabolite produced by several fungal species belonging to the genera *Aspergillus* and *Penicillium* (Samson et al., 2010). Many ecological factors affect OTA production by these species, including water activity (a_w), temperature and modified atmosphere (Mitchell et al., 2004; Cairns-Fuller et al., 2005; Pateraki et al., 2007; Natskoulis et al., 2009; Alborch et al., 2011), pH and type of substrates (Mühlencoert et al, 2004; Khalesi and Khatibb, 2011). Trace metals have also been shown to affect OTA production by *Aspergillus ochraceus* in synthetic medium (Steele et al., 1973; Mühlencoert et al., 2004). Zn^{2+}, Fe^{3+} and Mg^{2+} were shown to affect production of fusaricidin by *Paenibacillus polymyxa* in liquid culture (Raza et al., 2010). The effects of Zn^{2+}, Cu^{2+}, and Fe^{2+} on aflatoxin biosynthesis in *Aspergillus flavus* (Cuero et al, 2003) and on OTA production by *A. ochraceus* (Steele et al., 1973) were reported although a_w was not taken into account. The effect of Mg^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{2+} on 3-Acetyldeoxynivalenol (3-ADN) production by *Fusarium graminearum* has also studied (Vasavada and Hsieh, 1988). They reported that Mg^{2+} and Zn^{2+} supported toxin production with the highest yield (60% and 76% increase, respectively) at 1000µM. Also, a noticeable increase was observed in 3-ADN at low concentration of Fe^{2+} resulting in a 95% increase at 5µM concentration. In contrast, Mn^{2+} inhibited 3-ADN production at all tested concentrations. Jackson et al. (1989) examined the effect of Zn, Fe, Co and Mn on *Fusarium moniliforme* (=*F.verticillioides*) growth and fusarin C biosynthesis in submerged cultures. They found that zinc (26 - 3,200 ppb) inhibited fusarin C biosynthesis and increased dry weight accumulation. However, Mn (5.1 ppm) increased fusarin C with no effect on dry weight accumulation. Iron (10.0 ppm) and cobalt (9.0 ppm) had no effect on fusarin C biosynthesis or dry weight accumulation. Tiwari et al. (1986) also tested the effect of different metal ions including Fe, Mn and Co on aflatoxin B1 (AFB_1) production and the biomass of *A. parasiticus*. Iron salts decreased AFB_1 production to different levels but a mixed trend was observed depending on the magnesium salt concentration. Co salts stimulated AFB_1 production at all concentrations, with a maximum increase of 233% in production at 300µM of cobalt sulphate. They also found a negative correlation between AFB_1 production and vegetative growth of the species.

Recently, Mouhamed et al. (2015) evaluated the antifungal potential of ZnO and Fe_2O_3 nanoparticles in comparison with some commercial antifungal feed additives in inhibiting the growth of *A. ochraceus* and *A. niger* strains. They observed double the inhibition zones by using these metals when compared with commercial antifungals. Previous studies have only used liquid broth assays and not considered interactions with environmental factors such as water stress. This is particularly important as both food and feed are often intermediate moisture products and thus the efficacy under different water availability conditions should be considered.

The objectives of this study were to examine the effects of interactions between three metal ions (Mg^{2+}, Fe^{2+} and Co^{2+}; 150, 500 and 1000 µM) and water activity (a_w; 0.97, 0.92) on (a) growth and (b) OTA production by 2 strains each of *A. westerdijkiae* and *A. carbonarius* on a conducive solid YES medium.

MATERIAL AND METHODS

Fungal cultures

The ochratoxigenic strains, *A. westerdijkiae* CBS 121986 and CBS1121971 (CBS Culture collection) and *Aspergillus carbonarius* (ITAL 197; ITAL 204) were cultivated on potato dextrose agar (PDA) plates for 5 days at 28°C. Spore suspension of 10^6 spore ml^{-1} from each strain was prepared for inoculation. The tested strains were screened for OTA production with *A.westerdijkiae* strains producing high amounts of OTA (9-86 µg g^{-1} media) and the *A. carbonarius* strains, low OTA producers (0.7-1.5 µg g^{-1} media) at both a_w levels.

The *A. carbonarius* strains were isolated from coffee and kindly supplied by Dr M. Taniwaki (ITAl, Campinas, Brazil). The CBS strains were kindly supplied by Dr. R. A. Samson, CBS, Utrecht, Holland.

Preparation of metal ion media

Yeast Extract Sucrose agar (YES, sucrose 150, yeast extract 20, agar 20 g and 885ml H_2O, pH 6.2) was used for the metal ion experiments. The a_w of the YES medium was determined using the AquaLab 3TE (Decagon Devices, Inc., Pullman, Washington, USA) and found to be 0.97 a_w. This basal medium was modified to 0.92 a_w by using a glycerol/water solution during the preparation step. Stock solutions (0.2 M) of 3 different metal salts (Magnesium sulphate $MgSO_4.7H_2O$, Ferrous sulphate Fe $SO_4.7H_2O$ and Cobalt chloride $CoCl_2$) were prepared. The corresponding volumes from stock solution of each salt were added to YES medium (0.97 and 0.92 a_w) separately to obtain 150, 500 and 1000 µM concentrations. Controls without any metal ion addition were also prepared. The treatments were autoclaved and the molten media poured into 9 cm Petri plates.

YES treatment plates were centrally inoculated with 3 µl of a spore suspension ($1x10^6$ spores ml^{-1}) of the strains of *A. westerdijkiae* and *A. carbonarius* strains. Three replicates of each strain were incubated at 25°C for 10 days. Growth rates for all treatments were determined by daily measurement of colony diameters in two directions at right-angles to each other over periods of 7-10 days. Linear regression of the colony radius (mm) against time was used to determine the relative growth rates (mm day^{-1}). After 10 days, 5 discs of fungal culture were removed from these cultures using a surface sterilized cork borer (0.8 cm diameter), transferred to a 2 ml Eppendorf tubes, weighed, and frozen at -20°C until OTA analysis by HPLC.

OTA extraction

One ml of methanol was added to each Eppendorf tube containing culture plugs and shaken well for 1 h. The supernatant was collected with a sterile 1 ml syringe and filtered using a 0.22 µm nylon filter (Jaytee Biosciences Ltd.,Herne Bay, UK) into a sylanized HPLC vial for later analysis.

HPLC conditions for OTA analysis

The HPLC system used for OTA analyses was an Agilent 1200 series (Agilent, Berks., UK) with a fluorescence detector (FLD G1321A), an auto sampler ALS G1329A, FC/ALS therm G1330B, Degasser G1379B, Bin Bump G1312A and a C18 (Cronus, Nucleosil 100 5 micron, 150 x 4.6 mm) column joined to a pre-column (security guard, 4x3mm cartridge, Phenomenex Luna). The mobile phase was acetonitrile (57%):water (41%):acetic acid (2%) using an isocratic flow rate of 1ml min^{-1} at 333 nm excitation, 460 nm emission wavelengths. The run time for samples was 15 min with OTA being detected at 3.8 min.

Statistical analyses

Statistical significance was determined using Statistica Version 10 (StateSoft, Tulsa, OK, USA). The means of growth rate, OTA concentrations were for the different strains were compared using analysis of variance (ANOVA, two way analyses) ($P<0.05$). Fisher's LSD method ($\alpha=0.05$) was applied to compare differences between treatments.

RESULTS

Effect of metal ions (Mg^{2+}, Fe^{2+} and Co^{2+}) and water activity interaction on the growth of *A. westerdijkiae* and *A. carbonarius* strains

Figure 1 and 2 show the effect of the different metal ions at 150, 500 and 1000 µM on radial growth of the two strains each of *A. westerdijkiae* and *A. carbonarius* at the two a_w treatments.

In general, a_w significantly ($P=0.05$) affected the growth rate regardless of metal ion treatment or concentration (Table 1). With more available water (0.97 a_w), the growth rate of strains of both species, in all metal treatments, increased. Although, the growth rate of *A. carbonarius* strains (5.4-11.6 mm day^{-1}) was significantly higher than those of *A. westerdijkiae* strains (3.9-6 mm day^{-1}), *A. carbonarius* strains were more sensitive to changes in a_w change than the *A. westerdijkiae* ones.

ISSN: 0975-8585

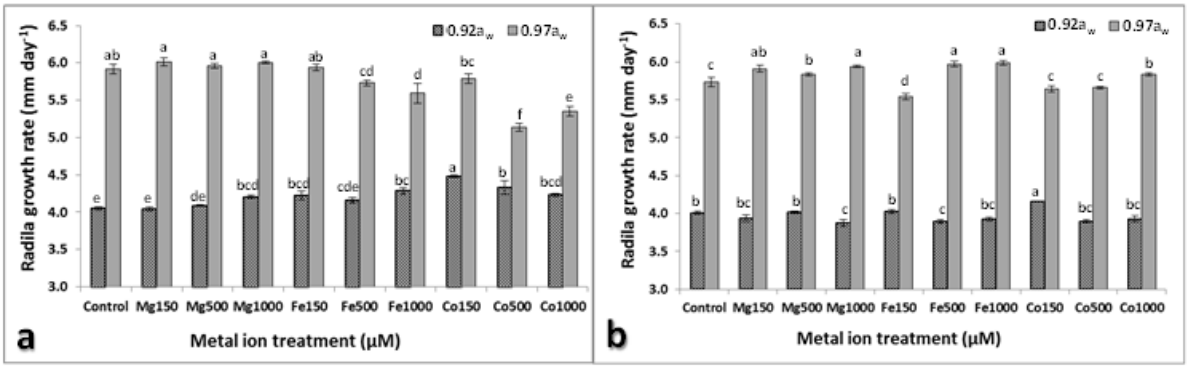

Figure 1 a,b: Effect of metal ions on growth rate of (a) *A. westerdijikiae* CBS 121986 and (b) *A. westerdijkiae* CBS 112791 within 7 days on YES media at 0.92 a_w and 0.97 a_w. Key to treatments: Mg, Magnesium; Fe, Iron; Co, Cobolt.

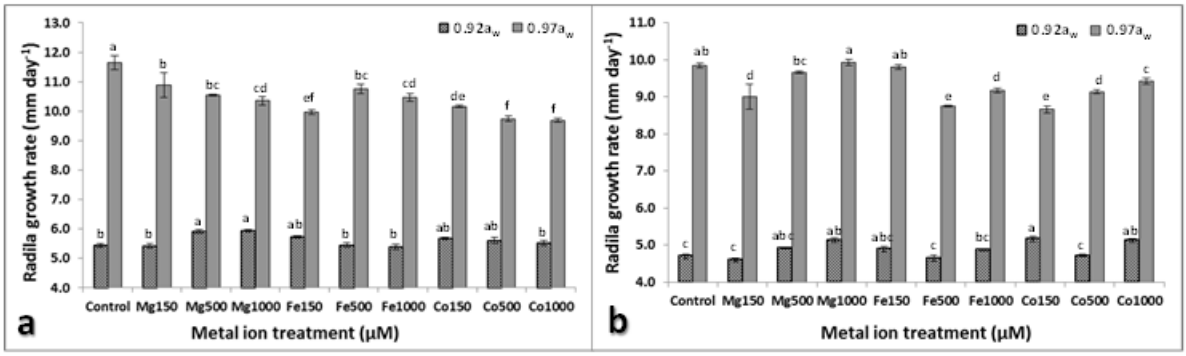

Figure 2 a,b: Effect of metal ions on growth rate of (a) *A. carbonarius* ITAL197 and (b) *A. carbonarius* ITAL204 within 7 days on YES media at 0.92 a_w and 0.97 a_w. Key to treatments: Mg, Magnesium; Fe, Iron; Co, Cobolt.

Table 1. Analysis of variance of the effect of metal ions, water activity (a_w) and their interaction on growth of *A. westerdijkiae* and *A. carbonarius* on YES medium.

A. carbonarius 121986					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	1981.914	1	1981.914	170544.1	0.000000
Metal ions	1.236	9	0.137	11.8	0.000000
a_w	47.154	1	47.154	4057.6	0.000000
Metal ions x a_w	2.642	9	0.294	25.3	0.000000
Error	0.697	60	0.012		0.000000
***A. westerdijkiae* 112971**					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	1910.335	1	1910.335	449686.7	0.000000
Metal ions	0.261	9	0.029	6.8	0.000001
a_w	67.499	1	67.499	15889.1	0.000000
Metal ions x a_w	0.853	9	0.095	22.3	0.000000
Error	0.255	60	0.004		
A. Carbonarius ITAL197					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	5135.078	1	5135.078	71679.16	0.000000
Metal ions	5.591	9	0.621	8.67	0.000000
a_w	463.644	1	463.644	6471.89	0.000000
Metal ions x a_w	8.399	9	0.933	13.03	0.000000
Error	4.298	60	0.072		
A. Carbonarius ITAL204					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	4046.491	1	4046.491	108735.6	0.000000
Metal ions	5.344	9	0.594	16.0	0.000000
a_w	398.516	1	398.516	10708.8	0.000000
Metal ions x a_w	3.997	9	0.444	11.9	0.000000
Error	2.233	60	0.037		

SS: sum of squares, DF: degree of freedom, MS: mean square, P: probability at confidence 0.95.

ISSN: 0975-8585

Effect of metal ions (Mg^{2+}, Fe^{2+} and Co^{2+}) and water activity interactions on OTA production by *A. westerdijkiae* and *A. carbonarius* strains

The effect of the different metal ion concentrations x aw interactions on OTA production by strains of both species are shown in Figures 3 and 4. This clearly shows that there was a significant (P=0.05) effect on OTA production. There were much bigger overall differences between effects of metals in terms of OTA production than for growth. For example, OTA production by *A. westerdijkiae* CBS 121986 was stimulated at both 0.92 and 0.97 a_w by increasing Mg^{2+} concentration. However, treatments with other metals such as Fe^{2+} resulted in a gradual decrease in OTA production. The metal ion Co^{2+} at all concentrations gave the same significant decrease when compared with control. At 0.97 a_w, the treatments which significantly increased OTA production were Mg^{2+} 500 and 1000 µM, whereas other treatment had no effect on OTA production.

Interestingly for the two strains of *A. carbonarius* (see Figure 4) the metal ion treatments had no effect on OTA production at 0.92a_w when compared with the control. At 0.97 a_w, only the Mg^{2+} at all concentrations showed a significant increase in OTA production (14 fold and 6 fold respectively) when compared with the controls.

Statistically, the metal ion factor was the main one affecting on OTA production (Table 2). Although, A. *westerdijkiae* strains grew slower than those of *A. carbonarius*, they were able to produce higher amounts of OTA on YES. Surprisingly, OTA production by *A. westerdijkiae* strains at 0.92 a_w was higher than that produced at 0.97 a_w in all metal ion treatments. In contrast. OTA production by *A. carbonarius* strains at 0.92 a_w was lower than that produced at 0.97a_w. In general, the Mg^{2+} metal ion was able to stimulate OTA production, especially at a 1000µM which appeared to be a threshold for this stimulation observed, regardless of a_w level tested.

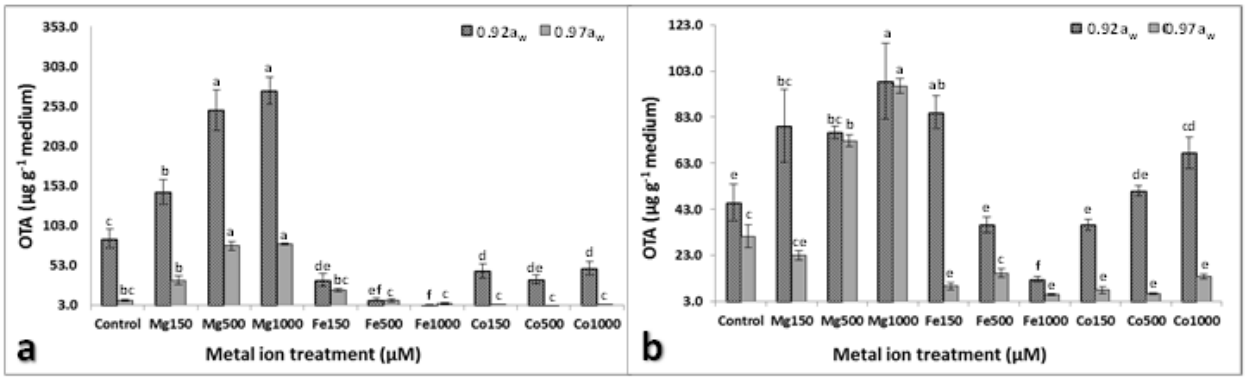

Figure 3 a,b: Effect of metal ions on ochratoxin A production by (a) *A. westerdijikiae* CBS 121986 and (b) *A. westerdijkiae* CBS 112791 after 10 days on YES media at 0.92 a_w and 0.97 a_w. Key to treatments: Mg, Magnesium; Fe, Iron; Co, Cobolt.

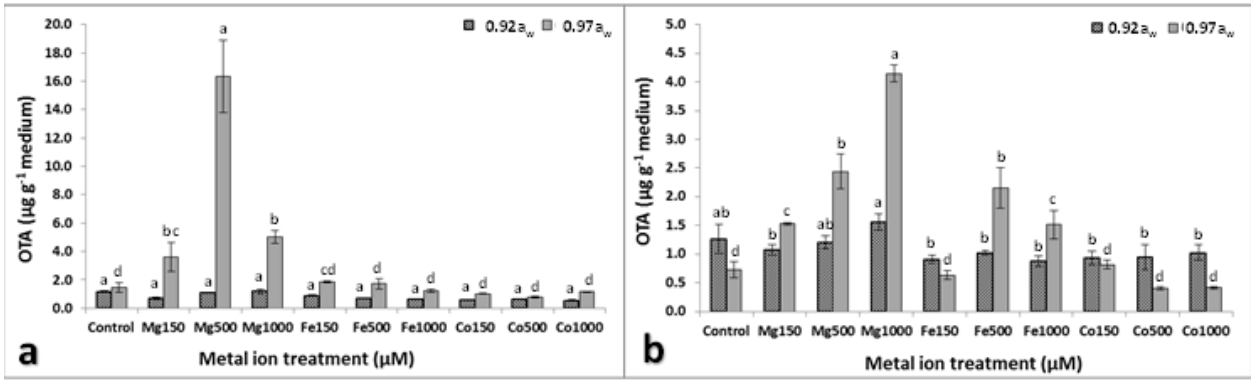

Figure 4 a,b: Effect of metal ions on ochratoxin A production by (a) *A. carbonarius* ITAL197 and (b) *A. carbonarius* ITAL204 after 10 days on YES media at 0.92 a_w and 0.97 a_w. Key to treatments: Mg, Magnesium; Fe, Iron; Co, Cobolt.

ISSN: 0975-8585

Table 2. Analysis of variance of the effect of metal ions, water activity (a_w) and their interaction on OTA production by *A. westerdijkiae* and *A. carbonarius* strains on YES medium.

A. westerdijkiae 121986					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	275397.0	1	275397.0	862.5847	0.00
Metal ions	287123.7	9	31902.6	99.9238	0.00
a_w	94393.8	1	94393.8	295.6556	0.00
Metal ions x a_w	85518.1	9	9502.0	29.7617	0.00
Error	19156.2	60	319.3		
A. westerdijkiae 1121971					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	152033.2	1	152033.2	999.4155	0.000000
Metal ions	49145.2	9	5460.6	35.8960	0.000000
a_w	18754.8	1	18754.8	123.2875	0.000000
Metal ions x a_w	11712.0	9	1301.3	8.5545	0.000000
Error	9127.3	60	152.1		
A. Carbonarius ITAL197					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	359.4672	1	359.4672	224.4504	0.000000
Metal ions	426.4817	9	47.3869	29.5882	0.000000
a_w	135.7726	1	135.7726	84.7761	0.000000
Metal ions x a_w	381.9037	9	42.4337	26.4955	0.000000
Error	96.0927	60	1.6015		
A. Carbonarius ITAL204					
Effect	**SS**	**DF**	**MS**	**F**	**P**
Intercept	130.8929	1	130.8929	1207.290	0.000000
Metal ions	32.4329	9	3.6037	33.238	0.000000
a_w	3.1324	1	3.1324	28.891	0.000001
Metal ions x a_w	19.2132	9	2.1348	19.690	0.000000
Error	6.5051	60	0.1084		

SS: sum of squares, DF: degree of freedom, MS: mean square, P: probability at confidence 0.95.

DISCUSSION

There has been no previous data available on the impact of metal ions on growth and OTA production by ochratoxigenic strains in relation to different environmental stresses, such as water availability. In general, no correlation between growth and OTA production was observed. There was less influence on growth of the tested *Aspergillus* species than on OTA production. Overall, there was a strong influence of metal type and concentration on the amount of OTA produced by both *A. westerdijkiae* and *A. carbonarius* strains on YES media.

Comparisons between the effect of Mg, Fe and Co on OTA production and that on growth were very different. Concerning to the influence on growth, the growth of the tested strains was relatively similar in the different concentrations of metal ions tested. There appeared to be a more significant impact of water stress than the concentration of the metal ions examined. Previous liquid culture studies suggested a significant reduction (30-80%) in fungal biomass of *A. parasiticus* in the presence of Fe (170, 510 µM) and Mg (170um) (Tiwari et al., 1986). However, Steele et al. (1973) found an increase in the dry biomass *A. ochraceus* with the addition of $FeCl_3$ (7.5-150µM) when compared to the controls. Vasavada and Hsieh (1988) studying *Fusarium graminearum* found an increase in the biomass when the cultures were supplemented by Mg and Fe up to 50mM in the media. Tiwari et al. (1986) found 154 and 53% enhancement in the dry biomass of *A. parasiticus* in the presence of 150 and 450um $CoCl_2$ respectively. It was suggested by Mouhamed et al. (2015) that the mechanism of action of Fe_2O_3 nanoparticles in inhibiting *A. ochraceus and A. niger* was by damaging the cell membrane and pitting of the cell wall leads to leakage and finally cell death. Lemire et al. (2013) discussed the mechanisms of antimicrobial activity of different metals in details which cause discrete and distinct types of injuries to microbial cells as a result of oxidative stress, protein dysfunction or membrane damage.

Regarding to OTA production, both strains of *A. westerdijkiae* strains were able to produce much higher amounts of OTA when compared with *A. carbonarius* strains. A water stress condition (0.92 a_w) was favorable for OTA production by *A. westerdijkiae* strains when compared with 0.97 a_w. In contrast, 0.97a_w was more favorable for OTA production by *A. carbonarius* strains. Previous studies by Mühlencoert *et al.,* (2004) examined the effect of Zn and Fe metal ions on OTA production by *Aspergillus ochraceus* NRRL 3174 on Modified Adye–Mateles (AM) synthetic medium at different pH levels. They observed noticeable effects of

metal ions on OTA production at pH 6.5 when compared with those at pH 5. At pH 6.5, 0.2 mg l^{-1} Zn increased OTA production by 50%, whereas, Fe at 0.12 mg l^{-1} resulted in a 40% decrease in the amount of OTA.

More recently, Alborch et al. (2011) found that the OTA concentration produced by *A. carbonarius* at 0.98 a_w was significantly higher than at 0.96 and 0.92 a_w ($p<0.05$). However, they did not examine the impact of different metal ions and environmental factors. However, in the present study, addition of Mg^{2+} (150, 500 and 1000 µM) to the medium at 0.92 a_w for *A. westerdijkiae* and 0.97 a_w for *A. carbonarius* strains stimulated OTA production. Mouhamed et al. (2015) observed 70 and 85% reduction in OTA production by *A. ochraceus* and *A. niger* respectively in YES medium treated with 25 ppb Fe_2O_3 incorporated as nanoparticles. Previous studies by Tiwari et al. (1986) carried out in broth culture with no environmental stress parameters found that 160µM Mg^{2+} gave 57% inhibition of AFB_1 production by *A. parasiticus.* However production was stimulated by 25% at 640 µM concentration. They also found that 170 or 510µM Fe^{2+} inhibited AFB1 production by 75%. In the present study the range of this metal ion used (150-1000 µM) also inhibited OTA production. The only stimulatory effect of Fe^{2+} at low concentrations (150µM) in 0.92 a_w with *A. westerdijkiae* CBS 112791 (85%) and at 500 µM, 0.97a_w with *A. carbonarius* ITAL204 (195%). Contrasting results have been obtained previously, with Mühlencoert et al. (2004) finding a 40% decrease in OTA production with $Fe_2(SO_4)_3$ at 0.12 mg l^{-1} (0.25µM) in YES broth medium and Steele et al. (1973) finding that 1.2-24 mg l^{-1} of $FeCl_3$ (7.5-150µM) supported OTA production by *A. ochraceus* (10-50mg l^{-1}) in broth media.

None of the previous studies evaluated the effect of $CoCl_2$ either on growth or OTA production by these species, especially interactions with water stress conditions. The present study found an increase in OTA production at 0.92 a_w with the presence of Co^{2+} at 1000µM for *A. westerdijkiae* CBS 112791 (≈45%). However, for all other strains/species examined this metal ion either had no effect or an inhibitory effect.

In conclusion, this study has demonstrated that the addition of Mg, Fe and Co resulted in relatively limited impacts on growth of these strains and species. However, there were differential effects on OTA production. In most cases there was a stimulation of OTA production at 0.92 a_w for strains of *A. westerdijkiae* and at 0.97 a_w for strains of *A. carbonarius* by especially by Mg^{2+} at 150, 500 and 1000µm.

Acknowledgement

Dr. Y. Sultan is grateful for a post-doctoral fellowship from the Egyptian Government.

REFERENCES

[1] Alborch, L., Bragulat, M.R., Abarca M.L. and Cabañes, F.J. (2011): Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus carbonarius* on maize kernels. International Journal of Food Microbiology, 147:53-57.

[2] Cairns-Fuller V., Aldred D. and Magan N. (2005): Water, temperature and gas composition interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum* on wheat grain. Journal of Applied Microbiology, 99: 1215-1221.

[3] Cuero, R., Ouellet, T, Yu, J. and Mogongwa, N. (2003): Metal ion enhancement of fungal growth, gene expression and aflatoxin synthesis in *Aspergillus flavus*: RT-PCR characterization. Journal of Applied Microbiology 2003, 94: 953-961

[4] Jackson, M.A., Slininger, P.J. and Bothast, R.J. (1989): Effects of Zinc, Iron, Cobalt, and Manganese on *Fusarium moniliforme* NRRL 13616 Growth and Fusarin C Biosynthesis in Submerged Cultures. Applied and Environmental Microbiology, 55: 649-655.

[5] Khalesi, M. and Khatib, N. (2011): Mini-review: The effects of different ecophysiological factors on ochratoxin A production. Environmental toxicology and pharmacology, 32: 113-121.

[6] Lemire, J., Harrison, J.J. and Turner, R.J. (2013): Review: Antimicrobial activity of metals: Mechanisms, molecular targets and applications. Nature Reviews Microbiology, 11:371-384.

[7] Mitchell, D., Parra, R., Aldred, D. and Magan, N. (2004): Water and temperature relations of growth and ochratoxin A production by *Aspergillus carbonarius* strains from grapes in Europe and Israel. Journal of Applied Microbiology, 97: 439-445.

[8] Mouhamed, A.E., Hassan, A.A., Hassan, M.A., El Hariri, M. and Refai, M. (2015): Effect of Metal Nanoparticles on the Growth of Ochratoxigenic Moulds and Ochratoxin A Production Isolated From Food and Feed. International Journal of Research Studies in Biosciences. 3: 1-14.

[9] Mühlencoert, E., Mayer, I., Zapf, M.W., Vogel, R.F. and Niessen, L. (2004): Production of ochratoxin A by *Aspergillus ochraceus*. European Journal of Plant Pathology, 110: 651-659.

[10] Natskoulis, P.I. Tassou, C.C. Magan N. and Panagou, E.Z. (2009): Effect of temperature and water activity on growth and ochratoxin A production boundaries of two *Aspergillus carbonarius* isolates on a simulated grape juice medium. Journal of Applied Microbiology, 107:257-268.

[11] Pateraki, M., Dekanea, A., Mitchell, D., Lydakis, D. and Magan, N. (2007): Influence of sulphur dioxide, controlled atmospheres and water availability on in vitro germination, growth and ochratoxin A production by strains of *Aspergillus carbonarius* isolated from grapes. Postharvest Biology and Technology, 44: 141-149.

[12] Protasowicki, M. (2005): Heavy Metals. In:. Dąbrowski W.M.and Sikorski Z.E. (Eds), Toxins in Food. CRC Press, Boca Raton, Florida 33431.

[13] Raza, W., Yang, X., Wu, H., Huang,Q., Xu, Y. and Shen, Q. (2010): Evaluation of metal ions (Zn^{2+}, Fe^{3+} and Mg^{2+}) effect on the production of fusaricidin-type antifungal compounds by *Paenibacillus polymyxa* SQR-21. Bioresource Technology, 101: 9264-9271.

[14] Samson, R.A., Houbraken J. and Thrane U. (2010): Food and Indoor Fungi. CBS Laboratory manual.

[15] Steele, J.A., Davis, N.D., and Diener, U.L. (1973): Effect of Zinc, Copper, and Iron on Ochratoxin A Production. Applied microbiology, 25:847-849.

[16] Tiwari, R.P., Mittal, V., Bhalla, T.C., Saini, S.S., Singh, G. and Vadehra, D.V. (1986): Effect of Metal Ions on Aflatoxin Production by *Aspergillus parasiticus*. Folia Microbiologica. 31, 124-128.

[17] Vasavada, A.B. and Hsieh, D.P.H. (1988): Effects of Metals on 3-Acetyldeoxynivalenol Production by *Fusarium graminearum* R2118 in Submerged Cultures. Applied and Environmental Microbiology, 54: 1063-1065.

[18] Weinberg, E.D. (1970)*:* Biosynthesis of secondary metabolites: roles of trace-metals. Advances in Microbial Physiology, 4:1-44.