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WATER RELATIONS OF *ASPERGILLUS NIGER* STRAINS OBTAINED FROM CHILLIES: EFFECTS ON LAG PHASES, GROWTH AND OCHRATOXIN A PRODUCTION

Diyaa Aljaza

Medical Mycology Group, Department of Pathological Analyses, Faculty of Science, Thi-Qar, Nasiriyah, Iraq.

Abstract

Contamination of spices with ochratoxin A (OTA) has resulted in EU legislative limits for this mycotoxin. Chillies are particularly susceptible to contamination with OTA. *Aspergillus niger* as fungal contaminant and OTA producer, plays an important role in chillies quality. This study has examined two *A. niger* strains obtained from chillies in Iraq. The effect of interactions between temperature (15-37°C) and water activity (0.90-0.995 a_w) on lag phases prior to growth, mycelial growth and OTA production was examined on a 10% chilli-based medium. The lag phases prior to growth were delayed markedly by lower temperatures (15-20°C) and a_w (0.90-0.928 a_w). Optimum growth of *A. niger* strains was at 35°C and 0.995 a_w and slowest growth was at 15°C in 0.955 a_w for both strains examined. The optimum temperature x a_w conditions for OTA production were at 25-30°C and 0.995 a_w by two strains. OTA was produced in lower amounts at 35 and 37°C and 0.955 a_w. However, no production of OTA occurred at 15 and 20°C at 0.90 and at 0.928 a_w respectively. Statistical analysis showed that temperature and a_w had a significant effect on lag time prior to growth ($P<0.05$) for the strains. There was also a significant effect of temperature x a_w on relative growth and OTA production by the two strains ($P<0.05$). These results are play an important role in a predicting of the safety conditions for drying and storage of chillies to prevent growth of *A. niger* and thus OTA contamination in the final chillies products.

Key words: *Aspergillus niger*; OTA; water activity; temperature; growth; lag phase; chilli

Introduction

Moulds are significant microorganisms widely distributed in nature especially in raw foods, among of them is chilli. In general, *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. are the most common fungal genera in food products (Reddy *et al.,* 2010). Some of their species can growth and produce mycotoxins in foods commodities under suitable conditions of temperature and humidity (Bennett *et al.,* 2003). Ochratoxins are secondary metabolites produced by fungi belonging to several fungal genera such as *Aspergillus* and *Pencicillium* spp. (Gopinandhan *et al.,* 2008). Fungi are very sensitive to environmental factors therefore, it is thus very important to understanding their requirements from a biotic factor such as temperature, O_2, water activity (a_w), pH and biotic factor nutrients, for their production of mycotoxins (Magan *et al.,* 2010).

Various studies on the effect of environmental factors on growth and mycotoxins production by *A. niger* have been carried out, but not in relation to chilli. For example, *In vitro* Cabrera *et al.,* (2014) found that the optimum temperature for growth and minimum lag phase duration by *A. niger* isolated from dried figs on culture media was at 30ºC. Parra *et al.,* (2004) *in vitro* the highest growth of *A. niger* was at 35ºC and 0.965 a_w. Passamani *et al.,* (2014) showed that the highest growth of *A. niger* in Brazilian grapes was between 24-37ºC in 0.95 a_w. Alborch *et al.,* (2011) found the optimum growth of *A. niger* in maize kernels was at 25-40ºC and 0.98 a_w. Recent study was by Akbar *et al.,* (2016) reported that the optimum growth of *A. niger* on coffee at 30-35ºC and 0.98-0.95 a_w, respectively. However, few if any studies have been carried out on chilli Costa *et al.,* (2019).

The objective of this study: a) *in vitro* study the efficacy of temperature x a_w and their interaction on lag time prior to growth and relative growth of two strains of *Aspergillus* section *Nigri* ($DAJA_2$ & $DAJA_3$) isolated from Iraqi chillies b) *in vitro* study effect of temperature

***Author for correspondence :** E-mail : diyaamt71@gmail.com

x a_w and their interaction on OTA production by *A. niger* strains.

Materials and Methods

Collection of chillies samples

A total of 7 various chillies samples (Red chillies early stage, Red chillies mid stage, Red chillies ripen stage, Red chillies post-harvest stage, Red chillies powder, Green chillies powder and Green chillies post-harvest) were brought from different (south and middle) regions of Iraq, whole samples were stored at 4ºC before analysis and then tested for fungal populations and ecology studies.

Media preparation

Using 10% chilli powder as the main nutrient source media were prepared for studies at different temperatures and a_w levels. For this, 10g of crushed chillies with 2% technical agar Nº3 (Oxoid) were prepared in distilled water. The medium was mixed well and autoclaved at 121ºC for 1 hr and then cooled to 50ºC and then poured into 9 cm Petri dishes. The a_w was modified with glycerol to 0.995-0.90 a_w. Studies were conducted at 15-37ºC. All media were checked for a_w accuracy using the Aqualab TE4 machine (Labcell Ltd, Medstead, UK).

Fungal species

Two strains of *Aspergillus* section *Nigri* ($DAJA_2$ and $DAJA_3$) were isolated from the Iraqi chillies previously used in this study.

Spore suspensions preparation

Take amount of fungal growth from 7 days old plates by sterile loop and transport to 9 ml of sterile water supplemented with 0.05% (w/v) Tween 80 for prevent the growth randomly.

Inoculation and the growth measurements

The spore suspensions of the stains were prepared as described previously. The treatment plates were inoculated centrally with a spore suspension on the 10% chilli media. The treatments and replicates were measured in two directions daily at right angles to each other for a period of 10 days. After ten days incubation, growth rate of each strain was calculated by plotting the diameter of the colonies (mm) vs time (days) in Microsoft Excel. From the regression lines the relative growth rate and the lag phases (λ, days) prior to growth were calculated.

OTA analyses

Samples evaluated by the agar plug technique (Bragulat *et al.,* 2001) 8 agar plugs were removed by 4 mm sterile cork-borer from each fungal plate 10 days old and transfer to pre-weighted 2 ml sterile Eppendorf tube and reweighted again. The agar plugs were extracted with 0.5 methanol, followed by derivatization and quantification using HPLC-FLD.

Statistical analyses

The effects of two a biotic factors temperature and water activity were examined by using the Shapiro Wallis Test (Non-normality data). The statistically significant level was at $P<0.05$ for both factors and their interactions table 1.

Results

a) Effect of temperature x water activity on lag phases prior to growth of *A. niger* strains

The effect of both factors on the lag phases prior to growth are shown in Fig. 1. Overall, very long phases were observed by *A. niger* at low temperatures (15-20ºC) and a_w (0.955- 0.995 a_w). At higher temperatures and a_w levels the lag phases were very short especially, at 37ºC in 0.982-0.995 a_w. Statistical analyses showed that temperature had significant effect on lag time prior to growth ($P<0.05$) for two strains of *A. niger* except at 15ºC in all levels of a_w table 1.

Table 2 shows that the lag phase prior to growth was significantly affected by a_w except at 0.90 a_w for both strains and at 0.928 a_w for ($DAJA_1$) and at 0.955 a_w for ($DAJA_3$). On the other hand, the lag phase prior to growth of both strains had non-significant effected ($P>0.05$) by interaction of temperature x a_w table 3.

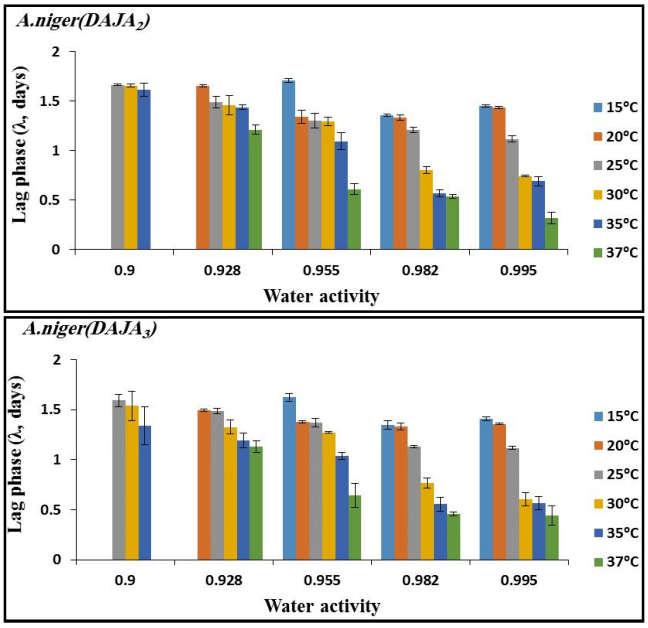

Fig. 1: Effect of different temperatures (15, 20, 25, 30, 35 & 37ºC) on lag phase of *A. niger* ($DAJA_2$, $DAJA_3$) at different a_w levels (0.995, 0.982, 0.955 & 0.90 a_w) after 10 days on a 10% chilli medium. Bars and standard deviation of the means.

b) Effect of temperature x water activity on growth of *A. niger strains*

Fig. 2 shows the effects of interacting conditions of a_w x temperature on the growth of two *A. niger* strains. The highest growth of the two strains of *A. niger* was at 35ºC in 0.995 a_w and the slowest growth at 15ºC in 0.955 a_w. No growth was observed at 0.90 a_w and 15, 20 and 37ºC and at 0.928 a_w in 15ºC.

However, there were some differences in growth patterns among the strains of *A. niger*, the growth rate of both strains was the best at 35ºC in all a_w levels except at 0.928, 0.955 and 0.982 a_w where the highest growth was at 37ºC compared with other temperatures.

Statistically, the growth rate was significantly affected by temperature at all a_w levels for two strains of *A. niger* table 1. There was also a significant effect of a_w on the growth rate for two strains of *A. niger* at all temperatures table 2. Table 3 shows that the growth rate was not significant affected by interaction of temperature x a_w.

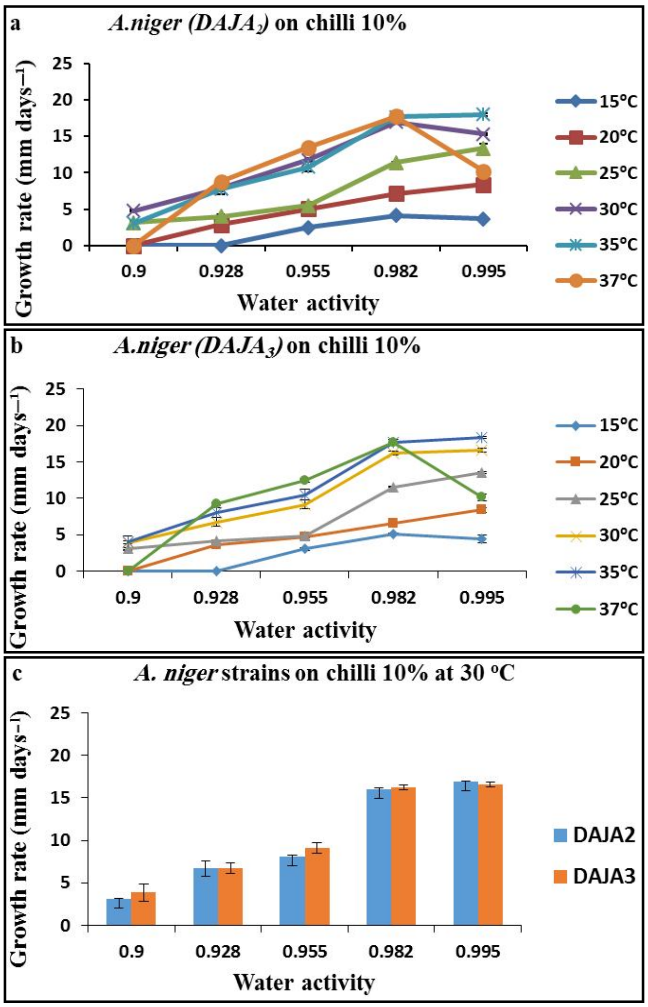

Fig. 2: (a-c) Effect of different temperatures (15, 20, 25, 30, 35 & 37ºC) on growth of *A. niger* ($DAJA_2$, $DAJA_3$) at different a_w levels (0.995, 0.982, 0.928, 0.955 & 0.90 a_w) over 10 days on a 10% chilli medium. Bars indicated SD of the means.

c) Effect of temperature x a_w and their interaction on OTA production by *A. niger* strains.

Fig. 3 a, b shows the effects of different temperatures and a_w levels on OTA production (ng g^{-1}) on 10% chilli medium after 10 days. The highest production of OTA was at 30ºC and 0.995 a_w by ($DAJA_2$) and at 25ºC and 0.995 by ($DAJA_3$), while the production was lowest at 35, 37ºC and 0.995 a_w by both strains, respectively. No production was found with 0.90 and 0.928 a_w at 15-20ºC.

Table 1 summarises the statistical analyses of the effect of temperature on OTA production by two strains of *A. niger* obtained from chillies. There was a significant effect ($P<0.05$) of temperature on OTA production for both strains except at 15 and 20ºC for ($DAJA_2$) and at 15ºC for ($DAJA_3$) at all a_w levels. Table 2 shows OTA production was significant affected by a_w for two strains at all temperatures. On other hand, table 3 shows OTA production effected by temperature and a_w but not affected by their interactions.

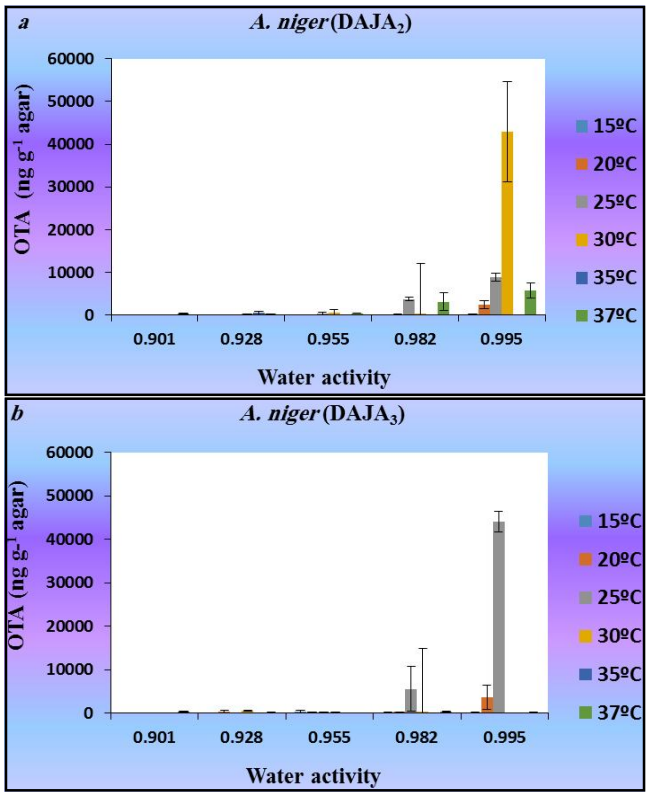

Fig. 3: Shows the effects of interacting conditions of a_w x temperature on OTA production by two strains of *A. niger* ($DAJA_2$ and $DAJA_3$) isolated from chilli at different temperatures (15-37ºC) and different a_w levels (0.995-0.90 a_w) after 10 days on 10% chilli medium. Bars indicate SD of the means.

Table 1: Shows statistical analyses of the effect of temperature on lag phase (λ, days) prior to relative growth and growth by two strains of *A. niger* ($DAJA_2$ & $DAJA_3$) isolated from Iraqi chillies by using Wilcoxon Test (non-normality data).

Strains	a_w	15 ºC			20 ºC			25 ºC			30 ºC			35 ºC			37 ºC		
		Lag Phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})
A. niger ($DAJA_2$)	0.90	*Ns*	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.928	*Ns*	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.955	*Ns*	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.982	*Ns*	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.995	*Ns*	*S*	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
A. niger ($DAJA_3$)	0.90	*Ns*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.928	*NS*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.955	*NS*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.982	*NS*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	0.995	*NS*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*

S mean there is significant effect ($P \leq 0.05$)
NS mean not significant ($P > 0.05$)

Table 2: Shows statistical analyses of the effect of water activity on lag phase (λ, days) prior to relative growth and growth by two strains of *A. niger* ($DAJA_2$ & $DAJA_3$) isolated from Iraqi chillies by using Wilcoxon Test (non-normality data).

Strains	Temperature	0.90 a_w			0.928 a_w			0.955 a_w			0.982 a_w			0.995 a_w		
		Lag Phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})	Lag phase	Gro-wth	OTA (ng g^{-1})
A. niger ($DAJA_2$)	15ºC	*Ns*	*S*	*S*	*S*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	20ºC	*Ns*	*S*	*S*	*S*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	25ºC	*Ns*	*S*	*S*	*S*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	30ºC	*Ns*	*S*	*S*	*S*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	35ºC	*Ns*	*S*	*S*	*S*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	37ºC	*Ns*	*S*	*S*	*S*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
A. niger ($DAJA_3$)	15ºC	*Ns*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	20ºC	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	25ºC	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	30ºC	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	35ºC	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*
	37ºC	*NS*	*S*	*S*	*NS*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*

S mean there is significant effect ($P \leq 0.05$)
NS mean not significant ($P > 0.05$)

Discussion

To my knowledge this is the first study for understanding the optimum conditions for OTA production by *A. niger* on chilli based-medium. The results of the ecological study showed that the growth rates of strains of *A. niger* isolated from Iraqi chilli was best at 35ºC and 0.995 a_w and the minimum temperature of growth was at 15ºC with 0.955 a_w level. In addition, there is not growth at 0.90 a_w in 15, 20 and 37ºC and at 0.928 a_w in 15ºC.

The optimum conditions of OTA production on chilli-based medium was at 25-30º and 0.995 a_w. It was interesting to note that while optimum growth was at 35ºC OTA production was much higher at 25-30ºC at the same level. The again suggests that optimum conditions for growth are not necessarily the same optimum for mycotoxin production as has been matrices have found optimum conditions for growth of *A. niger*. Similarly, Palacios-Cabrera *et al.,* (2004) showed that the best growth of *A. niger in vitro* on culture media at temperature > 30ºC. Alborch *et al.,* (2011) found the optimum temperature for growth of *A. niger* on maize kernels was between 24-40ºC at 0.98 a_w. Passamani *et al.,* (2014) reported the highest growth of *A. niger* on Brazilian grapes was between 24-37ºC and 0.95 a_w. Su-Lin *et al.,* (2006) Shown the optimum temperature for growth by *A. niger* at 35ºC and 0.98 a_w while the highest of OTA production at 15ºC and 0.95-0.98 a_w.

Concluded from this study: a) that strains of *A. niger*

Table 3: Summary statistical analyses for effects of a_w x temperature on Lag time (λ, days), relative growth and OTA production of *A. niger* strains by using Wilcoxon test (non-normality data).

Factors				
Strains	**a_w**	**tempe-rature**	**a_wx (ºC)**	**Response**
A. niger ($DAJA_2$)	*S*	*S*	*NS*	Lag phase (λ, days)
	S	S	NS	Growth rate (mm day^{-1})
	S	S	NS	OTA production (ng g^{-1})
A. niger ($DAJA_3$)	*S*	*S*	*NS*	Lag phase (λ, days)
	S	S	NS	Growth rate (mm day^{-1})
	S	S	NS	OTA production (ng g^{-1})

$P \leq 0.05$ mean there is at list one significant effect

obtained from Iraqi chillies grow best at 35ºC and 0.995 a_w b) The highest production of OTA between 25-30 ºC at 0.995 a_w c) this information can used for predicating of the relative risk of contamination with OTA during production and processing of chillies.

References

Alborch, L., M.R. Bragulat, M.L. Abarca and F.J. Cabames (2011). Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillus niger* and *A.carbonarius* on maize kernels. *International Journal of Food Microbiology,* **147:** 53-7.

Akbar, A.H. and N. Magan (2015). Growth and ochratoxin production by *Aspergillus* species in Coffee beans: Impact of climate change and control using O_3 (PhD thesis). Cranfield University, Cranfield, Beds., MK43 0AL, U.K.

Aysegul, M.I. and K.G. Funda (2014). Effect of Temperature on the Growth and Ochratoxin A Production of the *Aspergillus* section *Nigri* Members. *Journal of Food Safety,* **34:** 4.

Bennett, J.W. and M. Klich (2003). Mycotoxins (http:// cmr. Asm.org/ cgi/ pmidlookup? View=long&pmid=12857779). *Clinical Microbiological Review,* **16:** 497-516.

Bragulat, M.R., M.L. Abarca and F.J. Cabanes (2001). An easy method for fungi producing ochratoxin A in pure culture. *International Food Journal Food Microbial,* **71:** 139-144.

Costa, J., R. Rodriguez, E. Garcia-Cela, A. Medina, N. Magan, N. Lima, P. Battilani and C. Santos (2019). Overview of Fungal and Mycotoxin Contamination in *Capsicum* Pepper and in Its Derivatives. *Journal of Toxins,* **27:** 11010027.

Gopinandhan, T.N. and G.S. Kannan (2008). Influence of water activity on growth and ochratoxin A production by isolates of *Aspergillus ochraceus*. *Journal of Mycology PL Pathology,* **38:** 551-555.

Magan, N., D. Aldred, R. Hope and D. Mitchell (2010). Environmental factors and interactions with mycoflora of grain and grapes: effects on growth and deoxynivalenol and ochratoxin production by *Fusarium* culmorum and *Aspergillus* carbonarius. *Toxins,* **2:** 253366.

Palacios-Cabrera, H., M.H. Taniwaki, H.C. Menezes and B.T. Iamanaka (2004). The production of ochratoxin A by *Aspergillus ochraceus* in raw coffee at different equilibrium relative humidity and under alternating temperatures. *Journal of Food Control,* **15:** 531-535.

Passamani, F.R., T. Hernandes, N.A. Lopes, S.C. Bastos, W.D. Santiago, M.D. Cardoso and L.R. Batista (2014). Effect of temperature, water activity, and pH on growth and production of ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* from Brazillian grapes. *Journal of Food Protection,* **77:** 1947-52.

Parra, R., D. Aldred, B. David and N. Magan (2004). Water activity, solute and temperature modify growth and spore production of wild type and genetically engineered *Aspergillus niger* strains. *Journal of Enzyme and Microbial Technology,* **35:** 232-237.

Reddy, K.R.N., B. Salleh, B. Saad, H.K. Abbas, C.A. Abel and W.T. Shier (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Review,* **29:** 3-26.

Su-Lin, L.L., D.H. Ailsa and S.S. Eileen (2006). Effect of temperature and water activity on growth and ochratoxin A production by Australian *Aspergillus carbonarius* and *A. niger* isolates on a simulated grape juice medium. *International Journal of Food Microbiology,* **110:** 20-9-16.