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Research Article

Growth and Ochratoxin A production of black aspergilli isolated from Thai wine grapes

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Abstract

Ochratoxin A (OTA) is a mycotoxin, produced by filamentous fungi, toxic to humans and animals and naturally found in a wide range of different agricultural products, including wine grapes. Members of *Aspergillus* section *Nigri* (black aspergilli) are mainly responsible for OTA accumulation in wine grapes. The aims of this study were (i) to isolate ochratoxigenic black aspergilli from Thai wine grapes, (ii) to study their ability to produce OTA and (iii) to study the effect of temperature (20, 25, 30°C) and fungicides (Carbendazim and difenoconazole) on their growth and OTA production. Forty-four isolates of black moulds were found in 12 wine grape samples colleted from the central of Thailand in 2010 and identified as black *Aspergillus*. 61.36% of all isolates produced OTA with the range 0.1-12,168 ng/g culture. The isolate of *Aspergillus carbonarius* producing the maximum amounts of OTA was selected for the study of the effect of temperature and fungicides on growth and OTA production. The optimum growth was found at 30°C whereas the highest OTA level was observed at 20°C. Two types of fungicide at the assayed levels had effect on fungal growth and OTA production. Fungal growth and OTA production were slightly reduced when the concentration of the fungicides increased. These results showed that the OTA producing fungi were isolated from Thai wine grapes for the first time and the application of antifungal agents could reduce ochratoxingenic fungal contamination and OTA accumulation in wine grapes.

Keywords: *Aspergillus* section *Nigri*, Ochratoxin A, Thailand, tropical wine, contamination.

Introduction

Ochratoxin A (OTA) is a mycotoxin, a product of secondary metabolism of filamentous fungi. OTA is produced by various species in the genera *Aspergillus* and *Penicillium*. This toxin contaminates different agricultural products including cereal grain, nuts, cocoa, coffee beans, spices, dried fruits, beer and wine [1]. Furthermore, the contamination of OTA has also been reported in animal products such as beef, pork, chicken [2, 3] and processed products including milk, cheese, sausage, ham and bacon [4, 5, 6]. This mycotoxin has clearly demonstrated nephrotoxic, teratogenic and immonutoxic properties in animals and is recognized as a possible cause of Balkan Endemic Nephropathy (BEN) in South-Eastern Europe [7]. The International Agency for Research on Cancer (IARC) classified OTA as a possible human carcinogen (group 2B) [8].

The presence of OTA in grape juice and wine was reported for the first time in 1996 by Zimmerli and Dick [9]. Since then, the occurrence of OTA in wine has been reported in several countries in Europe, South America and Australia [10, 11, 12], especially grapes and wine from the Mediterranean countries such as France, Italy, Greece and Spain, where the OTA contamination in wine is higher than other regions [13]. The European Commission has fixed the maximum limits for OTA in grape juice and wine at 2.0 µg/kg [14].

Aspergillus section *Nigri* (black aspergilli) is considered to be principal source of OTA contamination in grapes and wine in various regions around the world especially *A. carbonarius* and the species included in the *A. niger* aggregate [15]. *A. carbonarius* is the major OTA producer isolated and observed more frequently from grapes [16, 17]. Whereas, *A. niger* aggregate is more commonly found in grapes but the ability to produce OTA is lower than that of *A. carbonarius*. Their production levels were about 1,000 times less than *A. carbonarius* [18, 19, 20, 21]. In addition, *A. tubingensis*, a member of *A. niger* aggregate, isolated from grapes has also been found to produce OTA [22].

Several factors influence fungal contamination and OTA accumulation in grapes. Weather is considered as a significant effect, particularly temperature that plays an important role in growth and OTA production of *Aspergillus* section *Nigri* [15]. High occurrences of ochratoxigenic black aspergilli were mainly found in vineyards located in hot and dry climatic zones [23]. Health of grapes is also an important factor that effects OTA contamination in grapes and wine. Damaged berries were found to contain more OTA than healthy berries. The air movement, insects and worms cause the distribution of black fungal spores from soil to the surface of grape berries. Then, the spores develop and form OTA particularly on damaged berries [15]. The use of fungicides is the best effective way to prevent fungal growth. Cyprodinil, an anilinopyrimidine type fungicide, was effective in reducing OTA contamination and OTA production by *A. carbonarius* colonizing on grape medium and grape berries [24]. Valero *et al*. [25] reported that application of cyprodinil and fludioxonil in vineyards before harvest inhibited growth and OTA production of *Aspergillus* section *Nigri*.

Currently, wine is becoming more popular in Thailand. Several wine-producing vineyards are located in the north, eastern and central Thailand. Thai wine production is mainly for domestic consumption and some of the wine products are exported to neighboring countries in Indochina and Asia. Export to the countries forming the major wine consuming markets such as United Kingdom, United States, Germany and France is currently small, but growth is increasing. In 2008, the value of Thai wine exports in the global market was about 450 million Baht, an increase of 25% over the previous year (data from the Information and

Communication Technology Center, Office of the Permanent Secretary Ministry of Commerce in cooperation with Thai Customs Department). Therefore, the scientific research on wine in Thailand is becoming more necessary. Much research has focused on development of the fermentation processes to obtain a taste similar to European wine. However, there is no research on OTA contamination and ochratoxigenic fungi in wine grapes, which directly relates to consumer safety and is a current interest in many countries around the world.

The aims of this study were (i) to isolate ochratoxigenic black aspergilli from Thai wine grapes, (ii) to study their ability to produce OTA and (iii) to study the effect of temperature and fungicides on their growth and OTA production.

Materials and Methods

Samples

Twelve wine grape samples were collected during the harvest season (February, 2010). Samples of 1 kg of bunches were randomly collected from a vineyard located in Pak Chang District of Nakhon Ratchasima Province situated in the northeast of Thailand. The samples were taken to the laboratory in closed plastic bags, transported in cooled boxes. Mycological analyses were immediately done and the remaining samples were frozen at -20°C.

Mycological Analysis

Isolation of fungi from grape sample by direct plating

Twenty-five berries were randomly selected from each wine grape sample and aseptically put directly onto the surface of DRBC (Dichloran Rose Bengal Chloramphenicol agar) culture medium (5 berries/plate). The plates were incubated at 25°C for 5-7 days.

Enumeration of total fungi in grape sample

Dilution plating method was used to examine the total fungi in wine grape samples. Wine grapes (50 g) were put into a stomacher bag. Then, 450 ml of sterile physiological water (0.85% NaCl) containing 0.01% Tween 80 were added and homogenized by a stomacher for 1 min. The suspension was diluted appropriately and 0.1 ml of each dilution was spread on PDA plate (Potato Dextrose Agar) in duplicate. The plates were incubated at 25°C for 5-7 days. The results were expressed in Colony-Forming Units (CFU)/g of wine grape.

Black spore-producing filamentous fungi (from 2.2.1 and 2.2.2.) were isolated on MEA (Malt extract agar) and CYA (Czapek yeast extract agar) medium and identified to genus and species level by morphological characterization including macroscopic characters; colour, size and colony appearance and microscopic characters; conidia, conidiophore, conidial heads, using the identification keys of Samson *et al.,* [26] and Pitt and Hocking, [27].

Determination of ochratoxigenic potential of Aspergillus section Nigri isolated

OTA production

OTA production of the isolated black aspergilli was determined on PDA medium. Inoculates were prepared by growing the strains on PDA medium at 25°C for 5 days. Conidia suspensions of each isolate were prepared in a physiological water (0.85% NaCl) containing 1% Tween 80 and adjusted to 10^6 conidia/ml. The adjusted suspension of 5 µl was dropped at the centre of PDA medium plate. The plates were incubated at 25°C for 7 days.

Extraction and quantification of OTA from culture medium
After 7 days of incubation, 3 agar plugs of 5 mm in diameter were removed from middle area of the colony. The plugs were weighed and extracted with methanol/formic (25:1) under sonication for 15 min. After evaporating the solvent under a nitrogen stream at 40°C, the dried extracts were re-suspended in the mobile phase of HPLC (acetonitrile:deionized water:acetic acid, 45.5:49.5:1) and filtered through a syringe filter (Minisart SRP 4, 0.45 μm, Sartorius, Germany). The filtered extract was analyzed for OTA determination by HPLC with C18 (25 x 4.6 mm, 5 μm) and fluorescence detector by using the method of Dachoupakan *et al.* [21].

Effect of temperature on growth and OTA production
The isolated black aspergilli producing the maximum of OTA level was cultivated on PDA medium at 25°C for 5 days. Conidia suspension of each isolate was prepared in a physiological water (0.85% NaCl) containing 1% Tween 80 and adjusted to 10^6 conidia/ml. An adjusted suspension of 5 μl was dropped at the centre of PDA medium plate. The plates were incubated at 20, 25 and 30°C (the average lowest temperature, the average temperature and the average maximum temperature respectively at Pak Chong District, Nakhon Ratchasima Province during harvest period in January-February) for 7 days. Diameter of the fungal colony was measured at 3, 5 and 7 days. The OTA production was determined by extraction and quantification of OTA from fungal culture medium (2.3.2).

Effect of fungicide on growth and OTA production
Carbendazim (benzimidazole antifungal agent) and difenoconazole (triazole antifungal agent) were used in this study. They were diluted in water as the concentration recommended by manufacturer. Each emulsion was diluted 10, 100 and 1000 times with water and added to PDA medium. The final concentrations of carbendazim in PAD medium were 25, 2.5 and 0.25 μg/ml and 12.5, 1.25 and 0.125 μg/ml were the final concentrations of difenoconazole.

The isolated black aspergilli producing the maximum of OTA level was cultivated on PDA medium at 25°C for 5 days. Conidia suspension of each isolate was prepared in a physiological water (0.85% NaCl) containing 1% Tween 80 and adjusted to 10^6 conidia/ml. An adjusted suspension of 5 μl dropped at the centre of PDA medium plate added with different concentrations of carbendazim and difenoconazole. The plates were incubated at the temperature favoring for OTA production (result from 2.4) for 7 days. Diameter of the fungal colony was measured at 3, 5 and 7 days. The OTA production was determined by extraction and quantification of OTA from fungal culture medium (2.3.2).

Results and Discussion

Study of Mycological Analysis of Wine Grape Samples
Twelve wine grape samples including various grape varieties: Syrah, Chenin Blanc, Cabernet Sauvignon, Viognier and Tempranillo, were examined for black aspergilli contamination. 75% of wine grape samples were contaminated by black fungi and 44 isolates of black aspergilli were isolated from these samples. After identification by morphological observation [26, 27], only 2 isolates of *A. carbonarius* and 42 isolates of *A. niger* aggregate, considered as principal possible producer of OTA in grapes [15, 16, 17, 18], were found (Table 1). The other filamentous fungal strains were also isolated and identified. It indicated that the grapes were also contaminated by *Penicillium* especially *Penicillium brevicompactum* that was the abundant genera found in grapes. However this species was never indexed as an OTA

producer in grapes [17, 28]. From the dilution plating method, the average total fungal count of all samples was low (1.75 x 10^2 cfu/g) and the counts of 8 samples were below 150 cfu/g.

Study of the Ability to Produce OTA of Black Aspergillus Isolated

All of black aspergilli isolates were tested for their ochratoxigenic ability. The results (Table 1) show that 27 isolates (61.36%) of all black aspergilli were able to produce OTA. All of *A.carbonarius* produced OTA with high level of OTA concentration (9,275-12,168 ng/g of culture) whereas 59.52% of *A. niger* aggregate have the ability to produce OTA (0.1-37.12 ng/g of culture). *A. carbonarius* is the specie more responsible for the OTA production than *A. niger* aggregate as was already shown in previous studies [16, 17, 29, 30]. Their production is actually 1,000 times superior to that of *A. niger* aggregate as observed in the studies of Serra *et al.* [17], Chulze *et al*. [11] and Dachoupakan *et al.* [21].

Table 1. Number of black aspergilli isolates and ochratoxigenic isolates found in Thai wine grape samples and their OTA production on PDA medium at 25°C for 7 days.

	Number of isolates	Number of OTA producing isolates	OTA production range (ng/g of culture)
A. carbonarius	2	2 (100%)	9,275-12,168
A. niger aggregate	42	25 (59.52%)	0.1-37.12

Study of the Effect of Temperature on Growth and OTA Production of Black Aspergilli

The strain of *A. carbonarius* producing the highest of OTA concentration was selected for this study. Figure 1 shows the colonial growth of *A. carbonarius* on PDA medium at 20, 25 and 30°C for 3, 5 and 7 days of incubation times. The optimum growth was observed at high temperature with the highest diameter recorded at 30°C. The fungal growth decreased with decreasing temperature whereas for OTA production the results show an opposite phenomenon. In addition, high OTA accumulation was obtained at low temperature with the maximal OTA level found at 20°C and the OTA content decreased with increasing temperature (Figure 2.). These results are related with Leong *et al.* [31] that *A. carbonarius* isolated from Australian wine grapes had a maximum growth at 30°C and 0.965 water activity, while the maximum of OTA production was at 15°C and 0.95-0.98 a_w. Furthermore, *A. carbonarius* isolated from European wine grapes had a maximum growth at 30-35°C and 0.93-0.987 aw and could produce maximum level of OTA at 15-20°C and 0.95-0.98 a_w [32].

Study of the Effect of Fungicides on Growth and OTA Production of Black Aspergilli

The growth of *A.carbonarius* presented in diameter of colony obtained in the different concentration of carbendazim and difenoconazole in PDA medium is shown in Table 2. At the optimum temperature for OTA production of *A. carbonarius*, 20°C, the fungal growth was highly influenced by two different fungicide classes. Colony diameters decreased with increasing fungicide concentration and no fungal growth was observed at the highest concentration levels. However, the growth was slightly favoured by the addition of a low concentration of carbendazim (0.25 µg/ml) in comparison with the control. For OTA production (Figure 3), the OTA content slightly decreased with increasing fungicide concentrations. The application of 1.25 µg difenoconazole/ml reduced significantly OTA production. At the highest concentration of fungicides, when the fungal growth was completely inhibited, no OTA production was found.

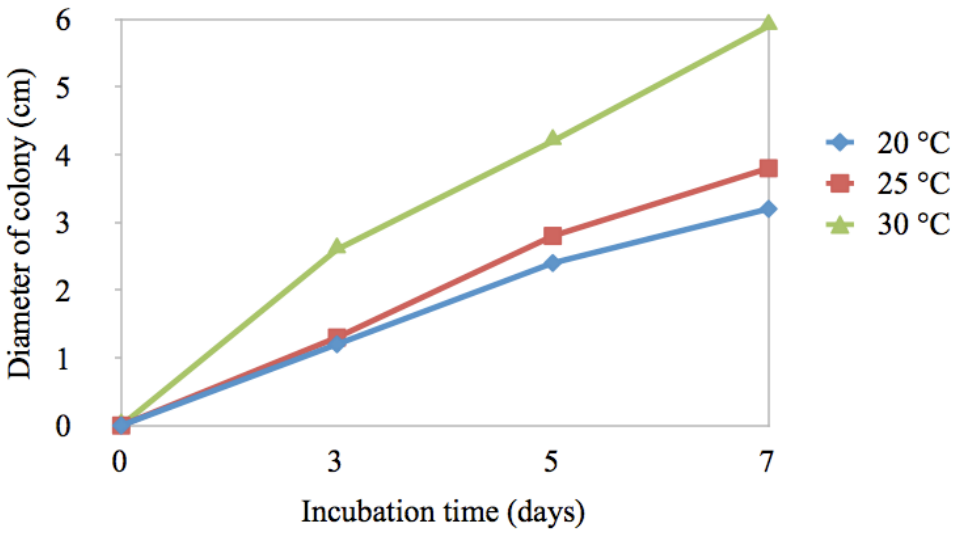

Figure 1. Colonial growth (diameter of colony on PDA medium at 20, 25 and 30°C) of *A. carbonarius* strain isolated from wine grapes at each incubation time.

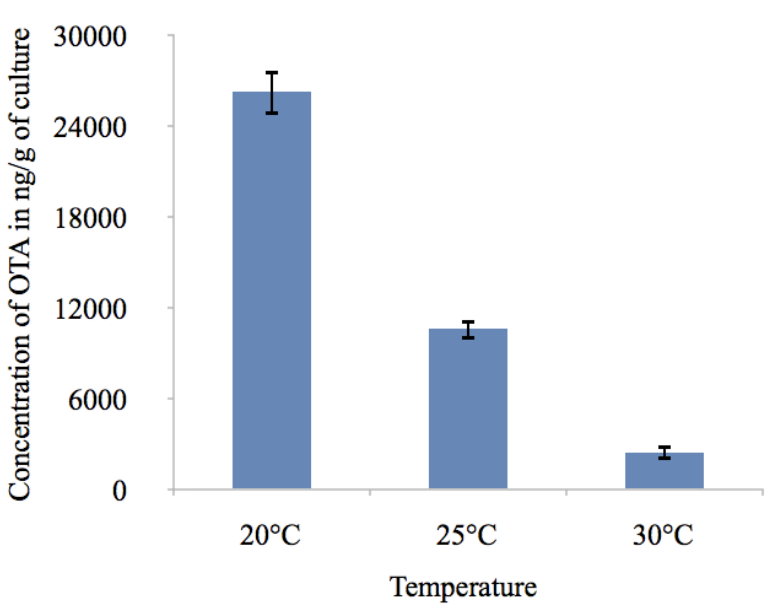

Figure 2. OTA production of *A carbonarius* strain isolated from wine grapes on PDA medium at 20, 25 and 30°C for 7 days.

Table 2. Effect of different concentrations of two fungicides in PDA medium on growth (diameter of colony in cm) of *A. carbonarius* at 20°C at each incubation times.

Fungicides	Incubation time (days)		
	3	5	7
Carbendazim (µg/ml)			
0	1.2	2.4	3.2
0.25	1.5	2.4	3.4
2.5	0.4	0.7	1.1
25	0	0	0
Difenoconazole (µg/ml)			
0	1.2	2.4	3.2
0.125	1.1	1.9	2.8
1.25	0.4	0.7	1.2
12.5	0	0	0

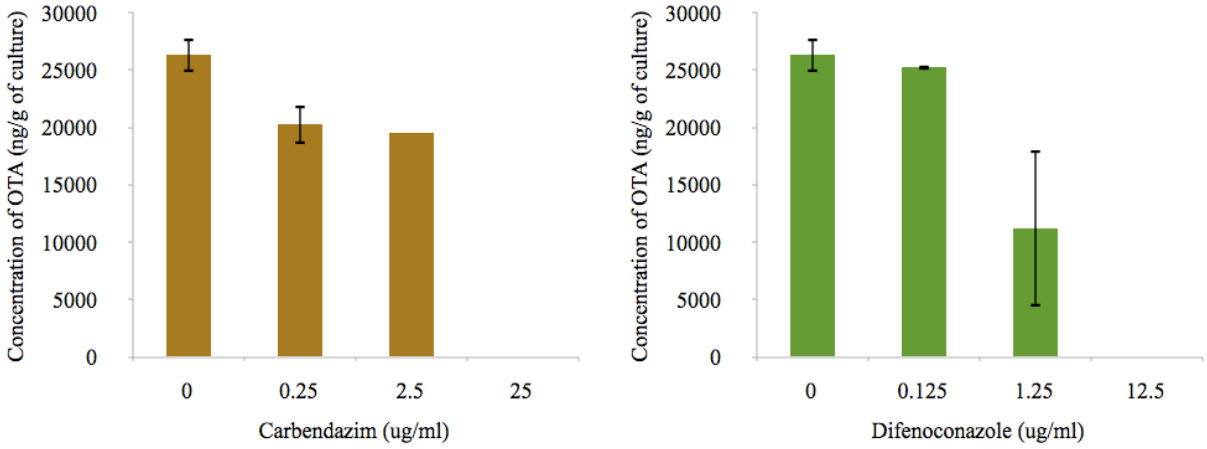

Figure 3. Effect of different concentrations of two fungicides in PDA medium on OTA production of *A. carbonarius* at 20°C for 7 days.

In this study the application of carbendazim showed a slightly negative effect on *A. carbonarius* growth and its OTA production except at the highest level tested (25 µg/ml) that completely inhibited growth and OTA production. Several *in vitro* studies have been realized on the influence of carbendazim, a systemic fungicide that is the most commonly used to control fungal infection in grapevine, on black aspergilli growth and OTA accumulation. It has been shown that carbendazim was not effective controlling the *A. carbonarius* population in vineyards [33] and this also agrees with the study of Medina *et al.* [34]. For difenoconazole, a triazole fungicide that is widely used for controlling fungal diseases in cereals, grapevines, banana and peanut [35], there is not much study conducted on the influence of this fungicide on ochratoxigenic aspergilli in wine grapes. Difenoconazole effect from our results showed an obviously negative influence on *A. carbonarius* growth and OTA production at mild concentration level tested. It could be used to control ochratoxigenic black aspergilli population and OTA contamination in wine grapes.

Conclusion

From this study it is concluded that the OTA producing black aspergilli exist in Thai wine grapes with low occurrence of *A. carbonarius* and high incidence of *A. niger* aggregate. The growth of *A. carbonarius* is favoured by high temperature (30°C), whereas its ability to produce OTA is increased at mild temperature (20°C). Two fungicides tested are both effective inhibiting growth and reducing OTA production of *A. carboanrius* when high concentrations were applied.

A limited number of samples is the constraining factor of this study. For future studies, the number of wine grape samples will be firstly regarded with consideration given to the different origins of samples. Molecular techniques will be used to study the genetic relatedness of black aspergilli isolates from different viticultural regions in Thailand. Finally, the combined effect of various factors on growth and OTA production of ochratoxigenic fungi will be evaluated.

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References

1. SCOOP European Commission (2002). Task 3.2.7. Assessment of dietary intake of ochratoxin A by the population of EU member states. Rome, Italy.

2. Jorgensen, K. (1998). Survey of pork, poultry, coffee, beer and pulses for ochratoxin A. ***Food Additives and Contaminants,*** 15, 550-554.

3. Walker, R. (1999). Mycotoxins of growing interest, ochratoxin. Presented at the 3rd Joint FAQ/WHO/UNEP International conference on mycotoxins, Tunis, Tunisia.

4. Skaug, M.A. (1999). Analysis of Norwegian milk and infant formulas for ochratoxin A. ***Food Additives and Contaminants,*** 16, 75-78.

5. Chiavaro, E., Lepiani, A., Colla, F., Bettoni, P., Pari, E. and Spotti, E. (2002). Ochratoxin A determination in ham by immunoaffinity clean-up and quick fluorimetric method. ***Food Additives and Contaminants,*** 19, 575-581.

6. González-Osnaya, L., Soriano, J.M., Moltó, J.C. and Mañes J. (2008). Sample liquid chromatography assay for analyzing ochratoxin A in bovine milk. ***Food Chemistry,*** 108, 272-276.

7. Vrabcheva, T., Usleber, E., Dietrich, R. and Märtlbauer, E. (2000). Co-occurrence of Ochratoxin A and Citrinin in Cereals from Bulgrian Villages with a History of Balkan Endemic Nephropathy. ***Journal of Agricultural and Food Chemistry,*** 48, 2483-2488.

8. IARC (1993). Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines, and Mycotoxins. IARC (International Agency for Research on Cancer) 56, 489.

9. Zimmerli, B. and Dick, R. (1996). Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. ***Food Additives and Contaminants,*** 13, 655-668.

10. Visconti, A., Pascale, M. and Centonze, G. (1999). Determination of ochratoxin A in wine by means of immunoaffinity column clean-up and high performance liquid chromatography. ***Journal of Chromatography A,*** 864, 89-101.

11. Chulze, S.N., Magnoli, C.E. and Dalcero, A.M. (2006). Occurrence of ochratoxin A in wine and ochratoxigenic mycoflora in grapes and dried vine fruits in South America. ***International Journal of Food Microbiology,*** 111, S5-S9.

12. Leong, S.L., Hocking, A.D., Pitt, J.I., Kazi, B.A., Emmett, R.W. and Scott, E.S. (2006). Australian research on ochratoxigenic fungi and ochratoxin A. ***International Journal of Food Microbiology,*** 111, S10-S17.

13. Battilani, P., Magan, N. and Logrieco, A. (2006). European research on ochratoxin A in grapes and wine. ***International Journal of Food Microbiology,*** 111, S2-S4.

14. European Commission (2005). Commission Regulation (EC) No 123/2005 of 26 January 2005. Amending Regulation (CE) No 466/2001 as regards ochratoxin A. ***Journal of the European Union,*** L25/3-25/5.

15. Varga, J. and Kozakiewicz, Z. (2006). Ochratoxin, A in grapes and grape derived products. ***Trends in Food Science and Technology,*** 17, 72-81.

16. Bau, M., Bragulat, M.R., Abarca, M.L., Minguez, S. and Cabañes, F.J. (2005). Ochratoxigenic species from Spanish wine grapes. ***International Journal of Food Microbiology,*** 98, 125-130.

17. Serra, R., Braga, A. and Venâncio, A. (2005). Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. ***Research in Microbiology,*** 156, 515-521.

18. Serra, R., Abrunhosa, L., Kozakiewicz, Z. and Venâncio, A. (2003). Black *Aspergillus* species as ochratoxin A producers in Portuguese wine grapes. ***International Journal of Food Microbiology,*** 88, 63-68.

19. Magnoli, C., Astoreca, A., Ponsone, L., Combina, M., Palacio, G., Rosa, C.A.R. and Dalcero, A.M. (2004). Survey of mycoflora and ochratoxin A in dried vine fruit from Argentina markets. ***Letters in Applied Microbiology,*** 39, 326-331.

20. Bellí, N., Bau, M., Marín, S., Abarca, M.L., Ramos, A.J. and Bragulat, M.R. (2006). Mycobiota and ochratoxin A producing fungi from Spanish wine grapes. ***International Journal of Food Microbiology,*** 111, S40-S45.

21. Dachoupakan, C., Ratomahenina, R., Martinez, V., Guiraud, J.P., Baccou, J.P. and Schorr-Galindo, S. (2009). Study of the phenotypic and genotypic biodiversity of potentially ochratoxinogenic black aspergilli isolated from grapes. ***International Journal of Food Microbiology,*** 132:14-23.

22. Perrone G. (2007). Biodiversity of *Aspergillus* Sect. *Nigri* from grapes in Europe. Presented at *Aspergillus* systematics in the genomic era an international workshop. Utrecht, The Netherlands, 12-14 April 2007.

23. Blesa, J., Soriano, J.M., Moltó, J.C. and Mañes, J. (2006). Factors affecting the presence of ochratoxin A in wines. ***Critical Reviews in Food Science and Nutrition,*** 46, 473-478.

24. Bellí, N., Marín, S., Sanchis, V. and Ramos, A.J. (2006). Impact of fungicide on *Aspergillus carbonarius* growth and ochratoxin A production on synthetic grape-like medium and on grapes. ***Food Additives and Contaminants,*** 23, 1021-1029.

25. Valero, A., Marín, S., Ramos, A.J. and Sanchis, V. (2007). Effect of preharvest fungicides and interacting fungi on *Aspergillus carbonarius* growth and ochratoxin A synthesis in dehydrating grapes. ***Letters in Applied Microbiology,*** 45, 194-199.

26. Samson, R.A., Hoekstra, E.S. and Frisvad, J.C. (2004). Introduction to food borne fungi. 7th edition, Centraalbureau voor Schimmelcucutures, Netherlands.

27. Pitt, J.I. and Hocking, A.D. (2009). Fungi and food spoilage. 3rd edition, Aspen Publishers, Inc., the United Kingdom.

28. Abrunhosa, L., Paterson, R.R.M., Kozakiewicz, Z., Lima, N. and Venâncio, A. (2001). Mycotoxin production from fungi isolated from grapes. ***Letters in Applied Microbiology,*** 32, 240-242.

29. Bellí, N., Marín, S., Sanchis, V. and Ramos, A.J. (2004). Influence of water activity and temperature on growth of isolates of *Aspergillus* section *Nigri* obtained from grapes. ***International Journal of Food Microbiology,*** 96, 19-27.

30. Esteban, A., Abarca, M.L., Bragulat, M.R. and Cabañes, F.J. (2004). Effects of temperature and incubation time on production of ochratoxin A by black aspergilli. ***Research in Microbiology,*** 155, 861-866.

31. Leong, S.L., Hocking, A.D. and Scott, E.S. (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, 209-216.

32. 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.

33. Tjamos, S.E., Antoniou, P.P., Kazantzidou, A., Antonopoulos, D.F. and Papageorgiou, I. (2004). *Aspergillus niger* and *Aspergillus carbonarius* in Corinth raisin and wine-producing vineyards in Greece: population composition, ochratoxin A production and chemical control. ***Journal of Phytopathology,*** 152, 250-255.

34. Medina, A., Mateo, R., Valle-Algarra, F.M., Mateo, E.M. and Jiménez, M. (2007). Effect of carbendazim and physicochemical factors on the growth and ochratoxin A production of *Aspergillus carbonarius* isolated from grapes. ***International Journal of Food Microbiology,*** 119, 230-235.

35. Gopinath, K., Radhakrishnan, N.V. and Jayaraj, J. (2006). Effect of propiconazole and difenoconazole on the control of anthracnose of chilli fruits caused by *Colletotrichum capsici*. ***Crop Protection,*** 25, 1024-1031.