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Aflatoxin, cyclopiazonic acid and β-nitropropionic acid production by *Aspergillus* section *Flavi* from dried figs grown in Turkey

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RESEARCH ARTICLE

Abstract

Aspergillus section *Flavi* isolates from dried figs were screened for aflatoxins, cyclopiazonic acid (CPA) and β-nitropropionic acid (BNP) production on yeast extract sucrose, czapek yeast extract and potato dextrose agar media. According to molecular identification, *Aspergillus flavus* (n=51) was dominant followed by *Aspergillus parasiticus* (n=5) and *Aspergillus tamarii* (n=1). All isolates were toxigenic producing at least one of the mycotoxins. 98% of the *A. flavus* strains produced aflatoxins (0.3-1,620 ng/g) and CPA (0.07-398.2 μg/g), with 31% of them also producing BNP (7.9-1,571.2 ng/g). All isolates of *A. parasiticus* were aflatoxins producers (106.4-1,255 ng/g), while 2 of them (40%) also produced CPA at a very low level (0.05-0.06 μg/g). Regarding toxigenic profiles of *A. flavus* and *A. parasiticus*, 6 and 3 chemotypes were classified, respectively. *A. flavus* isolates producing both B type of aflatoxins and CPA, comprised the most represented chemotype followed by the chemotype consisting of aflatoxin B_1, CPA and BNP producers. Regarding *A. parasiticus* chemotypes, two can be considered atypical being producer of only the B type of aflatoxins and being producer of the B, G type of aflatoxins along with CPA. To the best of our knowledge, an *A. flavus* isolate producing CPA and BNP but not aflatoxin has been found for the first time.

Keywords: mycotoxins, contaminants, food safety

1. Introduction

Turkey is a leader in the world market of dried fig production as well as dried fig export, supplying 55.2% of the world's dried fig export volume. Approximately 75% of the export practices were carried out with European countries (FAO, 2011). However, Turkey has been faced with mycotoxin problems especially the presence of aflatoxins in its exported dried fig products for years. Studies on the mycoflora revealed that the dominant flora of Turkish dried figs consists of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium* spp. (Heperkan and Karbancioglu-Güler, 2009). *A. flavus* and *A. parasiticus* belonging to *Aspergillus* section *Flavi* are the most important aflatoxin producers from a public health and economical point of view. *Aspergillus nomius, Aspergillus pseudotamarii, Aspergillus bombycis* and *Aspergillus parvisclerotigenus* species are the less common aflatoxin producers in this section (Frisvad *et al.*, 2006; Klich, 2007).

Aflatoxins are considered to be the most toxigenic fungal metobolites with carcinogenic, mutagenic, teratogenic and immunosuppresive effects (CAST, 2003). They consist of a group of approximately 20 related secondary fungal metabolites although only four major aflatoxins, B_1, B_2, G_1 and G_2, are naturally found in foods (Richard, 2007). Aflatoxin B_1 is known to be a potent hepatocarcinogen and classified as carcinogenic to humans (group I) by the International Agency of Research on Cancer (IARC, 1993). *A. flavus* normally produces only the B type of aflatoxins while *A. parasiticus* produces both B and G type of aflatoxins (Klich, 2007). Surveys revealed the presence of aflatoxins in diverse food commodities such as maize (Amra, 2009; Oruc *et al.*, 2006; Torres *et al.*, 2015), cotton seed (Jaime-Garcia and Cotty, 2003), peanut (Gürse, 2006;

Kamika and Takoy, 2011), pistachio nut (Chun *et al.*, 2007; Set and Erkmen, 2010), nuts (Aycicek *et al.*, 2005; Bacaloni *et al.*, 2008; Bircan *et al.*, 2008; Gürse, 2006), Brazil nuts (Leite *et al.*, 2014), cereals (Ghali *et al.*, 2009), spices (Bircan *et al.*, 2008; Set and Erkmen, 2010), and dried fruits (Bircan *et al.*, 2008; Heperkan *et al.*, 2012; Iamanaka *et al.*, 2007; Juan *et al.*, 2008).

Cyclopiazonic acid (CPA), initially isolated from cultures of *Penicillium cyclopium* (Holzapfel, 1968), is an indole tetramic acid produced by *A. flavus* and *A. tamarii* in *Aspergillus* section *Flavi* (Burdock and Flamm, 2000; Frisvad *et al.*, 2006; Goto *et al.*, 1997; Vinokurova *et al.*, 2007). It is a specific and reversible inibitor of calcium dependent ATPase in skeletal muscle with the property of chelating metal ions. It is known that CPA is toxic to a variety of animals such as pig, chicken, rat, rabbit and dog, causing degeneration and necrosis of liver, lesions of the myocardium and producing some neurological symptoms. Moreover it has been implicated in acute mycotoxicosis in humans called 'Kodua poisoning' in some regions of North India with the consumption of contaminated kodo millet (Antony *et al.*, 2003). But the major importance of this toxin is co-occurence with aflatoxins and it was considered responsible with aflatoxins for the outbreak of 'Turkey X disease' in turkey poults in 1960 (Frisvad *et al.*, 2006). Co-occurence of CPA and aflatoxins has been detected in various food and food products, including peanut (Fernandez-Pinto *et al.*, 2001; Gonçalez *et al.*, 2008; Mphande *et al.*, 2004; Urano *et al.*, 1992a), dried figs (Heperkan *et al.*, 2012), milk (Oliveira *et al.*, 2008), sour lime (Bamba and Sumbali, 2005) and animal feeds (Martins and Martins, 1999).

Besides aflatoxins and CPA, *A. flavus* is also capable of producing another secondary metabolite namely β-nitropropionic acid (BNP, 3-nitropropionic acid, NPA, hiptagenic acid) (Bush *et al.*, 1951; Frisvad *et al.*, 2006; Gilbert *et al.*, 1977; Iwasaki and Kosikowski, 1973). *Aspergillus wentii*, *Aspergillus oryzae*, *Penicillium atrovenetum*, *Arthrinium seccharicola* and *Arthrinium phaeospermum* are also BNP producers (Bhatnagar and Garcia, 2001; Burdock *et al.*, 2001; Liu *et al.*, 1992; Orth, 1977; Wei *et al.*, 1994). Animal exposure to BNP is a result of the consumption of forage plants by livestock. In case of human exposure, the main source is *Aspergillus* spp. and *Penicillium* spp. which are used as a starter cultures for production of traditional fermented foods such as tane-koji, shoyu, miso and katsuobushi. It has been reported that with the consumption of soy sauce and miso, exposure to BNP in Japan can be as high as 5.5 mg/day (Scott, 2004). The toxicity of BNP is irreversible changes in brain upon receiving a threshold dose with reversible inhibition of fumarase and aspartase and irreversible, noncompetitive inhibition of succinic acid dehydrogenase (Szabo *et al.*, 2005). Unfortunately, survey on natural contamination of BNP is limited in the literature. But presence of BNP was reported in cheese (Iwasaki and Kosikowski, 1973).

The main objective of the present study was to obtain detailed information about the determination of *Aspergillus* section *Flavi* isolates in Turkish dried figs with their potential of producing aflatoxins, CPA and BNP. Moreover discussion of mycotoxin production patterns consisting of three toxins will be a new approach to the determination of mycotoxigenic potential.

2. Materials and methods

Fungal isolates and preparation of spore suspensions

The fifty-seven strains used in this study belong to three species of *Aspergillus* section *Flavi*, namely 51 *A. flavus*, 5 *A. parasiticus* and 1 *A. tamarii*, isolated from dried fig samples collected from orchards according to European Commission sampling procedures (EC, 1998) during the drying stage in seven different districts (Erbeyli, Germencik, Incirliova, Ortaklar, Selcuk, Soke and Torbali) in the Aegean Region of Turkey (Heperkan *et al.*, 2012). Enumeration, isolation and identification of the mycoflora were performed as explained in Heperkan *et al.* (2012). Moreover identification of all *Aspergillus* section *Flavi* members was confirmed by molecular methods using PCR amplification of the ITS1 and ITS4 rDNA regions. Species were identified by comparing partial 18S rDNA sequences (Oktay *et al.*, 2011). All pure isolates were maintained on a slant of malt extract agar at -18 °C for further studies. Production of aflatoxins, CPA and BNP by isolates was determined on yeast extract sucrose, czapek yeast extract and potato dextrose agar, respectively. Strains were inoculated at a central single point on petri dishes (diameter 6 cm) containing 5 ml of medium with 1 µl of spore suspension (1-5×10^6 spores/ml) and incubated at 25 °C for 7 days.

Instrumentation

The liquid chromatograph used for aflatoxin analysis, was an Agilent Technologies 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a fluorescence detector set at an excitation wavelength of 360 nm and emission wavelength of 420 nm, a quaternary pump, a vacuum degasser and a Rheodyne injector with a 100 µl loop (Rheodyne, Rohnert Park, CA, USA). The separation was achieved at room temperature on an ODS Hypersil C_{18} reversed phase column (250×4.6 mm, 5 µm particle size; Supelco, Bellefonte, PA, USA) and a post-column derivatisation with a Kobra® Cell (R-Biopharm AG, Darmstadt, Germany). For CPA determination, the chromatographic system consisted of an Agilent Technologies 1100 system equipped with a photodiode array detector set at 279 nm wavelength, a binary capillary

pump, a vacuum degasser, a column oven and a micro autosampler. The separation was achieved at 25 °C on a LiChrosorb-NH_2 column (250×2.1 mm, 5 µm particle size; Supelco). All data were processed by Chemstation 3D software (Agilent Technologies).

Aflatoxin quantification

Aflatoxin analysis was carried out according to Stroka *et al.* (2000). Content of the petri dish was extracted with methanol:water (8:2, v/v) and NaCl. The extracts were filtered through a filter paper (Whatman no. 4; GE Healthcare Life Sciences, Freiburg, Germany). The filtrate (1.5 ml) was diluted with 9 ml of phosphate buffered saline (PBS) and applied to a PBS-conditioned immunoaffinity column (AflaTest®; Vicam, Watertown, MA, USA) with a flow rate of <3 ml/min. Aflatoxin was eluted with 1.25 ml of methanol. The eluate was evaporated to dryness under nitrogen at 40 °C and the residue redissolved in 1.25 ml methanol and 1.25 ml water, then quantified by high-performance liquid chromatography (HPLC). The mobile phase consisted of distilled water:acetonitrile:methanol (6:2:3, v/v/v) with 119 mg/l of KBr and 110 µl/l of 65% HNO_3. The injection volume was 100 µl and flow rate was 1 ml/min. Detection limits for aflatoxins B_1 and G_1 were 0.05 ng/g, and for aflatoxins B_2 and G_2 were 0.03 ng/g. Average recoveries of three replicates for aflatoxins were 95, 101, 99.5 and 88.1% for aflatoxins B_1, B_2, G_1 and G_2, respectively.

Cyclopiazonic acid quantification

CPA analysis was carried out according to Hayashi and Yoshizawa (2005) and Urano *et al.* (1992b). Content of the petri dish was extracted with 2% methanol:$NaHCO_3$ (7:3, v/v). The extracts were filtered through a filter paper (Whatman no. 1). The filtrate (50 ml) was transferred to a separating funnel (Isolab, Wertheim, Germany) and defatted with 50 ml hexane for 1 min. The lower phase was transferred to another separating funnel and 50 ml KCl (10%) was added. The pH was adjusted to 2.0 with 6N HCl solution and extracted twice with 50 ml chloroform. The lower layer was collected and filtered through 10 g anhydrous Na_2SO_4. The eluate was evaporated to dryness using a rotary evaporator (Buchi, Flawil, Switzerland) at 40 °C and the residue was re-dissolved in 3-4 ml chloroform and then evaporated to dryness under nitrogen; the residue was redissolved in 2 ml of mobile phase and then quantified by HPLC. The mobile phase consisted of acetonitrile:amonnium acetate buffer (0.05 M, pH=5) (8:2, v/v). The injection volume was 20 µl and flow rate was 1 ml/min. Average recovery of CPA (three replicates) was 74%. The limit of detection (LOD) was 50 ng/g.

Thin layer chromatography analysis of β-nitropropionic acid

Analysis of BNP was carried out semiquantitatively by a modification of the methods of Wei *et al.* (1994) and Frisvad and Thrane (1987). An approximately 10 mm diameter agar plug was cut out, weighed and transferred into a flask containing 100 ml of potato dextrose broth. After 7 days at 25 °C, the broth was filtered through a filter paper (Whatman no. 1). The pH of the filtrate was adjusted to 2-3 with 6N HCl and was extracted twice with 100 ml of ethyl acetate. The upper layer was filtered through 10 g anhydrous Na_2SO_4 and the eluate was evaporated to dryness using a rotary evaporator at 40 °C. The residue was dissolved in 4 ml ethyl acetate, evaporated to dryness under nitrogen and re-dissolved in 2 ml of chloroform-methanol (2:1, v/v). Thin layer chromatography aluminum plates precoated with silicagel G60 were obtained from Merck (Darmstadt, Germany). After spotting, the plates were developed in the solvent mixture toluene:ethyl acetate:formic acid 90% (5:4:1, v/v/v) and dried at room temperature. Finally, plates were sprayed with diazotised *p*-nitroaniline reagent (6.5 ml of 2.5% $NaNO_2$ (in water) and 100 ml of 0.3% paranitroaniline (in 1 N HCl)). BNP appeared as a yellowish orange spot in daylight and was quantitated by visual comparison with standards and using Equation 1:

$$\mu g\ BNP/kg = \frac{S \times Y \times V}{X \times W} \quad (1)$$

Where, S = volume of BNP standart gives the same colour intensity with sample extract (µl); Y = concentration of BNP standart (µg/ml); V = dilution factor of sample extract (µl); X = volume of sample gives same colour intesity with the S amount of mycotoxin standart (µl); W = sample amount within the liquid transferred to a separation funnel (g).

3. Results

Mycotoxin production of isolates

Mycotoxin production of *Aspergillus* section *Flavi* isolates from dried figs are shown in Table 1. Fifty out of 51 strains of *A. flavus* (98%) were aflatoxins (range 0.3-1,620 ng/g) and CPA (range 0.07-398 µg/g) producers. However, 16 out of the 51 strains of *A. flavus* (32%) were BNP positive (range 7.9-1,571 ng/g). None of the *A. flavus* isolates produced more aflatoxin B_2 than aflatoxin B_1 and G type of aflatoxins.

80% of *A. parasiticus* strains produced B and G type of aflatoxins simultaneously, whereas 20% (1 isolate) of them produced only B type of aflatoxins atypically. Along with this atypical strain, another *A. parasiticus* strain was a CPA producer. Aflatoxin production level of *A. parasiticus* strains ranged from 106.4 to 1,255 ng/g whereas CPA ranged from 0.05 to 0.06 µg/g. *A. tamarii* (1 isolate) was determined to be a CPA producer (2.69 µg/g) but with no aflatoxins. However it should be noted that both *A. parasiticus* and

Table 1. Mycotoxin production of *Aspergillus* section *Flavi* isolates from dried figs.

Isolate code	Sampling region	Species	Aflatoxin (ng/g)				Cyclopiazonic acid (µg/g)	β-nitropropionic acid (ng/g)
			B_1	B_2	G_1	G_2		
1	Germencik	*A. flavus*	1,020.67	5.38	–[1]	–	8.68	ND[2]
2	Germencik	*A. flavus*	1,110.51	13.62	–	–	7.91	293.3
3	Germencik	*A. flavus*	164.24	0.48	–	–	35.98	ND
4	Torbali	*A. flavus*	1.13	–	–	–	2.23	279.3
5	Germencik	*A. flavus*	448.91	13.34	–	–	38.12	ND
6	Erbeyli	*A. flavus*	1,251.55	10.08	–	–	9.13	ND
7	Germencik	*A. flavus*	3.30	0.58	–	–	8.65	ND
8	Incirliova	*A. flavus*	503.30	16.40	–	–	31.24	ND
9	Germencik	*A. parasiticus*	32.56	0.33	72.64	0.91	–	ND
10	Germencik	*A. flavus*	1,106.70	17.35	–	–	22.19	523.7
11	Selcuk	*A. flavus*	1,213.56	3.15	–	–	16.30	ND
12	Incirliova	*A. flavus*	1,314.72	5.18	–	–	8.71	ND
13	Erbeyli	*A. flavus*	1.79	–	–	–	10.45	261.9
14	Erbeyli	*A. flavus*	1,067.65	8.55	–	–	9.01	392.8
15	Incirliova	*A. flavus*	945.47	15.08	–	–	9.21	ND
16	Soke	*A. flavus*	1,446.40	7.35	–	–	10.00	ND
17	Selcuk	*A. flavus*	1,311.06	4.76	–	–	21.78	785.6
18	Germencik	*A. flavus*	239.70	0.84	–	–	47.60	1047.5
19	Incirliova	*A. flavus*	45.11	0.56	–	–	10.83	ND
20	Selcuk	*A. flavus*	912.25	10.51	–	–	6.43	ND
21	Ortaklar	*A. flavus*	29.98	1.77	–	–	3.15	ND
22	Ortaklar	*A. flavus*	0.78	–	–	–	3.87	ND
23	Torbali	*A. flavus*	251.12	2.15	–	–	24.92	ND
24	Selcuk	*A. flavus*	0.30	–	–	–	6.87	183.3
25	Ortaklar	*A. flavus*	1,166.33	19.70	–	–	4.78	ND
26	Ortaklar	*A. parasiticus*	242.28	2.94	54.49	0.24	–	ND
27	Incirliova	*A. flavus*	1,570.60	10.98	–	–	398.16	ND
28	Germencik	*A. flavus*	11.97	0.85	–	–	35.93	7.9
29	Germencik	*A. tamarii*	–	–	–	–	2.69	ND
30	Soke	*A. flavus*	1,606.83	12.96	–	–	18.16	13.1
31	Soke	*A. parasiticus*	478.05	5.36	185.06	2.52	0.059	ND
32	Germencik	*A. flavus*	1,163.09	11.16	–	–	38.31	10.5
33	Erbeyli	*A. flavus*	1,447.28	17.99	–	–	8.55	ND
34	Germencik	*A. flavus*	1,370.58	15.13	–	–	6.24	ND
35	Incirliova	*A. flavus*	394.23	5.08	–	–	33.75	ND
36	Selcuk	*A. parasiticus*	539.06	3.39	–	–	–	ND
37	Torbali	*A. flavus*	960.02	7.18	–	–	30.78	ND
38	Torbali	*A. flavus*	2.09	–	–	–	4.29	144.0
39	Torbali	*A. flavus*	1,239.35	12.03	–	–	50.41	ND
40	Germencik	*A. flavus*	281.26	3.27	–	–	20.71	ND
41	Germencik	*A. flavus*	1,328.45	2.72	–	–	6.56	ND
42	Torbali	*A. flavus*	624.48	8.50	–	–	8.45	ND
43	Torbali	*A. parasiticus*	793.80	6.39	452.94	1.85	0.052	ND
44	Germencik	*A. flavus*	436.74	1.91	–	–	7.14	34.9
45	Germencik	*A. flavus*	867.76	11.55	–	–	4.60	69.8
46	Selcuk	*A. flavus*	495.05	6.83	–	–	4.40	ND
47	Soke	*A. flavus*	759.92	15.53	–	–	4.39	ND
48	Incirliova	*A. flavus*	1.82	–	–	–	0.072	ND
49	Germencik	*A. flavus*	1.07	–	–	–	–	ND
50	Germencik	*A. flavus*	267.93	5.79	–	–	21.71	ND
51	Ortaklar	*A. flavus*	312.93	4.95	–	–	21.44	ND
52	Incirliova	*A. flavus*	–	–	–	–	13.44	1571.2
53	Incirliova	*A. flavus*	1.42	–	–	–	0.088	ND
54	Ortaklar	*A. flavus*	1.70	–	–	–	0.084	ND
55	Ortaklar	*A. flavus*	628.32	5.69	–	–	32.50	ND
56	Ortaklar	*A. flavus*	10.01	0.99	–	–	0.103	ND
57	Ortaklar	*A. flavus*	1.38	–	–	–	11.14	18.3

[1] < limit of detection.

[2] Not detected.

A. tamarii strains can be the producer of aflatoxins just below the LODs.

Prevalence of mycotoxins produced by *A. flavus* isolates is shown in Table 2. Most of the isolates (35%) produced aflatoxins between 100 and 1000 ng/g while 33% of them were able to produce aflatoxins at high levels (>1000 ng/g). The level of CPA produced by *A. flavus* strains was higher than of aflatoxins, in fact 53% and 43% of them were able to produce CPA at moderate and high levels of <10 and 10-100 µg/g, respectively. 16% of the isolates were able to produce BNP in the range of 100-1000 ng/g while only 2 of them (4%) produced BNP at a high level (Table 2).

Classification of isolates by toxigenic profiles

The classification of *Aspergillus* section *Flavi* isolates regarding aflatoxins, CPA and BNP production are shown in Table 3.

Aspergillus flavus strains were classified into 6 different chemotypes based on mycotoxigenic profiles. Isolates able to produce both B type of aflatoxins and CPA comprised the most represented chemotype (e) followed by the chemotype (f), which contains aflatoxin B_1, CPA and BNP producers. To the best of our knowledge, an *A. flavus* isolate producing CPA and BNP but not aflatoxin has been found for the first time.

Regarding toxigenic profiles of *A. parasiticus* strains, 3 chemotypes were categorised. Two of them were considered atypical because of the ability to produce only B type of aflatoxins (g) and CPA along with B and G type of aflatoxins (i).

4. Discussion

Among 57 strains screened, they were all toxigenic, producing one or more of the toxins. The previously reported percentages of aflatoxigenic isolates of *Aspergillus*

Table 2. Frequency of mycotoxins produced by *Aspergillus flavus* isolates.

Toxin level	Number of toxin producing strains (%)[1]		
	Aflatoxins (ng/g)	Cyclopiazonic acid (µg/g)	β-nitropropionic acid (ng/g)
None	1 (2%)	1 (2%)	35 (68%)
<10	10 (20%)	27 (53%)	1 (2%)
10-100	5 (10%)	22 (43%)	5 (10%)
100-1000	18 (35%)	1 (2%)	8 (16%)
>1000	17 (33%)	0	2 (4%)

[1] Percentage of 51 *A. flavus* strains.

Table 3. Mycotoxigenic profiles of *Aspergillus* section *Flavi* strains.

Species	Toxin profile	Aflatoxins[1]				CPA[2]	BNP[3]	Number of strains (%)[4]
		AFB_1	AFB_2	AFG_1	AFG_2			
A. flavus (n=51)	a	–	–	–	–	+	+	1 (1.8%)
	b	+	–	–	–	–	–	1 (1.8%)
	c	+	–	–	–	+	–	4 (7.1%)
	d	+	–	–	–	+	+	5 (8.9%)
	e	+	+	–	–	+	–	30 (53.5%)
	f	+	+	–	–	+	+	10 (17.9%)
A. parasiticus (n=5)	g	+	+	–	–	–	–	1 (1.8%)
	h	+	+	+	+	–	–	2 (3.6%)
	i	+	+	+	+	+	–	2 (3.6%)

[1] AFB_1, AFB_2, AFG_1, AFG_2 = aflatoxin B_1, B_2, G_1, G_2.
[2] CPA = cyclopiazonic acid.
[3] BNP = β-nitropropionic acid.
[4] Percentage of the 56 isolates.

section *Flavi* are quite variable depending on geographic and commodity origin of the strains (Riba *et al.*, 2010; Rodrigues *et al.*, 2009; Sanchez-Hervas *et al.*, 2008; Vaamonde *et al.*, 2003). Although 98% of the *A. flavus* isolates recovered in this study were aflatoxigenic, a number of authors have reported that not all *A. flavus* are capable of producing aflatoxin. Studies on *A. flavus* strains isolated from dried foods (dried beans, maize, macaroni, pecans), various cereal grains, smoked-dried meat products, peanuts, soybeans and animal feeds, which are less susceptible to aflatoxin contamination, reported low incidence (5-51%) of aflatoxigenic *A. flavus* strains (Cvetnic and Pepeljnjak, 1998; Martins and Martins, 1999; Trucksess *et al.*, 1987; Vaamonde *et al.*, 2003). Studies on *A. flavus* isolates from Brazil nut, cocoa bean, maize, wheat, wheat derivatives, black and white sultana and dried figs, showed a higher rate (64-100%) of aflatoxigenic *A. flavus* strains in concordance with our results (Arrus *et al.*, 2005; Giorni *et al.*, 2007; Iamanaka *et al.*, 2007; Lisker *et al.*, 1993; Riba *et al.*, 2010; Sanchez-Hervas *et al.*, 2008; Vaamonde *et al.*, 2003).

Of the 51 strains of *A. flavus* analysed, 35 and 33% of them were found to be moderately and highly aflatoxigenic with mean levels of aflatoxins ranging from 100-1000 ng/g and >1000 ng/g, respectively. *A. flavus* isolates producing higher levels of aflatoxins on culture media ranging from 0.004 to 234.6 µg/g have been reported in the literature (Cvetnic and Pepeljnjak, 1998; Lisker *et al.*, 1993; Riba *et al.*, 2010; Trucksess *et al.*, 1987). Atypical *A. flavus* strains capable of producing both B and G type of aflatoxins were not recovered in contrast to studies conducted on *A. flavus* isolates from cocoa beans (Sanchez-Hervas *et al.*, 2008), maize (Giorni *et al.*, 2007), peanut (Pildain *et al.*, 2004), wheat, soybean (Vaamonde *et al.*, 2003) and peanut soil (Barros *et al.*, 2006). Besides aflatoxins production, 98% of *A. flavus* strains were able to produce CPA. This high incidence of *A. flavus* isolates producing CPA (93-100%) was also found in studies on raisins, peanut and wheat (Romero *et al.*, 2005; Vaamonde *et al.*, 2003). On the other hand, in some studies, a lower percentage of CPA producing *A. flavus* strains than in our findings was observed. Lisker *et al.* (1993) reported 22.5% of *A. flavus* isolates from peanut were CPA producers while Cvetnic and Pepeljnjak (1998) indicated only 5.2% of *A. flavus* strains from various grain and smoked meat products were CPA producers.

Recently, it was reported that only 10% of *A. flavus* strains from wheat and wheat derivatives were able to produce CPA (Riba *et al.*, 2010). In this study, the equal percentage of aflatoxins and CPA producers indicates that CPA is as common a metabolite as aflatoxins of *A. flavus.*

The level of CPA produced by *A. flavus* strains was higher than aflatoxins producing moderate (<10 µg/g) and high level (10-100 µg/g). Several authors reported that the level of CPA produced in culture media ranging from 0.2 to 240.7 µg/g in concordance with our results (Cvetnic and Pepeljnjak, 1998; Sanchez-Hervas *et al.*, 2008).

28% of the total strains were able to produce BNP moderately; among these none of them was *A. parasiticus* nor *A. tamarii*. The reported levels of BNP produced by reference *A. flavus* strains in different culture media were 1 µg/g and 17 µg/g (Gilbert *et al.*, 1977; Iwasaki and Kosikowski, 1973). In this study, it was found that only 4% of *A. flavus* strains produced BNP higher than 1 µg/g. In a study from Egypt, of the 15 *A. flavus* strains from silage samples, 7, 9 and 1 isolates were aflatoxins, CPA and BNP producers, respectively (El-Shanawany *et al.*, 2005). In another study, 7 out of 16 *A. oryzae* strains used as a starter cultures, were BNP producers in the range of 1.9-43.6 µg/ml and 3 of them were CPA producers. However, none of the strains produced aflatoxins (Orth, 1977).

Research studies on *A. parasiticus* isolates revealed also different but comparable results throughout the world. Usually *A. parasiticus* isolates were reported to be more uniform in producing both B and G type of aflatoxins (Horn *et al.*, 1996). There are a few reports showing the presence of nontoxigenic *A. parasiticus* isolates (Rodrigues *et al.*, 2009; Tran-Dinh *et al.*, 1999; Vaamonde *et al.*, 2003). In the present study, it was found that all *A. parasiticus* strains were strongly aflatoxigenic producing with total aflatoxins in the range of 106.4-1,255 ng/g. Razzaghi-Abyaneh *et al.* (2006) and Rodrigues *et al.* (2009) also showed that all *A. parasiticus* isolates from soil and almonds were highly aflatoxigenic similar with our result.

Comparing aflatoxin production ability of *A. flavus* and *A. parasiticus* strains, 22% of *A. flavus* strains were able to produce aflatoxins higher than *A. parasiticus* strains. Additionally, a single *A. tamarii* isolate produced neither aflatoxins nor BNP but CPA, at a level of 2.7 µg/g. In the literature there is no report on production of BNP by *A. parasiticus* or *A. tamarii* species, which supports our results. However as it is in the case of atypical *A. parasiticus* strains it should be noted that *A. tamarii* strain can be the producer of aflatoxins but just below the LODs.

Regarding mycotoxin production patterns of the isolates, 9 different chemotypes were found in this study. This classification was done similarly in previous studies from Argentina (Vaamonde *et al.*, 2003), Iran (Razzaghi-Abyaneh *et al.*, 2006), Italy (Giorni *et al.*, 2007), Spain (Sánchez-Hervás *et al.*, 2008), Portugal (Rodrigues *et al.*, 2009) and Algeria (Riba *et al.*, 2010). However, studies on determining chemotypes of *Aspergillus* section *Flavi* strains, aflatoxins and CPA production ability of the isolates from different food commodities (peanut, cocoa beans, almonds) and soil were evaluated. This study differs from previous studies not only in origin of the isolates but also in containing results on BNP in addition to aflatoxins and CPA. The highest

proportion of the chemotypes represented by *Aspergillus* section *Flavi* strains produced B type of aflatoxins and CPA together, agreeing with Vaamonde *et al.* (2003), Pildain *et al.* (2004) and Giorni *et al.* (2007). This result suggests that production of CPA is correlated with production of B type of aflatoxins. But no strains produced more aflatoxin B_2 than aflatoxin B_1 unlike the studies conducted in Iran and Spain (Razzaghi-Abyaneh *et al.*, 2006; Sanchez-Hervas *et al.*, 2008). The chemotype with second highest proportion represented by *A. flavus* strains consisted of AFB, CPA and BNP producers. Since BNP production of *A. flavus* strains were determined on isolates from dried figs, it was thought that BNP could be common in other agricultural products as well. However, there are few studies to verify this.

Moreover, it has been reported that *A. parasiticus* species have more uniform characteristics for production of B and G type aflatoxins but not CPA (Klich, 2007), in concordance with our results. However, rare chemotypes (aflatoxin type B and G, and CPA) which was consisted of atypical *A. parasiticus* strains was found.

5. Conclusions

In conclusion, these results reveal that toxigenic strains of *A. flavus* on dried figs are prevelant within the mycoflora of dried figs grown in Turkey. Because the samples taken for the study from the Aegean region of Turkey cover more than 85% of Turkey's dried fig production. As a consequence co-occurrence of aflatoxins, CPA and BNP may take place in dried figs or other food commodities in which *A. flavus* is one of the major contaminants. Further studies are needed to define natural coexistence of these toxins in different commodities. Because of synergistic or antagonistic interactions of these mycotoxins, toxicological studies should also be considered for potential health hazard.

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