Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [Special Issue V] 2014: 105-109

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

An investigation on Ochratoxin production patterns in the northern Iran aspergilli fungal culture media in laboratory conditions

Hedieh Jafarzadeh, Leila Modiri*, Arash Chaichi Nosrati
Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran
Email:leim_clinpathem@yahoo.com

ABSTRACT
Study of the ochratoxins, except in some localized areas of the world, has generally been of much lower priority. This situation seems to be changing, as evidenced by the recent efforts of public health officials to develop estimates of human risk from exposure to ochratoxin'A. Furthermore, the Commission on Food Chemistry, IUPAC, has embarked on a worldwide effort to document the natural occurrence of ochratoxin. It is the purpose of this paper to summarize recent information on the occurrence, toxicology and public health significance of human exposure to ochratoxin A. One sample group was taken by six plates including Malt extract agar(MEA),Yeast extract agar(YEA),Czapek,s agar(CZA), Czapeks Yeast extract agar(CZYA), Saboraudsdextrouse agar(SDA) and Potato dexterous agar (PDA), all with 100 ppm chloramphenicol, respectively. All plates were incubated at 25°C aerobically, then examined in the periods of 3,7,10 and 14 days to identify any growing's so that they were harvested, subcultured,marked and then cultivated in the conserving prepared plates.To detect ochratoxin levels in the fungal biomasses and the culture medium samples using the competitive ELISA Procedure as described by R-Bio-Pharm GmbH was used and measured at the absorbance of 450nmIn the studies we conducted on the abundance and distribution of isolates producing minimum, average, and maximum ochratoxin, the species A. carbonarius and A. melleus had maximum toxin production with the values 8.007 ppb and 6.145 ppb, and the species A. Candidus, A. sojae, and A. sp IV had produced minimum amounts of toxin with the values 1.967 ppb, 1.835 ppb, and 1.939 ppb, respectively.
Keywords:Ochratoxin, aspergillifungal, production patterns, culture mediain , northern, Iran

Received 19.06.2014 Revised 19.08.2014 Accepted 30.10. 2014

INTRODUCTION

Frank growth of fungi on animal hosts produces the diseases collectively called mycoses, while dietary, respiratory, dermal, and other exposures to toxic fungal metabolites produce the diseases collectively called mycotoxicosis. The mechanisms of pathogenesis of both primary and opportunistic fungi are complex, and medical mycologists have devoted considerable research energy trying to identify the factors that distinguish fungal pathogens from saprophytic and commensal species [2, 6]. On the other hand, there is relatively little evidence that mycotoxins enhance the ability of fungi to grow in vertebrate hosts. *Aspergillus fumigates* is case in point. It is the major species associated with aspergillosis and produces gliotoxins (inhibitors of T-cell activation and proliferation as well as macrophage phagocytosis). For this and other reasons, the current view is that while some mycotoxins are known pathogenicity factors in plants, their significance in human mycoses is not yet clear. Toxicologists tend to concentrate their efforts on hazardous chemicals such as polyaromatic hydrocarbons, heavy metals, and organic pesticides. Because they have devoted less effort to natural products, agriculturalists, chemists, microbiologists,and veterinarians who are often unfamiliar with the basic principles of toxicology have conducted most of the mycotoxin research. There has been a lot of reinventing of the wheel and sometimes an imprecise use of toxicology jargon [1,5,7]. In recent years the attention of most mycotoxinologists has been focused to a great extent on the continuing quest for better ways to control exposure to aflatoxins, resolution of the questions relating to aflatoxin's acute and chronic toxicity (i.e., carcinogenicity) for humans, and an ever-expanding study of the myriad metabolites of the Fusaria

(especially trichothecenes).Study of the ochratoxins, except in some localized areas of the world (e.g., Denmark, Sweden), has generally been of much lower priority. This situation seems to be changing, as evidenced by the recent efforts of public health officials in various countries (Germany, Canada, and United States) to develop estimates of human risk from exposure to ochratoxin'A. The main difficulty in developing such risk estimates seems to be the paucity of data on human exposure to this mycotoxin and comparative toxicology data for translating observations in animals to humans. It has always been somewhat of an enigma that human exposure to ochratoxin seems to be localized in some countries ofnorthern Europe and the Balkans and may possibly be responsible for a serious human nephropathy, but is not generally found in other areas of the world .Nevertheless, the International Program on Chemical Safety/World Health Organization, in its latest review of environmental contaminants, has recently selected ochratoxin as one of the mycotoxins for which sufficient new information indicates a need for further study and evaluation with respect to human health potential. Furthermore, the Commission on Food Chemistry, IUPAC, has embarked on a worldwide effort to document the natural occurrence of ochratoxin. It is the purpose of this paper to summarize recent information on the occurrence, toxicology and public health significance of human exposure to ochratoxin A [1,4].

MATEREALS AND MTHODS

From the May - October 2010-2011, sampling was done using settle plates from agricultural area fields and also per each processing plant ,according to "CBS" instructions from indoor and outdoor stations. One sample group was taken by six plates including Malt extract agar(MEA),Yeast extract agar(YEA),Czapek,s agar(CZA),Czapeks Yeast extract agar(CZYA), Saboraudsdextrose agar(SDA) and Potato dexterous agar (PDA), all with 100 ppm chloramphenicol, respectively. All plates were incubated at 25°C aerobically, and then examined in the periods of 3,7,10 and 14 days to identify any growing so that they were harvested, subcultured, marked and then cultivated in the conserving prepared plates. Finally, for macroscopic and microscopic morphology examinations,107 aspergillus colonies were cultivated and grown at 25°C in order to identify and rank the colonies, various conventional mycological methods were used based on the ICPA rules for morphologic and microscopic and macroscopic examinations. Afterward fungal biomasses and culture media were harvested then dried out in desiccators Then, the samples were converted to powder by pearl/vortex and the initial culture media separated so that to be passed from plesezec number 20 (8, 9). The samples were packaged in plastic pocket to be away from any moisture that may cause growing fungi and increasing the amount of Aflatoxin (3,8).

ELISA determination

To detect ochratoxin levels in the fungal biomasses and the culture medium samples using the Competitive ELISA Procedure as described by R-Bio-Pharm GmbH was used .
And measured at the absorbance of 450nm (7).

RESULTS AND DISCUSSION

Stages in the three regions of Gilan and Mazandaran and Golestan.

Section Flavi isolates from 24 species consist 25% of the 6 most abundant species listing;Wentii, Candidi, Fumigati, Flavipedes, Nidulantes and Ornati with 1 sample and 4.2% with the lowest frequency. Regional distribution of the number of samples and Aspergillus species studied in Gilan Province Gilan region of examination that these 24 isolates consisting; *A. flavus*, *A.*af.*flavus,A. niveus* each 13% of the species *A. parasiticus A. sojae, A. alliaceus, A. awamori, A. carbonarius, A. fumigatus, A. foetidusA. ostianus, S. ornate,* each individually one isolated and found to be 6%(Table.1).
Regional distribution of the number of samples and Aspergillus species studied in Mazandaran showed that, of 24 species in the Caspian region examination, the isolated strains of *A. flavus* and *A.*af.*flavus* each 16 %*A. wentii, A. niveus, A. terreus, S. ornata ,A. ostianus* each 8 percent and *A. sojae, A. parasiticus A. alliaceus, A. niger, A. awamori, A. carbonarius,* 4% were found to isolate and separateed(Figure 1).
Regional distribution of the samples and Aspergillus species studied in Golestan Province resumed that, of 24 species were isolated were the strains of *A. flavus ,A.*af.*flavus, A. fumigatus,* each 18 percent and *A. niveus, A. sojae, A. parasiticus A. alliaceus, A. ostianus*each isolate and 9% were found(Figure 2).
In the ranged conducted geographic area, there is an almost constant population in terms of both the relative dispersion and the species which can be isolated from there. This showed that, in best sampling conditions, about 10-15% of new samples can be found and 85-90% of previous samples are again found which represents the uniqe ecosystem in microbial, fungal, and Aspergillus terms. When the conditions are the same for all fungi and we grown all of them in a fixed medium, the probability that environmental effects cause changes in their toxin production behavior is minimized. If we observe any environmental effects on them in the laboratory, they result from their own genome, genetic ability, and genetic diversity. In toxin production by Aspergillus, it is believed that a repeated specific species of Aspergillus

tend to produce toxin, these groups are limited and toxin production is exclusive in some of them. This belief stems from sparse reports most of which are related to the studies done in Europe. In our comparison of toxin production status of Guilan with Mazandaran, it was found that the toxin production level in Mazandaran was reversely related to that in Guilan, that is, if the same species producing high toxin levels in Guilan is found in Mazandarn, it will produce a small amount of toxin. Also, Mazandaran's toxin production amounts produced by aspergilla have a reverse relationship with Golestan's but the relationship is not significant(> 0.05).The significance of the difference between Guilan and Mazandaran mean numerical toxin ratios were much lower that the significance of the difference between Golestan and Mazandaran. As obtained a significant difference between Golestan and Mazandaran the certainty reasons that can be mentioned, there are surface, middle, and high winds which move from west to east dispersing the aspergilla spores in the whole area therefore it could be seen an isolated species bouthin the south of Caspian Sea, and usually be observed up to the eastfar regions. But we realized in our investigations that some species are only seen in some particular areas. For example:*A. terreus*and *S. ornata* only exist in Gilan and Mazandaran but never found in Golestan,*A. wentii* exists in Mazandaran but never found in Golestan and Guilanves versa.

In the study of Guilan region, 24 species were isolated among which *A. flavus, A. niveus,* and *A.*af.*flavus* have the maximum frequency, each 13%.

In the study of Mazandaran region, 24 species were isolated among which *A. flavus, and A..flavus* have had the maximum frequency, each of 16%.

In the study of Golestan region, 24 species were isolated among which *A. flavus, A. fumigatus,* and *A. nomius* have had the maximum frequency, each 18%.

In the studies by performing ELISA on 24 species of interest culture media, the mean amount of the tested toxin production were as follows:

*A. wentii*from the subgenus Circumdati with 2 isolated samples valued as 3.05 ppb for toxin production.

A. flavus,with 11 isolated samples valued as 3.22ppb for toxin production.

A. sojae,with 6 isolated samples valued as 1.93 ppb for toxin production.

A. parasiticus,with 5 isolated samples valued as 2.72 ppb for toxin production.

A. alliaceus,with 1 isolated sample valued as 3.24 ppb for toxin production.

A. niger,with 1 isolated sample valued as 3.53ppb for toxin production.

A. awamori,with 2 isolated samples valued as 4.24ppb for toxin production.

A. carbonarius,with 2 isolated samples valued as 8.00 ppb for toxin production.

Also, in comparison of indoors with outdoors during the examination into dispersion of the isolates obtained from process in plants and any places of food processing, it could said that there is no significant relations ($p>0.05$) and it cannot regard a specific covered area as having unique characteristics and containing high population density of any one particular species with toxin production ability in a restricted range.

In the studies we conducted on the abundance and distribution of isolates producing minimum, average, and maximum ochratoxin, the species *A. carbonarius* and *A. melleus* had maximum toxin production with the values 8.007 ppb and 6.145 ppb, and the species *A. Candidus*, *A. sojae*, and *A. sp*IV had produced minimum amounts of toxin with the values 1.967 ppb, 1.835 ppb, and 1.939 ppb, respectively, noticeably our criterion for statistical comparison was based on the average.

Table 1-Frequency of isolated Section groups species

sec					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-	3	12.5	12.5	12.5
	wentii	1	4.2	4.2	16.7
	candidi	1	4.2	4.2	20.8
	Circumdati	3	12.5	12.5	33.3
	Famigati	1	4.2	4.2	37.5
	Flavi	6	25.0	25.0	62.5
	Flavipeds	1	4.2	4.2	66.7
	Nidulantes	1	4.2	4.2	70.8
	Nigri	4	16.7	16.7	87.5
	Oranati	1	4.2	4.2	91.7
	Terrei	2	8.3	8.3	100.0
	Total	24	100.0	100.0	

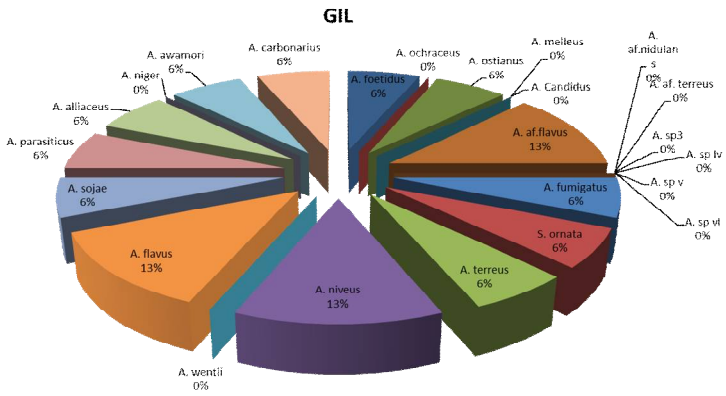

Figure 1-Regional distribution of the isolated Aspergillus species studied in Gilan province.

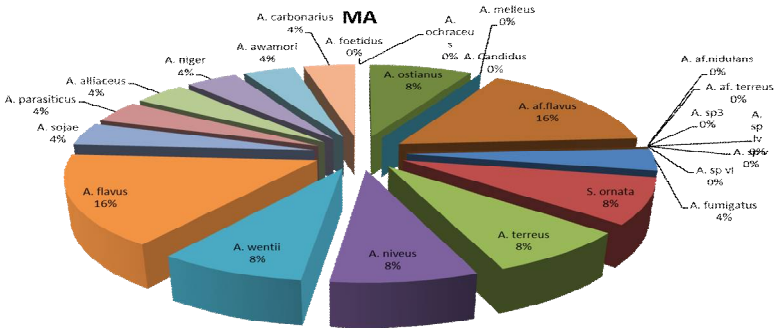

Figure 2- Regional distribution of the isolated Aspergillus species studied in Mazandaran province.

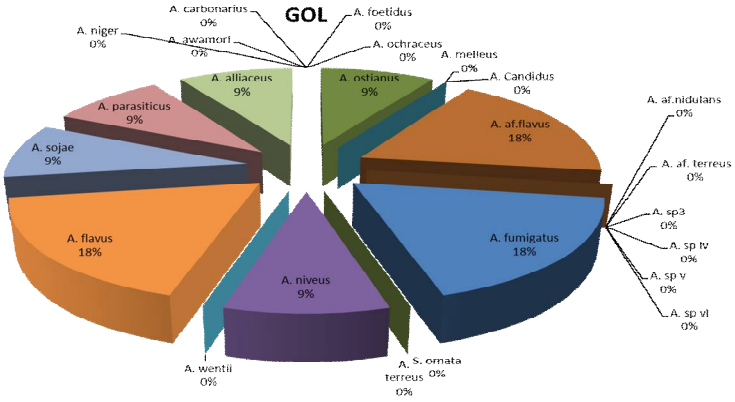

Figure 3- Regional distribution of the isolated Aspergillus species studied in Golestan province.

Table 2- Numerical average of toxin produced correlations in each three conducted geographic regions.

Correlations

			M	GIL	GOL
Spearman's rho	M	Correlation Coefficient	1.000	-.065	-.364
		Sig. (2-tailed)	.	.798	.137
		N	18	18	18
	GIL	Correlation Coefficient	-.065	1.000	.790(**)
		Sig. (2-tailed)	.798	.	.000
		N	18	18	18
	GOL	Correlation Coefficient	-.364	.790(**)	1.000
		Sig. (2-tailed)	.137	.000	.
		N	18	18	18

REFERENCES

1. Pohland, S. Nesheim, & L. Friedman.1992.Ochratoxin A: A review. Pure& Appl. Chern., Vol. 64, No. 7, pp. 1029-1046.
2. Calderone, R.A., & R.L. Cihlar. 2002. Fungal pathogenesis: principles and clinical applications. Marcel Dekker, Inc., New York, N.Y.
3. Desjardins, A.E.G.F., Spencer, R. D. ,Plattner, & M.N., Beremand. 1989. Furanocoumarin phytoalexins, trichothecene toxins and infection of Pastinac asativa by Fusarium sporotrichioides. Phtyopathology 79:170–175.
4. Kabar B., A.W. Dobson,& I. War. 2006. Strategies to prevent mycotoxin contamination of food and animal feed: A review. Critical Reviews in Food Science and Nutrition, 48: 593–619.
5. McGinnis, M. R., L. Sigler, & M. G. Rinaldi. 1999. Some medically important fungi and their common synonyms and names of uncertain application.Clin. Infect. Dis. 29:728–730.
6. Dixon, D. M. M. M. Mcneil, M. L. Cohen, B. G. Gellin, & J. R. Lamontagne.1996. Fungal infections.A growing threat. Public Health Rep.pp:226–235.
7. Rosi P., A. Borsari, G. Lasi, S. Lodi, A. Galanti, A. Fava. S. Girotti& F. Ferri. 2007. Aflatoxin M milk: Reliability of the immunoenzymatic assay. International Dairy Journal, 17: 429–435
8. Xiang Y., Y. Liu & M.L. Lee. 2006. High performance liquid chromatography Journal of Chromatography A, 1104 (1-2): 198–202.
9. Gary O. 2005. Aflatoxina and animal health. Iowa State University, Ames, Iowa, pp: 1–4.

CITATION OF THIS ARTICLE

Hedieh J, Leila M, Arash Chaichi N. An investigation on Ochratoxin production patterns in the northern Iran aspergilli fungal culture media in laboratory conditions. Bull. Env. Pharmacol. Life Sci., Vol 3 [Spl Issue V] 2014: 105-109