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Research Article

AN ASSAY OF CITRININ QUANTITY AND PRODUCTION PATTERNS IN THE CULTURE MEDIUM OF *ASPERGILLUS* ISOLATES OF IRANIAN NORTHERN STATES IN LABORATORY CONDITION

Omid Vahidnia* and Arash Chaichi-Nosrati
Department of Microbiology, Lahijan Branch, Islamic Azad University, Lahijan, Iran
**Author for Correspondence*

ABSTRACT
The present study was conducted in order to examine the amount of Citrinin produced in the liquid culture medium of *Aspergillus* isolates of the north of Iran by ELISA method. After sampling, cultivation was done in Czapek's medium containing 2% malt extract at room temperature for one week at 200 rpm. Then, filtration and separation of the fungal mass from the culture in order to measure the amount of the forgoing toxins by competitive ELISA method utilizing 2 gr medium dried in desiccator with the help of cell's physical grinding (centrifuge with glass pearl) and, after that, methanolic and acetonic extraction of culture medium in order to measure the amount of the target toxins using competitive ELISA method (r-biopharm: lRidascreen Fast Citrinin) were performed. The results showed that the produced toxins in 25 *Aspergillus* species had different significant amounts. Average Citrinin production in the isolates studied is different from 0-1655/91 ppb. Most isolates have produced toxin in the range of 0-500 ppb, which includes 20 *Aspergillus* species. The highest level of toxin production is for *A. niger* with 1655/91 ppb, and the least one is for *A. foetidus with*0/06 ppb.

Keywords: *Aspergillus, Citrinin, ELISA, Mycotoxin.*

INTRODUCTION
Many fungi have caused some diseases in animals and also human. Since these toxins are not easily recognizable, studying their characteristics is of great importance. Meanwhile, *Aspergillus*is known as one of the most important toxigenic fungi and exists in abundance in Iran's northern habitats, which are highly important habitats and the main source of nutrition and lots of foods. This microorganism produces a toxic metabolite named Citrinin. This toxin is produced by filamentous fungi such as *Aspergillus, Penicillium,* and *Monascus* and is known as a natural contaminant in grains, food, and biological fluids. These mycotoxins are mentioned as hepatonephrotoxins which their effects are particularly observed in monogastric animals including dogs and pigs. In poultry, Citrinin causes watery diarrhea, the increase in food intake, and weight loss due to kidney damage. Its effect on human is not fully specified, but kidney damage due to long-term ingestion of this toxin will probably be caused (Creppy, 2002).
Mycotoxins are small molecules known as secondary metabolites. Considering the structural diversity of these toxins, using a standard method toanalyze or diagnose it seems impossible, given that known *Aspergillus* regarding the produced toxins has not been extensively studied so far. On the other hand, with the increase infungalinfections and related damage, microbiologists' incentive toward fungal contamination in human habitats has increased. Since these toxins are not easily recognizable, studying their characteristics is of great importance. Based on the research by Raistrick *et al.*, in 1931, Citrinin was isolated from filtration of *Penicilliumcitrinum* culture for the first time (El-Adlouni *et al.*, 2006). During the research by Raistrick and Smith in 1940, antibiotical activity of Citrinin against a wide range of gram-positive bacteria was examined (Aziz *et al.*, 2006). In 1974, American Society for Microbiology studied the average production of Citrinin by *Penicillium citrinum* strain in semi-synthetic environment. In the studies carried out by Ueno and Kubota in 1976, it was reported that Citrinin has the ability to bind to DNA either in vivo or in vitro (Dietrich *et al.*, 1999). In research by Nishijima *et al.*, on 31 flour samples (wheat, rice, buckwheat, corn, rye) in Japan during 1980using TLC method, the highest and lowest levels of Citrinin production were reported (73 µg/kg and 27 µg/kg, respectively)(Nishijima, 1984). In 1984, Betina *et al.*, examined the activity of Citrinin while encountering bacteriophages, sarcoma, protozoa, and

animal and plant cells (Peraica *et al.*, 1999). In studies conducted by Saxena *et al.*, on 9 turmeric samples in India during 1984-1986 using TLC method, the highest and lowest levels of Citrinin production were reported (52 µg/kg and 42 µg/kg, respectively). In this regard, other studies have been conducted on other samples as follows: Study on 9 coriander samples, in which the amount of Citrinin production was 34µg/kg; Study on 9 fennel samples by TLC method, in which the highest and lowest levels of Citrinin production were reported 59µg/kg and 28µg/kg, respectively; Research on 8 samples of black and white pepper with Citrinin production amount of 50µg/kg;Study on 6 cardamom samples which has included the Citrinin production amountof 25µg/kg; Research on 8 cumin samples, in which the amount of Citrinin production was reported 22 µg/kg (Saxena, 1989). Based on the study by Reddy *et al.*, in 1988, the toxicity process of Citrinin was examined in the immune system of mice (Geiser *et al.*, 2007). In research carried out by Janardhana *et al.*, on 197 corn samples in India during 1994-1997 and using TLC method, the amount of Citrinin production has been reported 12µg/kg (Janardhana *et al.*, 1999). According to the studies of Blanc *et al.*, in 1995, producing Citrinin by different species of Monascus was studied (Chaichi *et al.*, 2006). In research by Curtui *et al.*, on 30 corn samples in Romania in 1997using ELISA method, the amount of Citrinin production has been reported 580µg/kg (Curtui *et al.*, 1998). In the studies of Dick (1988) on 4 wheat samples in Switzerland in 1997 by HPLC method, the highest and lowest levels of Citrinin production were reported 0.7µg/kg and 0.3µg/kg, respectively (Dick, 1988). in research done by Vrabcheva *et al.*, on 24 wheat bran samples in Bulgaria in 1998 using ELISA, the highest and lowest levels of Citrinin production were reported 230µg/kg and 5.9µg/kg, respectively; in another research on 37 wheat samples, the highest and lowest levels of Citrinin production are 420µg/kg and 20µg/kg, respectively (Vrabcheva *et al.*, 2000). In studies conducted by Abd-Allah *et al.*, on 30 rice samples in Egypt in 2002utilizing fluorometer method, the highest and lowest levels of Citrinin production have been reported 28.54µg/kg and 2.74µg/kg, respectively (Abd-Allah *et al.*, 2005).

Aspergillus is one of the most important fungi producing Citrinin, which can be broadly found in the north of Iran's habitats, as the main source of food. Since producing Citrinin by different genera has not been studied in Iran, and numerous species and the process of producing and secreting this toxin have remained unclear, the present research aimed at studying the production of Citrinin in the liquid culture medium of *Aspergillus* isolates and comparing the amount of the toxin produced in different species of *Aspergillus* in the north of Iran.

MATERIALS AND METHODS

The present study is of prospective, cross-sectional, observational type. The sampling was done following the instruction of sampling from open and close sites (firm) CBS. The samples were taken from each fifty square hectare of the field, a sample group with placing in open plates in the site. 6 plates having malt extract agar, yeast extract agar, Czapek yeast extract agar, Czapek agar, Sabouraud dextrose agar, and potato dextrose agar all mixed with 100 ppm chloramphenicol and 50 ppm tetracycline were used to take a sample group. All the plates were aerobically incubated at 25±2 °C. In the range of 3, 7, and 15 days, all the plates were always (and also daily) checked, identified, marked, and samples were taken by a sterile glass needle and cultured in prepared plates. In plates and tubes containing agar butt slant from growth substrates of malt extract agar, yeast extract agar, potato dextrose agar, corn meal agar, Sabouraud dextrose agar, and Czapekdox agar, all mold samples were recaptured and incubated with preplans. At last, some samples were taken from *Aspergillus*colonies and cultured in plates containing Czapekdox agar, Czapek yeast extract agar (with and without 20% sucrose), malt extract agar, and Czapekdox agar (with and without 20% sucrose). The samples were grown at 25±2 °C, and after 3, 7, or 15 days checking, and simultaneously, culture slide from each sample was provided on 20%-sucrose Czapek yeast extract and Czapekdox agar substrates in order to grow with former pattern (Abou-Zeid, 2012).

To provide extract from the obtained isolates of cultivation in liquid substrate to prepare and motivate the extract more and more, a full loop having 10^5 phialospore from the PBS mixture and conids of every grown isolate in Czapek extract agar plate were taken and re-cultured into a 50 mL Falcon tube containing the liquid substrate of Czapekdox broth with 1% malt extract agar. The re-cultured tubes were

incubated in the darkness-light period at 25±3 °C and 200 rpm. After seven days, floating or deposited mass in the liquid, which was mold fungal, infant small filament (Germ tube), was deposited by centrifugation at 3000 rpm for 15 minutes and removed from culture medium of fungus using sterile filter paper (Abou-Zeid, 2012).

Every provided culture medium was observed in a PBS tube and sampled into every 5mL Falcon tube of buffer, 1mL cold acetone was added, and the separation was done by centrifugation at 15-3000 rpm. The supernatant was separated from the larger deposits and kept in another tube after marking at -20°C. For synchronization, the size of protein of each mixture obtained from each *Aspergillus* isolate was measured by Bradford method and thick samples were diluted up to 0.5 mg/ml. The thick samples were diluted and the dilute samples were again concentrated by this method until all extract samples had 0.5 mg/ml protein (Abou-Zeid, 2012).

Finally, the one-sample Kolmogorov-smirnov test was used for statistical analysis of the normal distribution of the measured mean values of toxin in biomass and medium culture. We used NORMAL Q-Q PLOT test for the scattering distribution of the measured values of toxin in biomass and culture medium of the studied species, and in order to determine the numerical difference of Citrinin production amount between the medium and biomass the Willcoxon Singned Rank Test was used. Also, we used Excel and Office 2010 and SPSS 16 to analyze the findings.

RESULTS AND DISCUSSION

The relative distribution of the number of the studied fungal species is as follows:

Table 1: Distribution of the number of thestudied isolates belonging to the species of *Aspergillus* genus in examining the culture medium

			Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	**2.00**	**2**	8.0	8.0	8.0
	2	**3.00**	**9**	36.0	36.0	44.0
	3	**4.00**	**2**	8.0	8.0	52.0
	4	**5.00**	**3**	12.0	12.0	64.0
	5	**6.00**	**4**	16.0	16.0	80.0
	6	**7.00**	**2**	8.0	8.0	88.0
	7	**8.00**	**2**	8.0	8.0	96.0
	8	**17.00**	**1**	4.0	4.0	100.0
		Total	**25**	100.0	100.0	

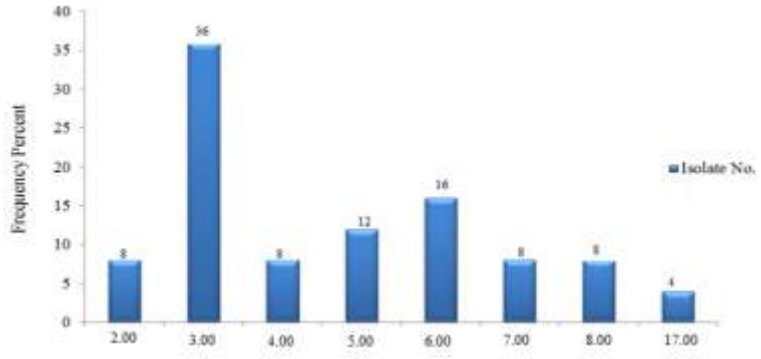

Figure 1: Distribution percentage of the studied isolates belonging to the species of *Aspergillus* genus

According to Figure 1, the frequency distribution percentages of *Aspergillus* species present in the culture in the order of most to least frequency from the set of 25 frequencies are as follows:
Sample 2, with 9 frequencies to the amount of 36%, has the highest frequency and includes the species below:
A.foetidus, A. ostianus, A. melleus, A. candidus, A. sp V, A. niveus, A. wentii, A. awamori, A.unguis
Sample 5 with 4 frequencies to the amount of 16% includes A. sp III, A. ornate, A. terreus, A. carbonarius. Sample 4 with 3 frequencies to the amount of 12% includes A. af.terreus, A. fumigatus, A. parasiticus. Sample 1 with 2 frequencies to the amount of 8% includes A.af. nidulans and A.alliaceus. Sample 3 having 2 frequencies to the amount of 8% includes A.ochraceus and A.niger. Sample 6 with 2 frequencies to the amount of 8% includes A. af.flavus and A. sp IV. Sample 7 with 2 frequencies to the amount of 8% includes A. flavus and A. sojae species. Sample 8 with 1 frequency to the amount of 4% includes A. sp IV. The frequency of the fungal species isolated from the culture medium is as follows:

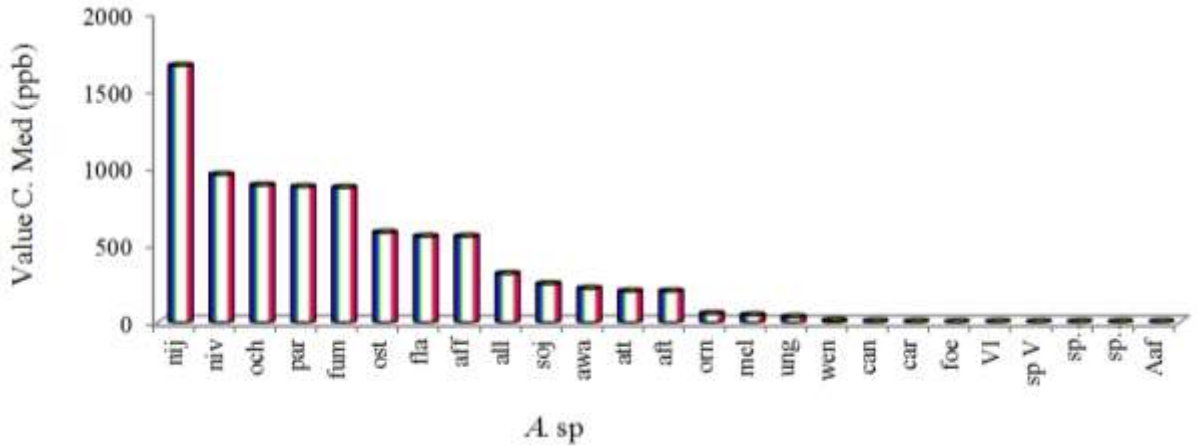

Figure 2: Average of the measured toxin and how Citrinin is produced by *Aspergillus* species identified in the culture medium of the studied samples

According to Figure 2, the average of Citrinin produced in the culture medium of *Aspergillus* species in the order of highest to lowest amount is as follows:
1- A. niger with 1655.91 ppb produced toxin
2- A. niveuswith 951.81 ppb produced toxin
3- A. ochraceus with 883.29 ppb produced toxin
4- A. parasiticus with 873.01 ppb produced toxin
5- A. wentii with 8.04 ppb produced toxin
6- A. candidus with 2.74 ppb produced toxin
7- A. carbonarius with 1.62 ppb produced toxin
8- A. foetidus with 0.06 ppb produced toxin
In other species including A.af.nidulansA. sp III, A. sp IV, A. sp V, A. sp VI, Citrinin has not been produced.
According to Figure 3, normal distribution curve of the average of measured values for Citrinin in the culture medium of the studied samples is described as follows:
The average amount of Citrinin in 25 different *Aspergillus* species measured in the medium is 326/74 ppb. The highest frequency exists in the range of 0-500 ppb which includes A.melleus, A.candidus A.foetidus, A.af.terreus, A.ornata, A.terreus, A.wentii, A.sojae, A.alliaceus, A.awamori, A.carbonarius A.unguis. The second frequency is in the range of 500-1000 ppb which includes A.ochraceus, A.af.flavus, A.fumigatus, A.niveus, A.flavus, A.parasiticus. In the range of 1000-1500 no frequency was observed. The lowest frequency exists in the range of 1500-2000 ppb which includes A.niger. Other studied species such as A.af.nidulans A. sp III, A. sp IV, A. sp V, and A. sp VI have lacked the ability to produce Citrinin.

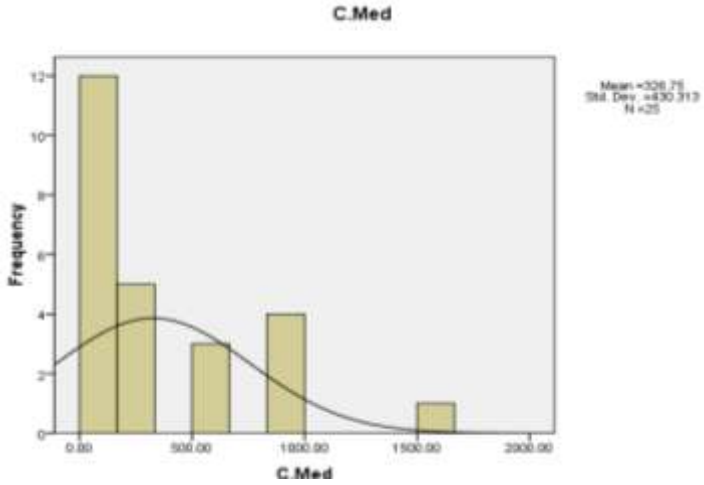

Figure 3: Normal distribution curve of the average of measured values for Citrinin in the culture medium of the studied samples

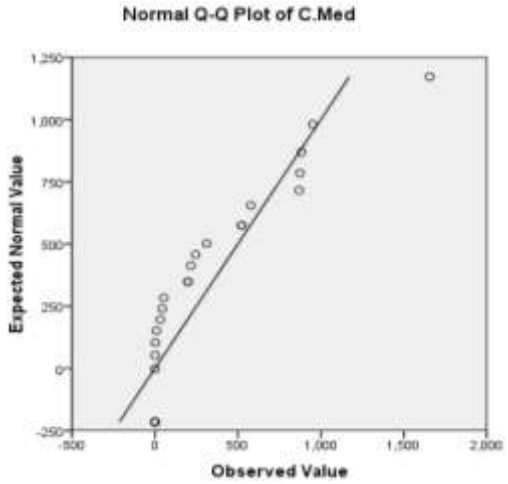

Figure 4: Scattering distribution of the measured amounts of toxin in the culture medium of the studied samples using NORMAL Q-Q PLOT method

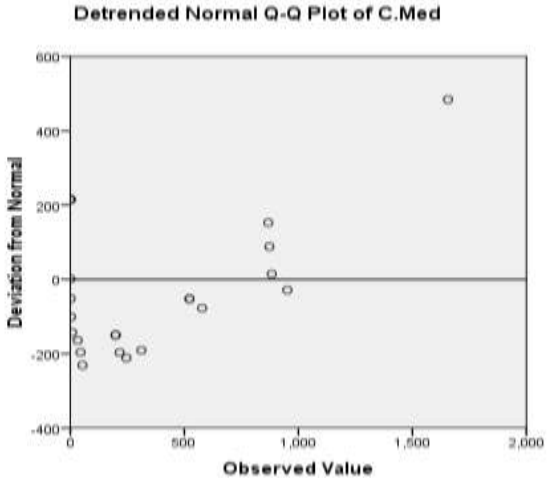

Figure 5: Scattering distribution of the measured amounts of toxin in the culture medium of the studied samples using NORMAL Q-Q PLOT method based on deviation from normal

Figures 4 and 5 show that the scattering distribution of the measured amounts in the culture medium is not significant, that is, it follows the normal distribution.

Table 2: statistical analysis of the normal distribution of the measured mean values of toxin in biomass and medium culture by one-sample test

One-Sample Kolmogorov-Smirnov Test

		C.Med	C.Bio
N		25	25
Normal Parameters[a]	Mean	3.2675E2	2.4572E2
	Std. Deviation	4.30313E2	4.30196E2
Most Extreme Differences	Absolute	.224	.284
	Positive	.217	.230
	Negative	-.224	-.284
Kolmogorov-Smirnov Z		1.119	1.420
Asymp. Sig. (2-tailed)		.163	**.036**

Table 2 shows that distribution of the measured mean values of toxin in biomass and medium culture is normal but, given $p<0.05$, the correlation is not significant.

Table 3: the correlation of the measured values of Citrinin in biomass and culture medium of the studied samples

Correlations

		C.Med	C.Bio
C.Med	Pearson Correlation	1	**.601****
	Sig. (2-tailed)		**.001**
	N	25	**25**
C.Bio	Pearson Correlation	.601**	1
	Sig. (2-tailed)	.001	
	N	25	25

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3 shows that the correlation of the measured values of Citrinin in biomass and culture medium of the studied samples is significant and parallel, in the sense that if the amount of Citrinin production increases in the culture medium, the amount of that increases in the biomass too, and vice versa.

Table 4: Numerical difference of Citrinin production amount between the medium and biomass by Willcoxon Singned Rank Test

Test Statistics[b]

	C.Bio - C.Med
Z	**-.784[a]**
Asymp. Sig. (2-tailed)	**.433**

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

Table 4 shows that the numerical difference of Citrinin production amount between the culture medium and biomass is not significant. The numeral increase or decrease of each one is independent of the other. In fact, an increase in the amount of Citrinin production in the medium causes a decrease in that in biomass, and vice versa.

Discussion and Conclusion

Mycotoxins with potential toxic effects and being carcinogenic are as one of the most important legal issues pursued in countries, and for this reason, regular tests are currently being done in order to control food and diagnose toxins seriously and broadly. This has resulted in serious restrictions on different types of using food products with the amount of toxin beyond the limit (Kogika *et al.,* 1996; Kononenko *et al.,* 2008).

A limited number of mycotoxins, such as aflatoxins, zearalenone, fumonisins, and ochratoxins are measured in import, export, and any transactions of products. But these toxins are not measured in food products used by animals in some countries (Herman. *et al.,* 2007).

In performed examinations in this study, statistical frequency related to Aspergillus species shows that the highest frequency is for sample 2 with 36% frequency. This frequency is of 9 species out of 25 studied ones which includesA. ostianus,A. melleus,A. candidus,A. sp V,A. niveus,A. wentii,A. awamori, and A.unguis.A. sp III, A. ornate, A. terreus, A. carbonarius species related to sample 5 with 16%, 4 frequencies, A. af.terreus,A.fumigatus, A. parasiticus species related to sample 4 with 12%, 3 frequencies, A.af.nidulans and A.alliaceus species related to sample 1 with 8%, 2 frequencies, A.ochraceusand A.niger species related to sample 3 with 8%, 2 frequencies, A. af.flavus and A. sp IV species related to sample 6 with 8%, 2 frequencies, A. flavusandA. sojae species related to sample 7 with 8%, 2 frequencies, and sample 8 with 4%, 1 frequency of A. sp IV species.

The isolates producing Citrinin in the range of 0-1655/91 ppb were examined, 17 Aspergillus species are in the range of 0-500 ppb with 16 species in the range of 0-250 ppb and 1 species in the range of 250-500 ppb. 3 and 4 species were found in the ranges of 500-750 and 750-1000 ppb, respectively. 1 species was observed in the range of 1000-2000 ppb. It should be noted that 5 of 25 studied species lacked the ability to produce the toxin but had the frequency of isolates.

Considering that the average of produced Citrininog Aspergillus in culture is 326/74 ppb and the studied range is 0-2000 ppb, it can be concluded that, statistically, the data have skewness to the right, are not significant, and follow the normal distribution. This conclusion was also confirmed by the statistical method of PLOT NORMAL Q-Q.

On the basis of the fact that in different areas of the world including the U.S., Canada, Europe, Asia, Australia, New Zealand, Latin America, Africa, and Middle East the range of minimum and maximum Citrinin production by HPLC is 0-500 ppb, and in Africa, Japan, and Middle East the recommended allowable maximum has been considered 200 ppb, the results of this study showing the average of 326/74 ppb poses a serious threat that can be considered a warning about controlling Aspergillus-contaminated food and agricultural products in the northern regions of Iran, as one of the largest areas of food and agricultural production in Iran. Compared to the research by Vrabcheva *et al.,* on 24 wheat bran samples in Bulgaria in 1998using ELISA, the highest and lowest levels of Citrinin production were reported 230µg/kg and 5.9µg/kg, respectively. Also, in another research on 37 wheat samples, the highest and lowest levels of Citrinin production are 420µg/kg and 20µg/kg, respectively. According to the results of this study, using ELISA, the highest level of Citrinin production in the culture medium of Aspergillus species was related to A. niger with the toxin production amount of 1655/91 ppb, and the lowest level of that was related to A. foetidus with the toxin production amount of 0.06 ppb. We can realize that the highest level of Citrinin production in the culture medium of Aspergillus species in north of Iran related to A. niger with the toxin production amount of 1655/91 ppb is several times greater than what Vrabcheva *et al.,* measured that can createa major concern in terms of food contamination by Aspergillus species.

Compared to the research by Curtui *et al.,* on 30 corn samples in Romania in 1997using ELISA method, the amount of Citrinin production was reported 580µg/kg, while the average amount of Citrinin production for 25 Apergillus species of the north of Iran was determined 326.74 ppb.

Considering the mentioned study, the toxin production in A. niger species with 1655.91 ppb produced toxin, A. niveus with 951/81 ppb produced toxin, A. ochraceus with 883/29 ppb produced toxin, A. parasiticus with 873/01 ppb produced toxin, and A. fumigatus with 868.25 ppb produced toxin is more than that of measured in the study by Curtui *et al.,* (1998). In addition to ELISA method, other studies are conducted using other methods such as HPLC, TLC, LC-MS/MS, and Fluorometer in order to measure the amount of Citrinin present in various food and agricultural samples. In the remainder, we review the results of these methods in different studied samples and the results of the present study.

Suggestions

✓ Global research on the placed Aspergillus in the north of Iran, which encompasses one of the world's oldest plant habitats, should be carried out to distinguish the characteristics of native and migratory species

✓ The products vulnerable to contamination by mycotoxins should be kept and transported in an appropriate environment and under careful and continuous inspection by technicians. As a result, it seems essential to build food factories in good conditions in terms of the distribution of Aspergillus.

✓ To examine and identify the mechanisms of Citrinin's production and release by Aspergillus

✓ Spectral analysis and examination of the genomic map for Citrinin production.

✓ To analysis the capacity of producing Citrinin among species under Aspergillus genus all around the world.

REFERENCES

Abd-Allah EF *et al.,* (2005). Natural occurrence of citrinin in rice grains and its biocontrol by Trichoderma hamatum. *Phytoparasitica* **33** 73-84.

Abou-Zeid AM (2012). Review on Citrinin: Synthetic Methods, Molecular Biosynthesis and Effect of Plant Extracts. *British Microbiology Research Journal* **2**(2) 108-122,

Adrene Pitter (1998). Natural occurrence of mycotoxin in foods and feeds. *An update review Revue de Medicine Veterinare* **149**(6) 479-492.

Alborzi S *et al.,* (2006). Aflatoxin M1 contamination in pasteurized milk in shiraz (south of Iran). *Food Control* **17**(7) 582-584.

Arbuckle MR *et al.,* (2001). Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus. *Scandinavian Journal of Immunology* **54** 211-9.

Aziz NH and Mattar ZA *et al.,* (2006). Contamination of grains by mycotoxin-producing molds and mycotoxins and control by gamma irradiation. *Journal of Food Safety* **26** 184-201.

Aziz NH *et al.,* (2006). Contamination of grains by mycotoxin-producing molds and mycotoxins and control by gamma irradiation. *Journal of Food Safety* **26** 184-201.

Barrett J (2000). Mycotoxins: of molds and maladies. *Environmental Health Perspectives* **108** 20-23.

Bennet JW and Klich M (2003). Mycotoxins. *Clinical Microbiology Reviews* **16** 497-516.

Beuchat LR (1987). Traditional fermented food products. In: *Food and Beverage Mycology* edited by Beuchat LR (New York: Springer) 2nd edition 224-53.

Bouakline A *et al.,* (2000). Fungal contamination of food in hematology units. *Journal of Clinical Microbiology* **38**(11) 4272-3.

Chaichi Nosraty A (2010). An investigation on taxonomic identification of aerial Aspergillus species in the North of Iran, an assay on protein pattern profiles of the genera, species and allergen antigens, *IMC 9 Proceedings, Edinburgh* 189.

Chaichi Nosraty A, Modiri L and Fayezi M (2006). An investigation on Tea garden air fungal Pollution in the north of Iran, Gilan province eastern region, ISI Web of Knowledge, ISI Current Contents connect, (International) *ISI Proceedings, IMC 8, Cairns* 143-47.

Chrétien P, Dauvin M, Hélin P, Ocwieja T, Absalon YB and Johanet C (1994). Comparison of indirect immunofluorescence on Crithidia luciliae of Farr test, and immunoenzymatic methods for the screening of anti-native DNA autoantibodies. *Annales de Biologie Clinique* **52** 645-50.

Cigic IK and Prosen H (2009). An Overview of Conventional and Emerging Analytical Methods for the determination of mycotoxin. *International Journal of Molecular Sciences* **10** 62-115. doi: 10.3390l/ijms 10010062.
Creppy ES (2002). Update of survey regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters* **127** 19-28.
Curtui V *et al.*, (1998). A survey on the occurrence of mycotoxins in wheat and maize from western Romania. *Mycopathologia* **143** 97-103.
Diaz DE *et al.*, (2001). The effect of inclusion of a clay type sequestering agent on milk production of dairy cattle consuming mycotoxins contaminated feeds. *Journal of Dairy Science* **84**(abstr) 1554.
Dick R (1988). Zum Vorkommen von Citrinin in Cerealien. *Mitteilungen Gebiete Lebensmitte Hygiene*. **79** 159-164.
Dietrich R, Usleber E, Märtlbauer E and Gareis M (1999). Nachweis des nephrotoxischen mykotoxins citrinin in lebensmitteln und mit Monascus spp. Hergestellten lebensmittelfarbstoffen. *Archiv für Lebensmittelhygiene* **50** 17-21.
Dragacci S *et al.*, (1996). Application of immunoaffinity colum clean up to the Aflatoxin M1 determinaton and survey in cheese. *Journal of Food Protection* **59**(9) 1011-1013.
Dragacci S *et al.*, (2001). Immunoaffinity colum cleanup with liquid milk: Collaborative study. *Journal of AOAC International* **84**(2) 437-443.
El-Adlouni C *et al.*, (2006). Preliminary data on the presence of mycotoxins (ochratoxin A, citrinin and aflatoxin B1) in black table olives "Greek style" of Moroccan origin. *Molecular Nutrition and Food Research* **50** 507-512.
El-Kady IA *et al.*, (1995). Natural occurrence of mycotoxins in different spices in Egypt. *Folia Microbiologica* (Praha) **40** 297-300.
Fokunang CN *et al.*, (2011). Mycotoxins: Quality Management, Prevention, Metabolism, Toxicity and Biomonitoring. In: *Health Management - Different Approaches and Solutions*. ISBN 978-953-307-296-8 117-142.
Gams W *et al.*, (1998). *CBS Course of Mycologhy* (Centraalbureu Voor Schimmelcultures. Baarn) 4th edition 1-165.
Geiser DM *et al.*, (2007). The current status of species recognition and identification in Aspergillus. *Studies in Mycology* **59** 1-10.
Gonzalez-Salgado A *et al.*, (2005). Discrimination of Aspergillus niger and other Aspergillus species belonging to section Nigri by PCR assays. *FEMS Microbiology Letters* **245**(2) 353-361.
Gregoire S *et al.*, (2002). Plant disease committee coparative tests where detection of pathogens is assessed by ELISA. *6th ISTA seminar on statistics June 2002.*
Hanika C (1983). Citrinin mycotoxicosis in the rabbit. *Food and Chemical Toxicology* **21** 487–493.
Herman J *et al.*, (2007). Mycotoxins and the pet food industry: Toxicological evidence and risk assessment. *International Journal of Food Microbiology* **119** 95–102.
Hope WW, Walsh TJ and Denning DW (2005). The invasive and saprophytic syndromes due to Aspergillus spp. *Medical Mycology* **43**(Suppl 1) 207-238.
Iheshiulor OOM (2011). Effects of mycotoxins in animal nutrition: a review. *Asian Journal of Animal Sciences* **5**(1) 19-33.
Jan Alexander, Diane Benford, Alan Boobis, Sandra Ceccatelli *et al.*, (2012). Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. *EFSA Journal* **10**(3) 2605. [82]. doi:10.2903/j.efsa.2012.2605.
Janardhana GR *et al.*, (1999). Mycotoxin contamination of maize grains grown in Karnataka (India). *Food and Chemical Toxicology* **37** 863-868.
Khongkhunthian P (2001). Aspergillosis of the maxillary sinus as a complication of overfilling root canal material into the sinus: report of two cases. *Journal of Endodontics* **27**(7) 476-478.
Kogika MM *et al.*, (1996). Experimental citrinin nephrotoxicosis in dogs. *Veterinary and human Toxicology* **35** 136–140.

Kononenko GP *et al.*, (2008). A survey on the occurrence of citrinin in feeds and their ingredients in Russia. *Mycotoxin Research* **24** 3-6.
Kumari CK (1987). Natural occurrence of citrinin and ochratoxin A in coconut products. *National Academy Science Letters-India* **10** 303-305.
López H *et al.*, (2005). Clinical disease activity and titers of anti-dsDNA antibodies measured by an automated immunofluorescence assay in patients with systemic lupus erythematosus. *Lupus* **14** 505-9.
Mirhendi H (2007). Identification of pathogenic Aspergillus species by a PCR-restriction enzyme method. *Journal of Medical Microbiology* **56**(Pt 11) 1568- 70.
Moallaei H *et al.*, (2006). Isolation of keratin-ophilic fungi from soil samples of forests and farm yards. *Iranian Journal of Public Health* **35** 62 – 9.
Molinié A *et al.*, (2005). Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B-1: development of a method for simultaneous extraction of ochratoxin A and citrinin. *Food Chemistry* **92** 391-400.
Nielsen KF *et al.*, (2009). Review of secondary metabolites and mycotoxins from the Aspergillus niger group. *Analytical and Bioanalytical Chemistry* **395**(5) 1225-42.
Nishijima M (1984). Survey for mycotoxins in commercial rations. In: *Toxigenic Fungi: Their Toxins and Health Hazards* edited by Kurata H and Ueno Y (Elsevier. Developments in Food Science, Amsterdam) **7** 172-189.
O'Brien Evelyn and Dietrich Daniel R (2004). Mycotoxins Affecting the Kidney. In: *Toxicology of the Kidney* 895-936.
Park CE *et al.*, (1992). Nonspecific reactions of a commercial enzyme-linked immunosorbent assay kit (TECRA) for detection of staphylococcal enterotoxins in foods. *Applied and Environmental Microbiology* **58** 2509-12.
Peraica M *et al.*, (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization* **77**(9) 754-766.
Pitt JI and Hocking AD (1997). *Fungi and Food Spoilage* (New York: Springer) 2nd edition 377-85.
Polisenska I (2010). Occurrence of ochratoxin A and citrinin in Czech cereals and comparison of two HPLC methods for ochratoxin A detection. *Food Additives and Contaminants* **27** 1545-1557.
Reddy K (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews* **29**(1) 3-26.
Robert A (2000). Biological and chemical terrorism: strategic plan for preparedness and response: recommendations of the CDC Strategic Planning Workgroup. *MMWR Morbidity and Mortality Weekly Report* **49** 1-14.
Sambrook J *et al.*, (2001). *Molecular cloning. A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press) 3rd edition.
Saxena J (1989). Screening of spices commonly marketed in India for natural occurrence of mycrotoxins. *Journal of Food Composition and Analysis* **2** 286-292.
Shi YC (2011). Beneficial effects of Monascus purpureus NTU 568-fermented products: a review. *Applied Microbiology and Biotechnology* **90** 1207-1217.
Siok Ghee *et al.*, (2006). Trends in detection of warfare agents Detection methods for ricin, staphylococcal enterotoxin B and T-2 toxin. *Journal of Chromatography* **1133** 1–12.
Suganuma T *et al.*, (2007). Some distinguishable properties between acid-stable and neutral types of alpha-amylases from acid producing koji. *Journal of Bioscience and Bioengineering* **104**(5) 353-62.
Sydenham EW (1996). Chromatographic and allied methods of analysis for selected mycotoxins. In: *Progress in Food Contaminants Analysis* edited by Gilbert J (Blackie Academic and Professional, London) 65-146.
Tabata S *et al.*, (2008). Investigation of ochratoxin A, B and citrinin contamination in various commercial foods. *Journal of the Food Hygienic Society of Japan* **49** 111-115.
Tangni EK *et al.*, (2006). Ochratoxin A and citrinin loads in stored wheat grains: Impact of grain dust and possible prediction using ergosterol measurement. *Food Additives & Contaminants* **23** 181-189.

Trucksess MW (2001). Rapid analysis (thin layer chromatographic and immunochemical methods) for mycotoxins in foods and feeds. In: *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium* (Wageningen, the Netherlands: Ponsen & Looyen) 29–40.
Vrabcheva T *et al.*, (2000). Co-occurrence of ochratoxin A and citrinin in cereals from Bulgarian villages with a history of Balkan endemic nephropathy. *Journal of Agricultural and Food Chemistry* **48** 2483-2488.
Wannemacher RW (1991). Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: *Mycotoxins and Animal Foods* edited by Smith JE and Henderson RS (CRC Press, Inc., Boca Raton, FL) 499-552.
Willson K and Walker J (2005). *Principles and Techniques of Practical Biochemistry* 5th edition.
Xu B *et al.*, (2006). Review on the qualitative and quantitative analysis of the mycotoxin citrinin. *Food Control* **17** 271-285.
Yaroglu T *et al.*, (2005). Aflatoxin M1 Levels in cheese samples from some provinces of Turkey. *Food Control* **16**(10) 883-885.
Zheng Z (2005). Validation of an ELISA test kit for the detection of ochratoxin A in several food commodities by comparison with HPLC. *Mycopathologia* **159** 265–72.
Zinedine A, Soriano JM, Molto JC and Manes J (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food and Chemical Toxicology.*