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Research Article

EVALUATION OF CITRININ TOXIN PRODUCED IN CELL EXTRACTS OF *ASPERGILLUS* ISOLATES IN NORTHERN IRAN

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ABSTRACT
As far as the productions of Citrinin toxin by *Aspergillus* have not been widely studied, so as to include multiple species and how the process of production and secretion of this toxin in *Aspergillus* is unknown, this research is conducted. In this study we examined the extent Citrinin toxin produced in the cell extracts of strains of *Aspergillus* with ELISA, in northern of Iran. After sampling, fungal isolates in Czapelc medium containing 2% malt extract with around 200 RPM at room temperature for a week, then isolation and filtration fungal populations by medium in order to measurement of the amount of toxin by the competitive ELISA, with dry fungal biomass utilization 2gr on the desiccator with physical comminution (grinding) of cells (centrifugation with Glass Pearl), and then. Methanol and Estonia extraction of biomass to measure the amount of toxin by competitive ELISA (r-biopharm: lRidascreen Fast Citrinin) is used. The toxin produced by 21 *Aspergillus* species had different significant levels. The average rate of Citrinin toxin production of the isolates examined was from 0 to 2009.29 ppb. Most isolates were in the range of 0-500 ppb toxin production, that including 12 species of *Aspergillus*. Most toxin production associated with *A. niger* was 2009.29 ppb and *A. wentii* with the lowest was 18.46 ppb. This study showed that *Aspergillus* can produce Citrinin toxins are native to northern Iran and food contamination with *Aspergillus* should have been investigated for the presence of toxin Citrinin, like other mycotoxins to ensure food safety.

Keywords: *Aspergillus, Citrinin, ELISA, Mycotoxin*

INTRODUCTION
Fungi are eukaryotic organisms belonging to kingdom Fungi. These organisms are heterotrophic and are facultative aerobic or anaerobic organisms. Fungi, by releasing various enzymes and changing organic materials to dissolve foods, can transform the materials inside, the cell through reactive absorption or active transformation (Gams *et al.*, 1998). Fungi also are able to produce metabolic products. Primary metabolites are regular cell metabolism products being found frequently in nature and in all the genera of a family. Secondary metabolites are normal cell metabolism products in all species of a family. So called secondary metabolites seem to don't favor the fungi cells themselves (Alborzi *et al.*, 2006).
Some fungi if growing on a food produce toxin under special condition. These are indeed specific metabolites of fungi. All species of a fungus don't produce toxin. For example some species of *Aspergillus* are toxigenous. On the other hand, one kind of fungus produces more than one toxin or a kind of fungus. Depending on the fact that fungi are grown on what material, they may produce different toxins. For example, kalvispes propora produce toxin when growing on rye plant, but it doesn't produce toxin or produce low amount of toxin on other food stuffs. Mycotoxins are able to increase the susceptibility of person against microbial disease, malnutrition and even other toxin. The number of people infected by mycotoxins is known. However, some believe that the number of these people is by far lower than individuals suffered from microbial disease (American Academy of Pediatrics, 1998). Interest of microbiologists toward the fungal infection in human ecologies has greatly increased in recent decades and in Iran it has been greatly addressed along with other researchers (Moallaei *et al.*, 2006). By increasingly improvement in the knowledge on human susceptibility and in response to human being hygienic requirements, more recognition on the microbes has increased and it is addressed more than ever (Chaichi, 2010). *Aspergillus* are among frequent and ubiquitous known microorganisms and achieved a prominent value in the diseases and food contamination.

Unfortunately, the ability and value of *Aspergillus* and its products is lower than its harm and damage (Chaichi *et al.,* 2006).

Aspergillus are a diverse genus of mycelium including several groups and species. Identification of these fungi is important from different aspects such as pathogenicity, toxin production and industrial aspect. *Aspergillus* is consisted of about 180 groups and species of mycelia (Khongkhunthian, 2001). These fungi belong to hyphomycetes from deyteromycets phylum. According to the fact that some of them produce ascus and ascapore in the reproductive phase, they are group in the phylum ascomycetes (Geiser *et al.,* 2007). *Aspergillus* are important from various industrial, medical and biochemical aspects. So far, 40 species were isolated from fungal infections. Among them are *Aspergillus fumigates, Aspergillus flavus, Aspergillus niger, Aspergillus tereus* and *Aspergillus nidolance* (Pitt and Hocking 1997). In addition *Aspergillus* are able to prude and release toxins such as aflatoxin, ocratoxin, patolin and citrinin in particular conditions which are important from view point of health and chronic and acute toxicities (Nielsen *et al.,* 2009). Enzymes such as amylase and products with industrial consumption such as citric acid, buric acid and lactic acid are produced by some of species of this genus (Suganuma *et al.,* 2007). The role played by *Aspergillus* alongside other microorganisms in the cycle of minerals is very prominent. Taking the medical and biologic importance of *Aspergillus*, it is obvious that recognition of these species is necessary to conduct and study on them (Mirhendi, 2007). *Aspergillus* are among most frequent environmental fungi: such that their conidia (spore) may be easily isolated on the surface of fruits, bread, cereals and in general anywhere in water, soil and air (Bouakline *et al.,* 2000). Based on new classification, genus *Aspergillus* is divided to various parts each part is containing various varieties including, *Flavus, Fumigatoi, Niger,* etc (Gonzalez-Salgado *et al.,* 2005). *Aspergillus* are fungi with binary nature of beneficial or seemingly harmful. Some of them are pathogen and in human and animals produce infection such as aggressive pulmonary *Aspergillus*, mycetoma, kartitis and auto mycosis; such that among 180 *Aspergillus* described so far, about 40 species have been isolated from human fungal infection (Hope, 2005). On the other hand, *Aspergillus* has been used long ago to produce and ferment some foods. Some of metabolites of this genus are important medically such as Levostatine taking from *Aspergillus tereus* or cilophangin drugs achivied by chemical changes of action candin B from *Aspergillus nidolance* (Beuchat, 1987). Mycotoxine is defined as compounds produced by fungi and are toxic for other organisms. Mycotxoins along with other fungal metabolites such as antibiotics, alkaloids and so on are compounds produced in the final steps of mycelium via mycetocytes and are known as secondary metabolites (Alborzi *et al.,* 2006). On this basis, present study was performed aiming to study the production of citrinin toxin in *Aspergillus* isolates of northern Iran in the cell extract and to compare the amount of toxin produced in various species of *Aspergillus.*

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 (American Academy of Pediatrics, 1998).

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 (American Academy of Pediatrics, 1998).

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 concentratedby this method until all extract samples had 0.5 mg/ml protein (American Academy of Pediatrics, 1998).

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

In the studies performed in present study, statistical frequency to the identified *Aspergillus* species in the biomass suggests that the highest frequency is related to the sample 3. With 28.6 %. This is frequency of 6 species among 21 studied species including *A.ostianus,A.fumigatus, A.niveus, A.parasiticus, A.awamori* and *A.niger*. species *A.foetidus, A.ochraceus, A.terreus, A.carbonarius* and *A.unguis* were related to sample 4 with 23.8 % and 5 frequencies, species *A.meleus, A.af.nidulans, A.sp VI* and *A.wentii* were related to sample 2 with 19 % and 4 frequency, species *A.candidus* and *A.alliaceus* were related to the sample 1 with 9.5 % and 2 frequencies, species *A.sp3.a f.A.nomius* and *A.sojae* were related to the sample 7 with 9.5 % and 2 frequencies, species *A.ornata* was related to the sample 6 with 4.8 % and 1 frequency, species *A.flavus* was related to sample 5 with 4.8 % and 1 frequency (table 1-4, figure 1-4).

Citrinin producing isolates were examined in 0-2500 ppb range, where in 0-500 ppb range, 12 *Aspergillus* specied are placed which 6 species are located in 0-250 ppb range and 6 species are located in 250-500 ppb. In each 500-750 ppb and 750-1000 ppb ranges. One species was found. In present study, 1000-2000 pb range had no species and in 2000-2500 ppb, one species was observed. It's worth mentioning that among 21 studies species, 6 species lacked the toxin production ability but have isolated isolates frequency.

According to the fact that mean citrinin produced by *Aspergillus* in the biomass was measured as 245.72 ppb and studied range is 0-2500 ppb, it can concluded that statistically data is skewed towards the right and is not significant and follows normal distribution. This conclusion was also verified using NoRMAL Q-Q-PLOT. According to the fact that in different parts, across the world such as America, Canada, Europe, Asia, Australia, new Zealand, Latin America, Africa and Middle East, maximum and minimum range of citrinin production using HPLC technique is 0-500 ppb, and in Africa, Japan and middle east maximum permitted level is 200 ppb, citrinin level based on the results of this study I,e,245.72 ppb is considered as a serious hazard which could be considered as an award to future control of foods and

agricultural products contaminated to *Aspergillus* in northern part of the country as one biggest areas of food and agriculture production in Iran. Because 9 species of *Aspergillus* among 21 species produce more than 200 ppb citrinin. These species are including *A.niger* ، *A.sojae* ، *A.ornata* ، *A.tereus* ، *A.fumigatus* ، *A.unguis* ، *A.parasiticus* ، *A.flavus* and *A.melleus*. This rate is more that maximum level recommended in Europe i.e. 100 ppb which according to this rate it will cover 2 species *A.alliaceus* and *A.ostianus* in addition to the above mentioned species. This permitted maximum level in china and FDA is defined as 20 µg/kg which accordingly will include 3 further species of *A.carbonarius* ,*A.foetidus* and *A.candidus* in addition to above mentioned species (figure 4-2) (Wannemacher, 1991).

In a comparative study by Vrabcheva, *et al.,* in Bulgaria during 1998 on 24 samples of wheat bran using ELISA, maximum citrinin production was related as 230 µg/kg and minimum citrinin production was 5.9 µg/kg.

In addition in another study on 37 wheat samples, maximum citrinin production rate was 420 µg/kg and maximum 20 µg/kg. results of this study indicated that maximum citrinin production in the biomass is related to *A.niger* with 2009.29 ppb toxin production and minimum citrinin production is related to *A.wentii* with 18.46 ppb toxin production using ELISA. It can be found that maximum citrinin production in cell extract of *Aspergillus* of northern Iran related to *A.niger* with 2009.29 ppb is multiple times higher than the rate measured by Vrabcheva, *et al.,* which may vcreat major concern in respect of food contamination by various *Aspergillus* species (figure 2.4) (Vrabcheva *et al.,* 2000).

In a comparative study by Kononenko *et al.,* in Russia during 2003-2006 on 148 samples of soya bean, oil cake, bean using ELISA, maximum citrinin production was reported as 30 µg/kg and minimum production rate was 14 µg/kg or in another study on 37 wheat bran, citrinin production rate was measured (50 µg/kg) and on 157 corn samples, maximum citrinin was 953 µg/kg and its minimum rate was 218 µg/kg. According to this study it can be found that even mean citrinin production of 21 *Aspergillus* species of northern Iran with 245.7 ppb is more than maximum citrinin measured in soya bean, oil cake, bean and wheat bran samples of this study area during 2003-2006 (figure 2.4) (Kononenko *et al.,* 2008).

In a comparative study by Dietrich, *et al.,* in Germany on 35 cider sample using ELISA, citrinin production rate was reported 0.13 µg/kg. In addition, in this regard further studies were performed on other samples including a study on 11 tomato juice samples with 0.12 µg/kg citrinin production, study on 5 cherry juice samples with 0.10 µg/kg citrinin production, study on 2 blackberry and mulberry with 0.20 µg/kg citrinin production. Based on this study it can be concluded that citrinin rate measured by Dietrich, *et al.,* is lower than minimum citrinin produced in cell extract of *A.wentii* with 18.46 ppb citrinin production (figure 2.4) (Dietrich, *et al.,* 1999).

In a comparative study by Curtui *et al.,* in Romania during 1997 on 30 corn samples using ELISA, mean citrinin production rate of 580 µg/kg was reported. While citrinin production by 21 *Aspergillus* species of northern Iran is 245.72 ppb. Only citrinin production, in two *A.niger* species with 2009.29 ppb citrinin production and *A.sojae* with 765.84 ppb citrinin production was more than mean production by Curtui *et al.,* (figure 2.4) (Curtui *et al.,* 1998).

In addition to ELISA, other studies have been performed using other techniques such as HPLC ،TLC ، LC-MS/MS and fluorometer to measured citrinin amount existing in various food and cultural samples. In the following, we will the results of these techniques in various samples with the results of present study.

In a comparative study by Polisenska *et al.,* (2007) in Czech Federation on 3 samples of barley using HPLC, maximum citrinin production rate was defined as 93.64 µg/kg and minimum rate was 1.82 µg/kg. in another study on 6 barely samples, maximum citrinin production was reported as 13.17 µg/kg and its minimum rate was 5.25 µg/kg. While in present study, maximum citrinin production was 2009µg/k and its minimum rate was 18.64 and in both cases it is more than results of Polisenska *et al.,* (figure 2.4) (Polisenska, 2010).

In a comparative study by Aziz *et al.,* in Egypt on 10 grapes samples using TLC, mean citrinin production was reported as 70µg/kg. in this regard, other studies were performed on other samples as follows: study

on 10 figure samples with 60 µg/kg mean citrinin, study on 10 pear samples with 50 µg/kg citrinin production.

While in this study, mean citrinin production by 21 *Aspergillus* species in northern Iran was defined as 245.72 ppb which in all cases is more than rated measured by Aziz *et al.,* (figure 2.4) (Aziz *et al.,* 2006).

In a comparative study by Tabata *et al.,* in Japan on 12 wheat samples using LC-MS/MS, citrinin production rate was measured as (0.19 µg/kg) and in another study on 2 rye samples, maximum citrinin production was 0.62 µg/kg and mininmum production was 0.55 µg/kg. While in this study maximum citrinin production was 2009.29 µg/kg, its minimum rate was 18.46 µg/kg and mean citrinin production by 21 *Aspergillus* species of northern Iran was defined as 245.72 ppb. In all cases, results of this study indicates much more citrinin production compared to the results of Tabata *et al.,* (figure 2.4) (Tabata *et al.,* 2008).

In a comparative study by Abd-Allah *et al.,* in Egypt (20020 on 30 rice samples using fluorometer technique, maximum citrinin production was reported as 28.54 µg/kg and its lowest rate was 2.74 µg/kg. while in this study maximum citrinin production was defined as 2009.29 µg/kg and its minimum rate was 18.46 µg/kg and in both cases was more than amounts measured by results of Abd-Allah *et al.,* (figure 2.4) (Abd-Allah *et al.,* 2005).

Unfortunately, due to shortage of information and low cases of toxicity by citrinin compared to other mycotoxins, concern toward the study of amount of this mycotoxin in food and agricultural products has been disregarded in Iran and then is no standard measured by organization of standard and control of food stuffs.

This is worth mentioning that taking the dangers and effects of mycotoxins on the economy and ability to contaminate the food and agricultural products leading to concerns on the food and society health, it is required that presence society or absence of this mycotoxin being examined continuously.

Relative distribution of the number of samples of each studied fungi species is as given in table in table below.

Table 1.4: Distribution of studied isolates belonging to the species of *Aspergillus* genus in the study of cell extract

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	2	9.5	9.5	9.5
	2	4	19.0	19.0	28.6
	3	6	28.6	28.6	57.1
	4	5	23.8	23.8	81.0
	5	1	4.8	4.8	85.7
	6	1	4.8	4.8	90.5
	7	2	9.5	9.5	100.0
	Total	21	100.0	100.0	

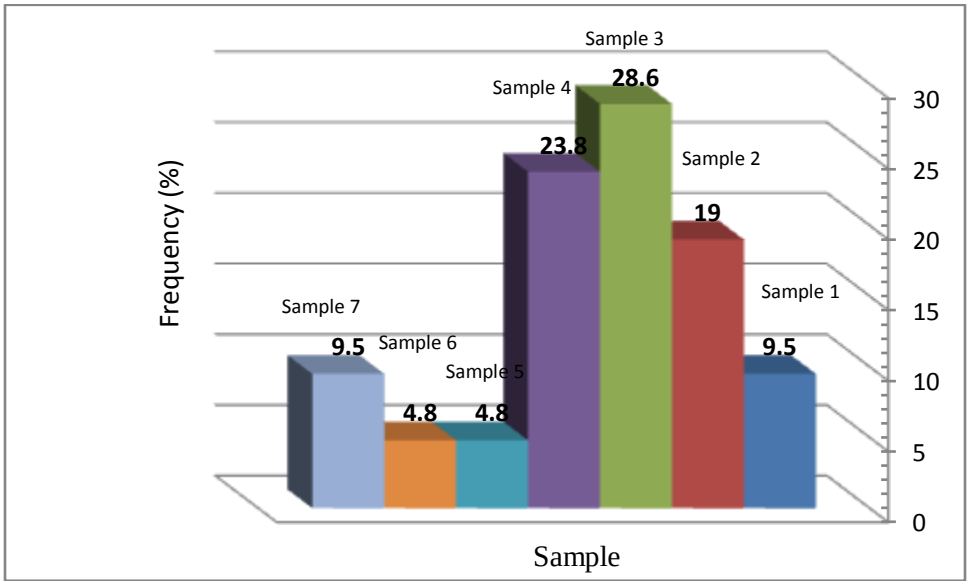

Figure 1.4: Distribution percent of studied isolates belonging to the species of *Aspergillus* genus

According to the figure 1-4 on percentage of frequency distribution of *Aspergillus* species present in the biomass, maximum and minimum frequency numbers of 21 frequencies are as follows, respectively:
Sample 3 having 6 frequencies with 28.6 % has minimum frequency number and is including: *A.ostianus, A.fumigatus, A.niveus, A.parasiticus, A.awamori, A.niger.*
Sample 4 having 5 frequencies with 23.8 % was including: *A.ochraceus, A.terreus, A.carbonarius, A.unguis, A.foetidus*
Sample 2 having 4 frequencies with 19 % was including: *A.meleus, A.af.nidulans, A.sp VI, A.wentii*
Sample 1 having 2 frequencies with 9.5 % was including: *A.candidus, A.alliaceus*
Sample 7 having 2 frequencies with 9.5 % was including: *A.sp3.a f.A.nomius, A.sojae*
Sample 5 having 1 frequency with 4.8 % was including: *A.ornata*
Sample 6 having 1 frequency with 4.8 % was including: *A.flavus*

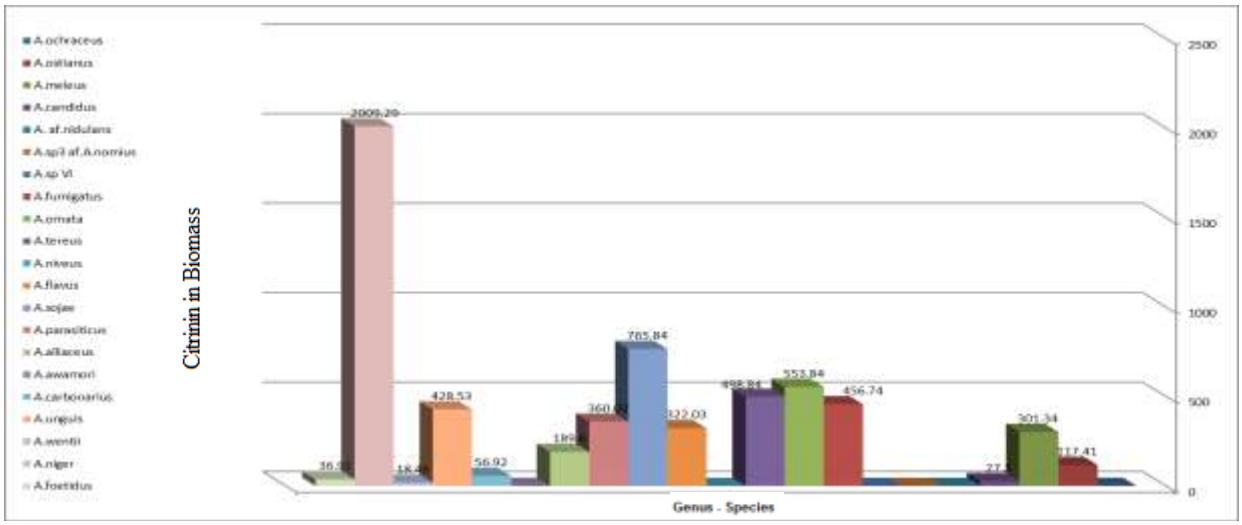

Figure 2.4: Study on the mean Citrinin production in each species in the biomass

According to the figure 2.4, mean citrinin produced in the biomass on 21 *Aspergillus* species is as follows respectively in the maximum order:

1- *A.niger* with 2009.29 ppb citrinin production
2- *A.sojae* with 765.84 ppb citrinin production
3- *A.ornata* with 553.84 ppb citrinin production
4- *A.tereus* with498.84ppb citrinin production
5- *A.fumigatus* with 456.74ppb citrinin production
6- *A.unguis* with 428.53ppb citrinin production
7- *A.parasiticus* with 360.03ppb citrinin production
8- *A.flavus* with 322.03ppb citrinin production
9- *A.melleus* with 301.34ppb citrinin production
10- *A.alliaceus* with 189.60ppb citrinin production
11- *A.ostianus* with 117.41ppb citrinin production
12- *A.carbonarius* with 56.92ppb citrinin production
13- *A.foetidus* with 36.91ppb citrinin production
14- *A.candidus* with 27.30ppb citrinin production
15- *A.wentii* with 18.46ppb citrinin production
16- In other species including *A.sp3 af.A.nomius, A. af.nidulans, A.ochraceus, A.sp VI, A.niveus, A.awamori* citrinin production was not occurred.

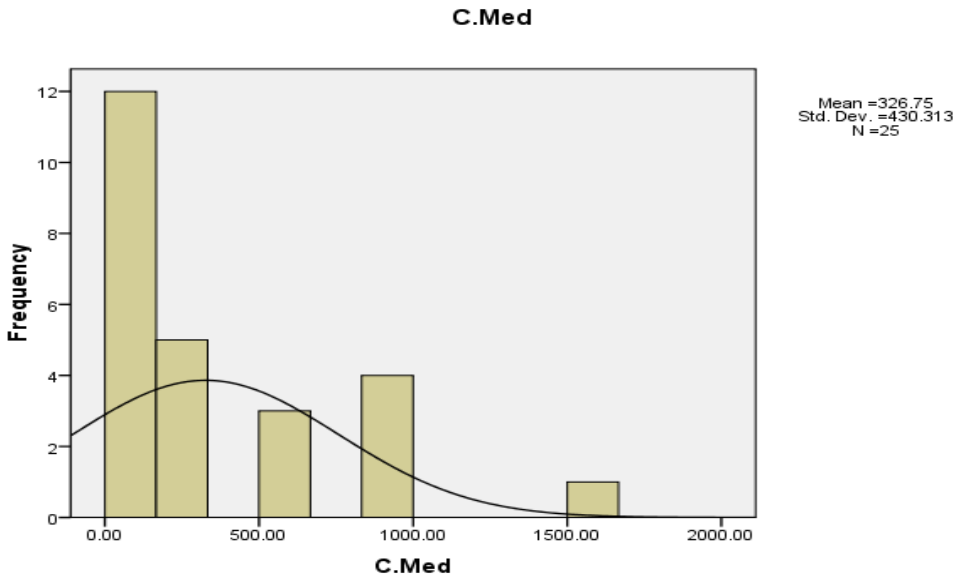

Figure 3.4: Normal distribution curve of mean citrinin measured amounts in the studied samples medium

According to the figure 3.4, normal distribution curve of mean citrinin amounts in the studied samples medium is as follows:

Mean citrinin amount in 25 various *Aspergillus* species measured in the medium was 326.75 ppb. Maximum frequency is located in 0-500 ppb range which is including *A.melleus, A.candidus A.foetidus, A.af.terreu, A.ornata, A.terreus, A.wentii, A.sojae, A.alliaceus, A.awamori, A.carbonarius A.unguis.*

Second frequency was located in 500 to 1000 ppb including *A.ochraceus, A.af.flavus, A.fumigatus, A.niveus, A.flavus, A.parasiticus.* in 1000 to 1500 ppb range, no frequency was observed. minimum frequency is located in 1500 to 2000 ppb range which is including *A.niger.* other studied species such as *A.af.nidulans, A.spv, A.VI, A.spv* lacked citring production ability.

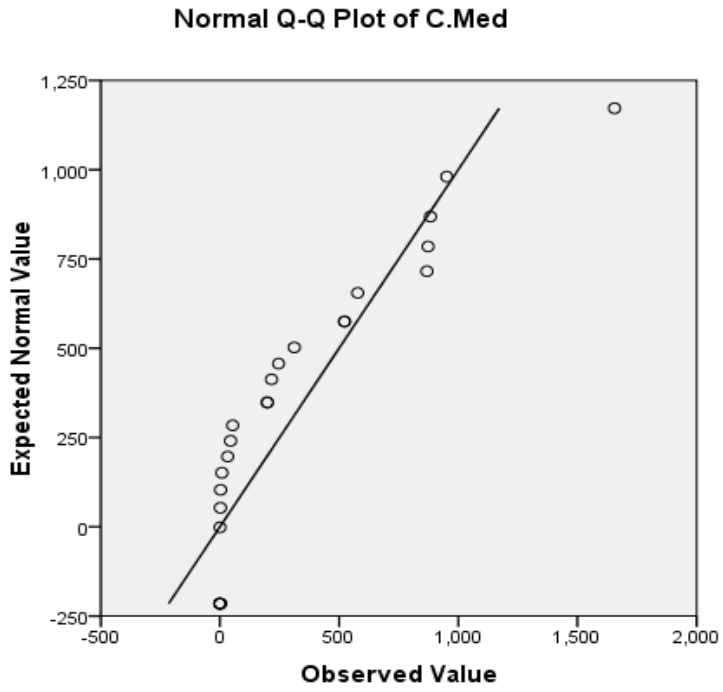

Figure 4.4: Frequency distribution of citrinin measured in the medium of studied specie using NORMAL Q-Q PLOT

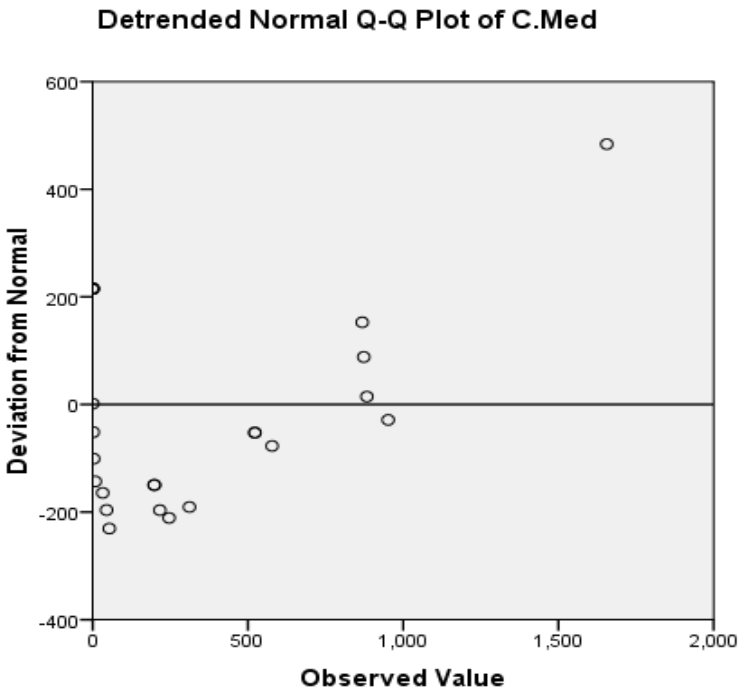

Figure 5.4: Frequency distribution of citrinin amount measured in the medium of studied species using NORMAL Q-Q PLOT based on deviation from normal

Figures 4.4 and 5.4 indicated that frequency distribution of citrinin measured in the medium is not significant i.e it follows normal distribution.

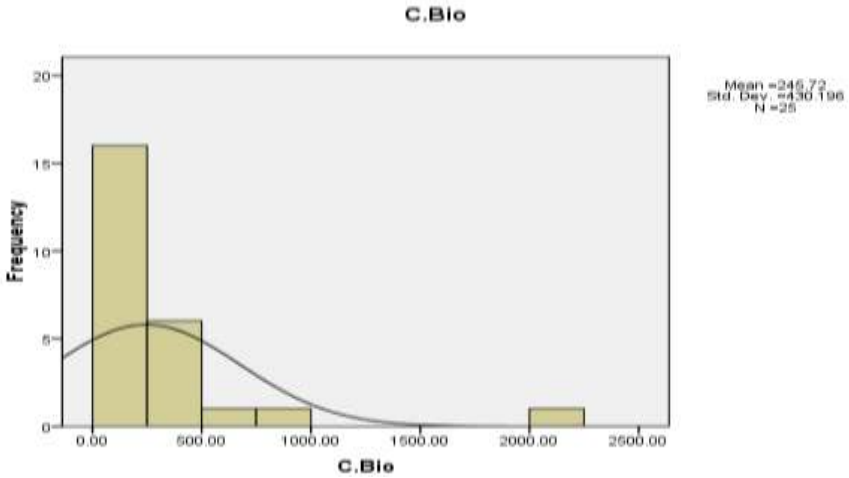

Figure 6.4: Curve of normal distribution of mean measure in the biomass of studied samples

According to the figure 6.4, curve of normal distribution of mean citrinin measured in the biomass of studied samples is as follows: mean citrinin rate in 21 different *Aspergillus* species in the biomass is 245.72 ppb.

Maximum frequency is in the range 0 to 250 ppb including *A.ostianus, A.candidus, A.alliaceus, A.carbonarius, A.wentii, A.foetidus*.

Second frequency is located in 250 to 500 ppb including *A.melleus, A.fumigatus, A.terreus, A.flavus, A.parasiticus, A.unguis.*

A.ornata is the only species found in the range of 500 to 750 ppb.

A.sojae is the only species found in the range of 750 to 1000 ppb.

In the range of 1000 to 2000 ppb there was no species.

Other studies specied including *A.sp3 af.A.nomius, A. af.nidulans, A.ochraceus, A.niveus, A.awamori, A.sp VI* lack citrinin production ability.

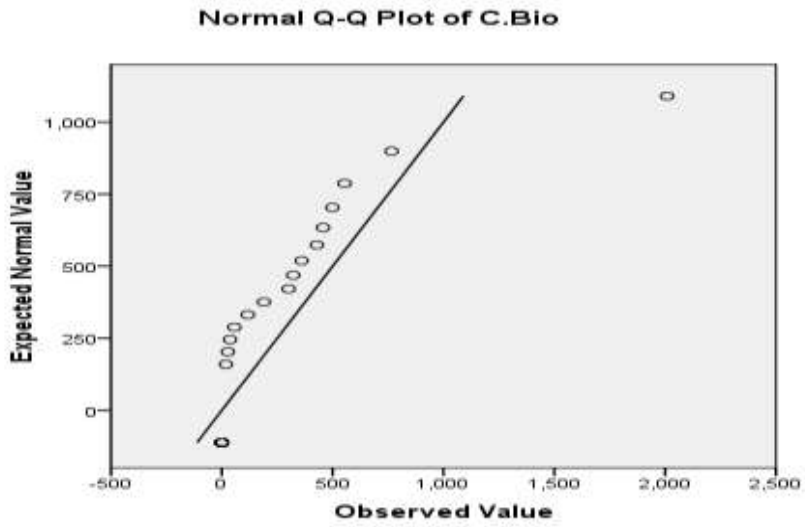

Figure 7.4: Frequency distribution of citrinin produced in the biomass of the species studied using NORMAL Q-Q PLOT

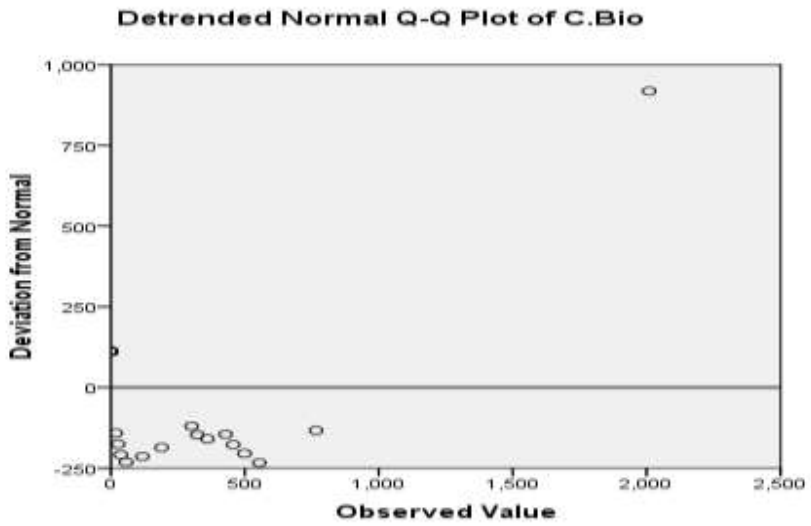

Figure 8.4: Frequency distribution of citrinin produced in the biomass of the species studied using NORMAL Q-Q PLOT based on deviation from normal

Figure 7.4 and 8.4 indicate that distributed of measured amounts in the biomass is significant i.e normal distribution is not followed.

Table 2.4: Statistical measurement of normal distribution of mean citrinin measured in the biomass and medium using one-sample Kolmogorov-smirnov 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 2.4 indicated that distribution of mean citrinin measured in the biomass and medium was normal but according to $p < 0.05$ level, correlation is not significant.

Table 3.4: Correlation of citrinin measured in the biomass and medium of 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 3.4 indicates that correlation of citrinin amounts measured in the biomass and the medium of studied samples is significant and convergent, meaning that by increase in citrinin production rate in the medium, citrinin production rate in biomass will increase and vice versa.

Table 4.4: Numerical difference of citrinin production between medium and biomass using Wilcoxon Signed Ranks 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 4.4 indicates that numerical difference of citrinin production between medium and biomass is not significant. Numerical increases or decrease rates are independent of each other. In fact, increase in citrinin production rate in medium decrease citrinin production rate in the biomass and vice versa.

Recommendations

Through studies must be performed on *Aspergillus* established in northern Iran with one of oldest plant ecosystem across the world so that characteristics of native and introduced species are distinguished.

Products susceptible to mycotoxin contaminations must be stored and handled in a suitable medium under continuous, exact control of technical experts. As a result, necessity to contrast food stuff plants in suitable conditions in respect of *Aspergillus* distribution seems necessary.

Studying and understanding the mechanism of production and dispersion of citrinin by *Aspergillus*.

Spectral analysis and study on genomic map of citrinin production.

Analysis of citrinin production power among *Aspergillus* species and subgenera across the world.

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