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Research Article

AN INVESTIGATION ON THE NORTHERN IRAN'S ASPERGILLUS SPECIES TOXIGENICITY, AN ANALYTICAL COMPARISON OF AFLATOXIN, OCHRATOXIN AND CITRININ IN THE FUNGAL BIOMASSES

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ABSTRACT
One of the most serious problems to confront the quality of food and feed, is the presence of mycotoxins which are produced by different species of the genus *Aspergillus.* Aflatoxin, Ochratoxin and Citrinin are of greatest concern as they are highly toxic and carcinogenic compounds. Samples collected by settling plates, in northern Iran and pure culture isolation performed till the toxin measurement to be done in cell extracts (biomass) which prepared by merging culture in separated prepared culture media incubation. The amount of toxin measured by extracting solutions using Direct Competitive ELISA. The greatest amount of Citrinin produced valued as *A.niger* (2009.3ppb), the highest amount of Ochratoxin produced by *A.melleus* (56.21ppb) and *A.parasiticus* with the highest amount of Aflatoxin (6.02 ppb) played a noble role among the all conducted isolates, in contrast the lowest Citrinin produced by *A.wentii* (18.46ppb) and the lowest Ochratoxin prepared by *A.alliaceus* isolates (4.02ppb)the same as lowest Aflatoxin in *A.awamori (*1.25ppb). According to maximum Citrinin limits (200ppb) and maximum Ochratoxin and Aflatoxin limits (5ppb) in food and feed including to Aspergilli and as the result of our measurements and the maximum amount of Citrinin respectively was (2009.3ppb) produced by *A.niger* infact was the most important species and the highest producer that were much more than the universal standard allowance, the greatest amount of Ochratoxin produced by *A.melleus* (56.21ppb) and the greatest amount of Aflatoxin produced by *A.parasiticus* (6.02ppb) were too much more than standard allowance. Since methods for controlling mycotoxins are largely preventive so we decided to elucidate the comparison between these toxins.

Keywords: *Aspergillus, Citrinin, Aflatoxin, Ochratoxin, Toxigenic Activity, Cell Extracts*

INTRODUCTION
Fungal growth is one of the main causes of food spoilage. It not only generates great economic losses, but also represents a threat to human and animal health, particularly through the synthesis of mycotoxins. Citrine is a polyketide mycotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. Some of the Citrinin producing fungi are also able to produce the mycotoxins Ochratoxin A or Aflatoxin. Citrinin is generally formed after harvest under storage conditions and it occurs mainly in grains cereals but can also occur in other products of plant origin and also in spoiled dairy products, no previous assessments could be identified However, in various documents on the assessment of Ochratoxin A, references made to the concomitant occurrence of Citrinin in a given food or feed material, meaningly When Citrinin was found in a sample, it always occurred with Ochratoxin A (Smith *et al.,* 2009). Citrinin has a conjugated, planar structure which gives its natural fluorescence, the highest fluorescence is produced by a non-ionized citrinin molecule at pH 2.5 (Franco *et al.,* 1996). For the growth of the citrinin producing fungi on grain it is necessary to have a humidity of at least 16.5 – 19.5% and is practically insoluble in cold water but soluble in aqueous sodium hydroxide, sodium carbonate, or sodium acetate; in methanol, acetonitrile, ethanol, and most other polar organic solvents (Xu *et al.,* 2006). One of the most serious problems to confront the quality of food and feed, is the presence of mycotoxins (EFSA 2011).

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Citrinin is a nephrotoxic mycotoxin produced by several species of the genera *Aspergillus*, and also has been reported that Citrinin may be implicated in human disease, in particular, there are limited evidence for the carcinogenicity that was concluded by the International Agency for Research on Cancer (IARC 1993). During research for antibiotic agents in the middle of the last century, interest in Citrinin arose when its broad antibacterial activity was identified, However, interest decreased when its mammalian toxicity was demonstrated (EFSA 2011). A large number of Citrinin derivatives have been isolated from different fungal species in search of antitumor compounds indicating that Citrinin might be a precursor of novel active compounds (Du *et al.*, 2010). Fungal growth and mycotoxin production during storage are generally spot processes significantly affected by crop variety agronomic practices and weather conditions during harvest post, harvest drying and cleaning storage and processing conditions as well as toxigenic potential of the mould species. Consequently, the distribution of mycotoxins in a lot of agricultural products is heterogeneous. Thus sampling is the largest source of variability associated with mycotoxin analysis and the most crucial step in obtaining reliable results (Köppen *et al.*, 2010).

Ochratoxin A (OTA) is a toxin naturally produced by several species of *Aspergillus* and *Penicillium*. These mould species are capable of growing indifferent climates and on different plants and so contamination of food crops with OTA can occur worldwide. This toxin has a nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties (Schilter *et al.*, 2005). Many studies have shown that cereals and cereal products are the main sources of OTA (Czerwiecki *et al.*, 2002; Kuiper-Goodman, 1996). It has also been detected in other products such as green coffee, milk, wine and grape juice (Serra *et al.*, 2004). OTA can be found in a wide range of human foods. The toxicology and human health risks of OTA have been assessed at both European and international levels by the European Commission Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), who have established tolerable intakes of OTA from food (JECFA 2001). The kidney is the major site of OTA-induced toxicity, where it acts principally on the middle (S2) and terminal (S3) segments of the proximal convoluted tubules (Jung and Endou 1989). OTA has been shown to be nephrotoxic in all monogastric species tested, although there are species differences in sensitivity to nephrotoxic effects (Kuiper-Goodman and Scott 1989). Once absorbed, OTA readily binds to serum albumin and is distributed in the blood predominantly in bound form (Galtier *et al.*, 1980). OTA binds strongly to human serum albumin and shares a common binding site with other known anionic compounds, including warfarin, naproxen and phenylbutazone, giving rise to the possibility of OTA–drug interactions (Il'ichev *et al.*, 2002). Clinical chemistry parameters have been used to monitor the effects of OTA on renal function. Studies have shown that OTA exposure can lead to increased urine volume, blood urea nitrogen (Hatey and Galtier 1977), urinary glucose, and proteinuria (Berndt and Hayes 1979) as well as to reductions in the activity of Ochratoxin A 313 enzymes in the kidney, such as alkaline phosphatase, leucine aminopeptidase, and γ -glutamyl transferase (Kane *et al.*, 1986). The biochemistry of OTA results primarily from its structural similarity to the essential amino acid, phenylalanine (Phe). The principal effect appears to be inhibition of protein synthesis, although secondary effects such as inhibition of RNA and DNA synthesis have also been implicated in its mechanism of action. The chemical structure of OTA consists of a 5'-chlorinated-3, 4-dihydro-3-methyliscumarin moiety linked to L-Phe (Creppy *et al.*, 1984). A number of epidemiological studies have identified OTA as a likely aetiological agent responsible for a fatal kidney disease primarily affecting rural populations in the central Balkan peninsula including Bosnia and Herzegovina, Bulgaria, Croatia, Romania, Serbia and Slovenia, the disease, referred to as Balkan endemic nephropathy (BEN), is characterized by tubular degeneration, interstitial fibrosis and hyalinization of the glomeruli and a slowly developing impairment of renal function with a progressive decrease in kidney size (Vukelic *et al.*, 1991). Although OTA is a contaminant of food and feed worldwide, it has been detected at high levels in a wide range of foods and in human blood samples taken from areas where BEN is endemic (Pfohl-Leszkowicz *et al.*, 2002). OTA has been implicated in the development of cancers of the human urinary tract because of the higher incidence of urinary tract tumours in humans in regionswhere BEN is endemic (Chernozemsky *et al.*, 1977). The mechanism by which OTA induces tumour formation remains controversial and is an area of active study. Formation of

DNA adducts is thought by some researchers to be an important event in the tumorigenicity of OTA and it is postulated that OTA or one of its metabolites may act through a genotoxic mechanism involving direct covalent binding to DNA. Although numerous studies have investigated the transformation of OTA into a reactive intermediate and its possible role in tumorigenesis (El Adlouni *et al.,* 2000). Data on the possible effects of OTA on the immune system are limited. However, a number of *in vivo* and *in vitro* studies suggest that OTA may affect both humoral and cell-mediated immunity, although the reported effects were generally observed at higher doses than those capable of causing nephrotoxicity.At very high doses (0.5–80 mg/kg body weight) OTA has been shown to cause gross changes to organs of the immune system, such as reductions in thymus size in mice, rats and chickens (Boorman e al.1984) and necrosis of cells in the spleen and lymph nodes in rats (Kanisawa *et al.,* 1977).

Aflatoxins are secondary metabolites produced by species of Aspergilli, specifically *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 is the most potent natural carcinogen known and is usually the major Aflatoxin produced by toxigenic strains. These molds are ubiquitous in nature and grow on a variety of substrates, thereby producing Aflatoxins. From the mycological perspective, there are great qualitative and quantitative differences in the toxigenic abilities displayed by different strains within each aflatoxigenic species. For example, only about half of *Aspergillus flavus* strains produce aflatoxins (Klichand and Pitt 1988), while those that do may produce more than 106 _g/kg (Cotty *et al.,* 1994). Aflatoxins are of great concern due to their biochemical and biological effects on living organisms so the occurrence of Aflatoxins, their biosynthesis, factors influencing their production, their effects on living organisms, and methods of detection and control in food were rutinly reviewed including some future areas of research involving mathematical modeling of factors influencing Aflatoxin production and alternative methods of control, were also discussed (Smith *et al.,* 2009). Many substrates support growth and aflatoxin production by aflatoxigenic molds. Natural contamination of cereals, Figure s, oilseeds, nuts, tobacco, and a long list of other commodities is a common occurrence, like the genetic ability to make aflatoxin, contamination is highly variable. Sometimes crops become contaminated with aflatoxin in the field before harvest, where it is usually associated with drought stress (Diener *et al.,* 1987). Aflatoxin is associated with both toxicity and carcinogenicit in human and animal populations (Eaton and Groopman 1994). The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. The liver is the primary target organ, with liver damage occurring when poultry, fish, rodents, and nonhuman primates are fed aflatoxin B1. The data on aflatoxin as a human carcinogen are far more damning than the data implicating it in acute human toxicities.Exposure to aflatoxins in the diet is considered an important risk factor for the development of primary hepatocellular carcinoma, particularly in individuals already exposed to hepatitis B. In classical epidemiology, several studies have linked liver cancer incidence to estimated aflatoxin consumption in the diet (Li *et al.,* 2001). There are substantial differences in species susceptibility. Moreover, within a given species, the magnitude of the response is influenced by age, sex, weight, diet, exposure to infectious agents, and the presence of other mycotoxins and pharmacologically active substances. Thousands of studies on aflatoxin toxicity Have been conducted, mostly concerning laboratory models or agriculturally important species (Newberne and Butler 1969). Cytochrome P450 enzymes convert aflatoxins to the reactive 8, 9-epoxide form (also referred to as aflatoxin-2, 3 epoxide in the older literature), which is capable of binding to both DNA and proteins, in molecular epidemiology, it is possible to demonstrate with more certainty the association between putative carcinogens and specific cancers. Biomonitoring of aflatoxins can be done by analyzing for the presence of aflatoxin metabolites in blood, milk, and urine; moreover, excreted DNA adducts and blood protein adducts can also be monitored (Richard *et al.,* 1999).

Although there are geographic and climatic differences in the production and occurrence of mycotoxins, exposure to these substances is worldwide, with much of the world food supply contaminated to some extent. Monitoring for the presence of mycotoxins is therefore needed. From time to time the presence of mycotoxins may render food commodities unsafe, requiring a variety of measures to reduce risk. As these toxicants can never be completely removed from the food supply, many countries have defined levels in food (tolerances, guideline levels, maximumresidue levels) that are unlikely to be of health concern.

Initially, the lack of aunified and transparent approach resulted in a wide range of guidelines and regulations regarding mycotoxins among various countries(Stoloff *et al.*, 1991). Scientefic evaluations have now generally become the basis for recommendations regarding the international regulation on mycotoxins (Aflatoxin, Ochratoxin and Citinin) by the Codex Committee on Food Additives and Contaminants CCFAC as well as the European Union. Regulations may include guidelines regarding maximum residue levels or procedural guidelines aimed at prevention by using a Hazard Analysis of Critical Control Points (HACCP), or a combination of these. The overall process needs to be transparent and should aid in the development of harmonized mycotoxin regulations control procedures.Ideally, such guidelines are acceptable to countries producing as well as those importing food commodities (CODEX 2001). Domestic animals are exposed to mycotoxins, significant amounts of latter shall be carried over into animal products. Based on a study carried out in order to determine the possible average of Aflatoxin, Ochratoxin A (OTA) and Citrinin (CIT) in cell extracts (biomass), using ELISA (Markov *et al.*, 2013).

MATERIALS AND METHODS

The following sample agenda for the closed (Indoor) and opened (Outdoor) positions based on the CBS rules was performed, fifty square acres of agricultural areas and processing plants samplified by settle plates in a group setting, were taken by six plates containing Malt extract agar, Yeast extract agar, Czapek yeast extract agar, Czapek agar, Saboraud dextrose agar and potato dextrose agar confounded with chloramphenicol (100ppm) were used to withdraw a sample group.All plates at 2 ± 25c° and were incubated aerobically and consistently in the range of 3, 7 and 15 days were checking to withdraw and the culture plates are subcultured in the tubes that containing agar slant bott from growth media of Malt extract agar, Yeast extract agar, Potato dextrose agar, Corn meal agar, Saburod dextrose agar, Czapek yeast agar, Czapek doxs agar and incubated with the previous program. Of Aspergillus colonies on selective agar plates that containing Czapek doxs agar, Czapek yeast extract agar (with and without %20 sucrose), Malt extract agar and Czapek doxs agar (with and without %20 sucrose) according to ICPA identification rules grown at 2 ± 25 c°, after 3, 7 or 14 days were reviewed and provided slide cultures for each sample on substrates of Czapek doxs agar and Czapek yeast extract %20 sucrose to provide normative growth model.

Preparing extracts obtained from isolates grown in liquid medium to perform more motivate and abundant extract of each isolate grown were taken on Czapek extract broth and a 50ml Falcon tubes containing liquid medium Czapek doxs broth that contains 2 percentage of malt extract to enhance growings, tubes with 200RPM at 3 ± 25c° and in the light - darkness were incubated then after a 7-14 days float or sink in a liquid mass on the same field small Germ tubes using centrifugation with 3000RPM for 15 min precipitated then separated with sterile filter paper from the growth medium till each to be harvested.The mass was dried for 48 hours in a desiccator and then 2g of biomass was harvested, dried and then 2g of each major mold was transferred in a 15ml falcon tubes three times in a row (every 1± 5min), mixed with 5 ml of liquid nitrogen and a stirring device glass tube (Pearls) and every 25 min combination. Falcon tubes with 5ml of sample buffer and added cold acetone 1ml, centrifuged with 3000RPM for 15min separation takes place supernatant of coarse sediment removal and other tubes were kept in the notation for synchronization, the size of each protein mixture obtained from Aspergillus isolates was performed and all samples were measured by the Bradford method to size 0.5mg/ml aligned the concentrated sample dilution and the diluted samples were again concentrated by this method until all the juice extract samples with 0.5 mg per ml of protein.

A competitive enzyme immunoassay for the quantitative analysis of Citrinin in cereals and feed (RIDA SCREEN® FAST assay) were used to quantification of mycotoxin citrinin formed by the *Aspergillus* species are able to produce Citrinin and/or Ochratoxin A, therefore both mycotoxins often appear together, is possible to detect this mycotoxins both rapidly and with accuracy. Firstly filtered the extracts through whatman No.1 filter, diluted 1 ml of the deionized water and used 50µl of the filtrate per well in the tests as the basis of the antigen-antibody reaction in the microtiter wells coated with the aimed mycotoxins followingly standards, respectively sample solution and anti- mycotoxins antibodies, were

added concommitantly. Free and immobilized mycotoxins compete for the mycotoxins antibody binding sites (competitive enzyme immunoassay) after a washing step secondary 100µl antibodies labeled with peroxidase were added to bind to the bound anti- mycotoxins antibodies while any unbound enzyme conjugated secondary antibodies were then removed in washing step, 100µl substrate/chromogen were added to the wells, bound enzyme conjugate (secondary antibodies labeled with peroxidase) converts the choromogen into a blue color product then turning by addition of the 100µl stop solution leads to a color change to yellow.The measurement is made photometrically at 450nm, at the absorbance lead inversely proportional to the mycotoxins concentration in the sample.

RESULTS

Aspergillus species that produced Citinin in biomass were *A.candidus* and *A.alliaceus* with one isolated, *A.nidulans*, *A.wentii*, *A.*sp*VI*, *A.melleus* with two isolated, *A.fumigatus*, *A.ostianus*, *A.niveus*, *A.niger*, *A.awamori*, *A.parasiticus* with three isolatesd and *A.ochraceus*, *A.terreus*, *A.carbonarius*, *A.unguis*, *A.foetidus* with four isolated and as well as *S. ornata* with five isolated, *A.flavus* with six isolates and also *A.sojae*, *A.af.nomius* species with seven isolated runned for analytical toxicology.

In the measurement of the Citrinin mean produced among the studied species, most of the toxin produced by *A.niger* (2009.3 ppb) and after that *A.sojae* (765.74ppb) and *S.ornata* (553.84ppb), *A.terreus* (498.84ppb), *A.fumigatus* (456.74ppb), *A.unguis* (428.53ppb), *A.parasiticus* (360.3ppb), *A.flavus* (322.3ppb), *A.melleus* (301.34 ppb), *A.alliaceus* (189.6 ppb), *A.ostianus* (117.41ppb), *A.carbonarius* (56.92 ppb), *A.foetidus*(36.91ppb), *A.candidus* (27.3 ppb), *A.wentii* (18.46ppb) and *A.ochraceus, A.awamori, A.VI, A.niveus, A.af.nidulans* and *A.af.nomius* species has not produced Citrinin in biomass (Figure 1).

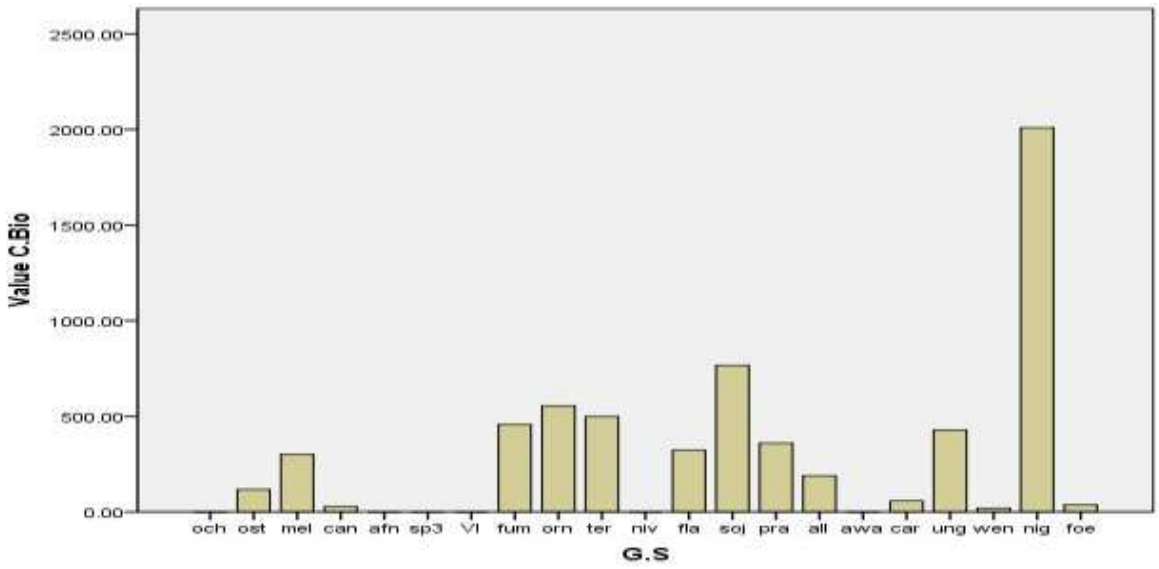

Figure 1: Measurement of Citrinin average amount in the biomass of each *Aspergillus* species

Aspergillus species that produced Aflatoxin and Ochratoxin in biomass were *A.*af *nidulans* and *A.*sp *VI*, *A.alliaceus* with two isolated, *A.melleus*, *A.awamori*, *A.niveus*, *A.ostianus* with three isolated, *A.ochraceus*, *A.candidus* with four isolated, *A.parasiticus*, *A. fumigatus* with five isolated, *S.ornata*, *A. carbonarius* with six isolated, *A.terreus*, A.sp3, *A.af.nomius* with seven isolated, *A.sojae* with eight isolated and also *A.flavus* species with seventeen isolated.

In the measurement of the Ochratoxin mean produced among the studied species, most of the toxin produced by *A.melleus* (56.21ppb) and after that *A.niveus* (11.41ppb) and *A.parasiticus* (10.73ppb), *A.terreus* (9.81ppb), *A.af.nomius* (9.80ppb), *A.af.nidulanc* (9.79ppb), *A.sojae* (9.78ppb), *A.ochraceus*

(9.76ppb), *A.ostianus* (9.75ppb), *A.VI* (9.74 ppb), *A.awamori* (9.73ppb), *S.ornata* (9.73ppb), *A.carbonarius* (9.69ppb), *A.flavus* (9.63ppb), *A.fumigatus* (9.09ppb) and *A.candidus* (8.50 ppb), *A.alliaceus* (4.02ppb), *A.unguis*, *A.niger*, *A.foetidus* and *A.wentii* species has not produced Ochratoxin in biomass (Figure 2).

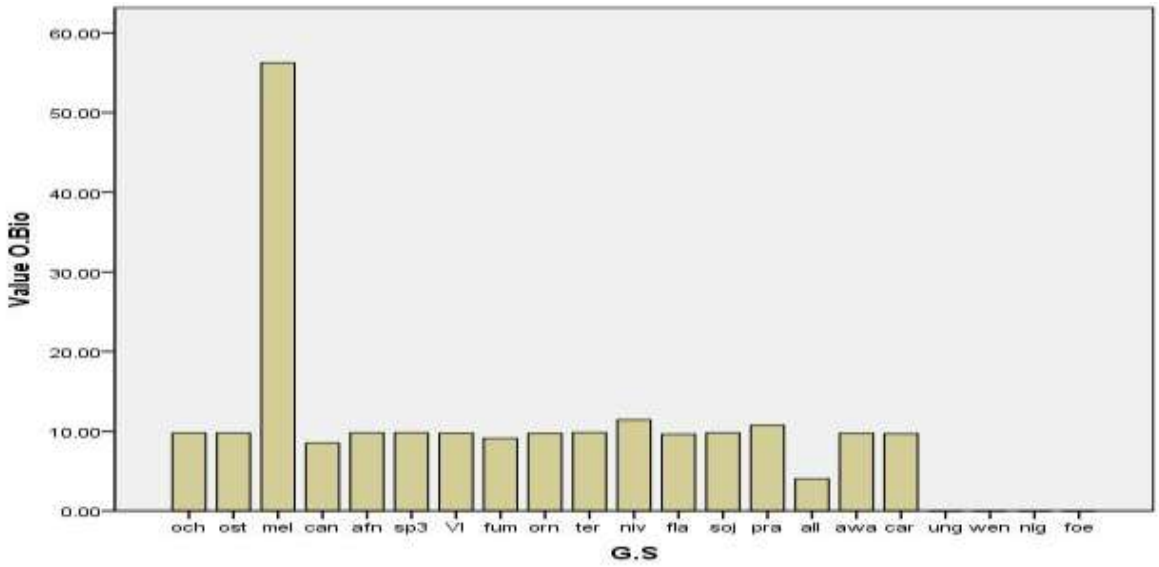

Figure 2: Measurement of Ochratoxin average amount in the biomass of each *Aspergillus* species

In the measurement of the Aflatoxin mean produced among the studied species, most of the toxin produced by *A.parasiticus* (6.02ppb) and after that *A.candidus* (4.75ppb) and *A.niveus* (4.52ppbppb), *A.alliaceus* (3.43 ppb), *A.melleus* (3.08ppb), *A.sojae* (2.41ppb), *A.fumigatus* (2.03ppb), *A.af.nomius* (1.82ppb), *S.ornata* (1.77ppb), *A.flavus* (1.77ppb), *A.terreus* (1.72ppb), *A.af.nidulanc* (1.71ppb), *A.carbonarius* (1.62ppb), *A.ochraceus* (1.60ppb), *A.VI* (1.56ppb) and *A.ostianus* (1.41ppb), *A.awamori* (1.25ppb), *A.unguis*, *A.niger*, *A.foetidus* and *A.wentii* species has not produced Aflatoxin in biomass (Figure 3).

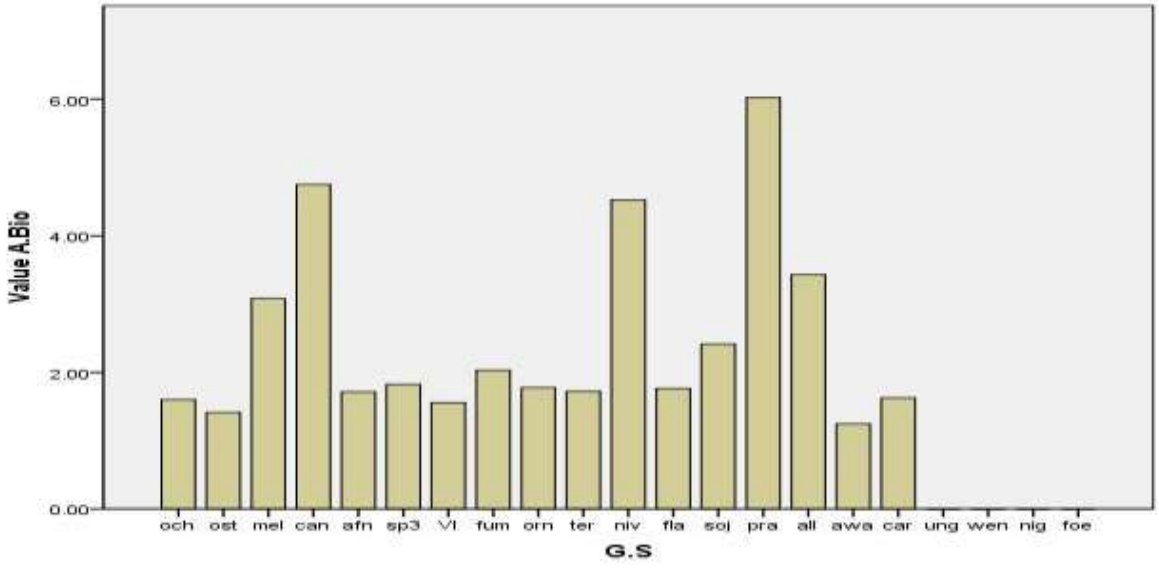

Figure 3.Measurement of Aflatoxin average amount in the biomass of each *Aspergillus* species

According to the measured Ochratoxin, Aflatoxin and Citrinin average amounts were produced by *Aspergillus* species, *A.parasiticus, A.candidus, A.niveus, A.alliaceus, A.melleus, A.sojae, A.fumigatus, A.af.nomius, S.ornata, A.flavus, A.terreus, A.af.nidulanc, A.carbonarius, A.ochraceus, A.VI, A.ostianus* and *A.awamori* that produced both of the Ochratoxin and Aflatoxin showed that there is a significant relationship enable them to produce more than one toxin at a time.And also sighnificant relationship has been seen in *A.melleus, A.parasiticus, A.terreus, A.sojae, A.ostianus, S.ornata, A.carbonarius, A.flavus, A.fumigatus, A.candidus* and *A.alliaceus* isolates that produced both of Citrinin and Ochratoxin as well. A sighnifican relationship in the isolates of *A.melleus, A.parasiticus, A.terreus, A.sojae, A.ostianus, S.ornata, A.carbonarius, A.flavus, A.fumigatus, A.candidus* and *A.alliaceus* that produced both of Citrinin and Aflatoxin were seen.

DISCUSSION

In studies conducted in this research and the results obtained by ELISA and relative distribution, the number of samples per obtained *Aspergillus* species isolates prepared for cell extracts (biomass), the most frequent were *A.ostianus, A.fumigatus, A.niveus, A.niger, A.awamori and A.parasiticus* respectively with a prevalence (28.6%) as the most frequent, according to measurements of mycotoxins averaged Citrinin, Ochratoxin and Aflatoxin in biomass, indicated that the most of species produced Citrinin was *A.niger* valued (2009.3 ppb), for Ochratoxin the highest concentration belonged to *A.melleus* at the rate of (56.21ppb) and for Aflatoxin, *A.parasiticus* with the value of (6.02 ppb) take the top in contrast the lowest Citrinin produced by *A.wentii* (18.46ppb) and the lowest Ochratoxin prepared by *A.alliaceus* isolates (4.02ppb) the same as lowest Aflatoxin in *A.awamori* (1.25ppb). According to maximum Citrinin limits (200ppb) and maximum Ochratoxin and Aflatoxinin limits (5ppb) in Europe, Asia, America eventualy Latin America, New Zealand, Africa, Canada and the Middle East in food products and animal feed including, wheat, corn, barley, rice, flour, Black wheat, oats, red rice, fruit and nuts, especially related to the genus *Aspergillus* and *Penicillium* and etc were determined, as the result of our measurements and the statistical analysis, maximum amount of Citrinin in the cell extracts, respectively was (2009.3ppb) produced by *A.niger* infact was the most important species and the highest producer that were much more than the universal and local standard allowance, the greatest amount of Ochratoxin produced by *A.melleus* (56.21ppb) and the greatest amount of Aflatoxin produced by *A.parasiticus* (6.02ppb) were more than standard allowance. Since methods for controlling mycotoxins are largely preventive we focused on those mycotoxins that are known or suspected to cause human disease.

According to the conducted research in relation to the measurement of Aflatoxin, Ochratoxin and Citrinin and the results of our analysis, It should be noted that the previous studies, Ochratoxin A produced by species of *A.carbonarius*, *A.ochraceus* and *A.niger* in grain samples were examined and According to measurements performed in our study, *A.niger* (2009.3 ppb), *A.carbonarius* (9.69 ppb), *A.ochraceus* (9.75 ppb) produced OTA in cell extracts (Abarca *et al.,* 2001). domestic animals are exposed to mycotoxins, significant amounts of the latter shall be carried over into animal products such as milk, eggs and meat. This study was carried out in order to determine the possible presence of aflatoxin B_1 (AFB_1), ochratoxin A (OTA) and citrinin (CIT) in game sausages (n = 15), semi-dry sausages (n = 25) and fermented dry-meat products (n = 50), randomly taken from individual producers and the Croatian market. AFB_1 and OTA were quantified using ELISA, while CIT was quantified using HPLC-fluorescence detector. Out of 90 samples, the fungi most frequently isolated from dry-cured meat products were of *Penicillium* species, while *Aspergillus* was isolated from only one sample. As much as 68.88% of the samples were positive for mycotoxins. Finally, the analysis of different types of meat products resulted in OTA identification in 64.44%, CIT identification in 4.44% and AFB_1 identification in 10% of the samples. The maximum OTA concentrations established in the commercial sausage samples equalled to 7.83 μg/kg, while that of AFB_1 amounted to 3.0 μg/kg. Generally, although OTA was detected in all three types of products in different percentage shares, mutual differences were not statistically significant (Molinié *et al.,* 2005; Markov *et al.,* 2013). In Spain, samples of aromatic and/or medicinal herbs sold screened, using an ELISA (LOD = 16.5 μg/kg) and found 61% of samples contaminated with Citrinin (up to 355 μg/kg in ginkgo leaves), in

two samples the highest concentrations of Citrinin contamination, the toxin co-occurred with OchratoxinA, Aflatoxin B1 and also the another studied, seed samples of medicinal plants known to have curative properties for various human diseases, from different storage centers in India, 20% samples were contaminated with Citrinin at a concentration between 10 and 760 µg/kg(Santos *et al.,* 2009). In a preliminary studied, on feed samples collected from different parts of Northern India were examined for the presence of Aflatoxigenic strains of *A. flavus/parasiticus* for detection of Aflatoxin B_1 (AFB_1) reported that out of 198 *A. flavus* and 15 *A. parasiticus* isolated strains, 76% and 86% respectively, were found to be toxigenic.Aflatoxin B_1 content of these feeds, as estimated by (TLC) and (ELISA) were very high (average 0.412 ± 0.154 ppm) in comparison to the permissible Indian regulation level (0.03 ppm). Seasonal variation of incidence and level of toxin in feed was recorded and it was high during monsoon/post monsoon period while in our analysied informations, *A.parasiticus* (6.02ppb) and *A.flavus* (1.77ppb) produced Aflatoxin in cell extracts (Dutta and Das 2001). The occurrence of 3 mycotoxins (Aflatoxin B1, Citrinin and Ochratoxin A) investigated in rice samples collected from 5 provinces of the central region of Vietnam, using HPLC with fluorescence detection (LOD = 0.11 and LOQ = 0.35 µg/kg). Citrinin was detected in 13% of the samples at concentrations up 0.42µg/kg(Nguyen *et al.,* 2007).One of the first comprehensive studies on the occurrence of Citrinin was done in grain samples associated with lung problems in farmers and silo operators were collected from farm storages and analysed. The grain had been stored under damp conditions, resulting in heating and spoilage. After development of an appropriate screening method, more samples were found to contain Citrinin (0.07 to 80 mg/kg). The contaminated samples included wheat, oats, barley and rye.All samples positive for Citrinin were also contaminated with Ochratoxin A. In Europe studies have been mainly carried out in South-Eastern European countries, where the occurrence of Citrinin has been linked in the past to the so-called Balkan endemic nephropathy (Scott *et al.,* 1972). In studied on wheat samples (for food use) from the Czech Republic analysed shortly after harvest. There was only one sample positive for Citrinin, which had a low content not exceeding the LOQ (1.5 µg/kg). The same samples had an Ochratoxin A content of 4.7µg/kg. The authors also analysed barley samples destined for malt production. One of the samples was offered to a malt house but not accepted due to a higher content of admixtures and impurities and a mouldy smell. This sample contained the highest Citrinin content (93.6µg/kg) and also contained Ochratoxin A (31.4 µg/kg). Barley and wheat for feed use were also analysed by these authors and Citrinin was found in only few barley samples up to a concentration of 13.2 µg/kg(Polisenska *et al.,* 2010). In one studied on Citrinin investigated in grains for food use with LC-MS/MS. Citrinin was detected in one wheat sample at a concentration of 0.19 µg/kg, together with Ochratoxin A, and in two buckwheat samples at concentrations of 0.55 and 0.62 µg/kg, also with Ochratoxin A. In one third rice samples(Tabata *et al.,* 2008). A methodology described for simultaneous extraction/purification of Ochratoxin A and Citrinin with a recovery for Citrinin of 80% and an LOD of 0.5 µg/kg. They confirmed that for breakfast cereals, if Citrinin was present its content was higher than that of Ochratoxin A. They analysed samples, of which 69% were contaminated with Ochratoxin A at 0.2-8.8µg/kg and 18% were contaminated with Citrinin in the range of 1.5 to 42 µg/kg. When Citrinin was found in a sample, it always occurred with Ochratoxin A.The sample with the highest Citrinin concentration (42µg/kg) contained Ochratoxin A at a concentration of 4.1µg/kg(Molinié *et al.,* 2005). The Enzyme linked immunosorbent assays (ELISA) for Citrinin detection have been reported in wheat, barley, maize, RMR, and other grains, with LODs ranging from 2 to15000 µg/kg that according to our analysis by ELISA methods, Citrinin is produced in the range of (18.46-2009.3ppb) in cell extracts by *A.niger* (2009.3ppb) and *A.wentii* is 18.46ppb (Duan *et al.,* 2009; Li *et al.,* 2010).

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