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COMPARING AFLATOXIN G_1, G_2, TOTAL AFLATOXIN AND OCHRATOXIN PRODUCTION SPECTRA AND QUANTITY IN CULTURE MEDIUM OF 24DIFFERENT ASPRERGILLUS SPECIES FROM IRANIAN NORTHERN

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ABSTRACT

Aflatoxin is a fungal toxin that commonly contaminates maize and other types of crops during production, harvest, storage or processing. Exposure to aflatoxin is known to cause both chronic and acute hepatocellular injury. From the first May to the last October 2011, sampling was done according to"CBS" instructions for indoor and outdoor stations.One sample group was taken from among 50000 meter square area fields and also per processing, plant using settle plates based on CBS rules too.The all samples prepared as mentioned above for an indirect Competition ELISA assay for fine quantitation of ochratoxin based on manufacturer instructions for all samples and standards of estimated and then corrected datas reflecting to standard curve obtained as ELISA reader calibrated by 450 nm UV the light for comparing the density of sample sand standard OP and preparing final results. The greatest ochratoxin mean was realted to the sepcies A.carbonarius and also the greatest aflatoxin G1 and total aflatoxin mean was realted to the sepcies *A.carbonarius*. Aflatoxin G2 Mean is zero.

KEY WORDS: Aflatoxin G_1, G_2, Asprergillus species, Culture medium, Northern Iran.

INTRODUCTION

Aspergilus is one of the oldest named genera of fungi. *Aspergillus* in agriculture, originally was considered a serious problem largely because of its prevalence in the biodeterioration of stored crops and as an opportunistic pathogen of field crops, particularly under high moisture conditions (Cawood et al., 1991). During the early 1960, the discovery of aflatoxins associated with massive deaths of poultry, trout and other domesticated animals species worldwide raised new awareness that these fungi posed threats to foods and feeds beyond their ability to rot plant materials (Cole et al., 1981). Research on aflatoxins led to a so-called ‘golden age’ of mycotoxin research during which many new fungal toxins were discovered from species of *Aspergillus* and other common moulds. In addition to aflatoxins, other important *Aspergillus* mycotoxins include ochratoxin,patulin and fumigillin (Goldblatt ,1969). Ochratoxins are fungal secondary metabolites produced by several species of *Aspergillus* and *Penicillium*. They consist of an isocoumarin moiety and a phenylalanine moiety linked by an amide bond (Reddy et al.,2010).Ochratoxin A was first isolated from A. Ochraceus(hence its name) in 1965, in South Africa (Stormer,1992). Ochratoxin A

(OTA), which is a potent nephrotoxin and nephrocarcinogenic mycotoxin, can occur in a wide range of unprocessed and processed food (Vander Merwe et al., 1965).

MATERALS AND METHODS

From the first May to the last October 2011, sampling was done according to"CBS" instructions for indoor and outdoor stations. One sample group was taken from among 50000 meter square area fields and alsoper processing, plant using settle plates based on CBS rules too. Six plates including Malt extract agar (MEA), Yeast extract agar (YEA), Czapek agar (CZA), Chapek Yeast extract agar (CZYA), Saborud dextrouse agar (SDA) and Potato dexterous agar (PDA), all with 100 ppm chloramphenicol and 50 ppm tetracycline were applied for one sample group. All the plates were incubated at $2\pm25c^{\circ}$ aerobically then examined in the periods of 3, 7 and 15 days. At last 107 colonies were cultivated for macroscopic and microscopic morphology examinations the study on morphology and macroscopic features, front and back colores, pigments , umbrella ,foundings and grown masses and also examining and micrometery was done bymicrophotometicestroscope and microscope, the samples with the help of slide culture prepared then were done by leicamicro analysis microscope hard and soft wares.

HPLC measuring

In order to do HPLC process, we first purified our sample of all Aflatoxins; we used imonoaffinty columns. According to the test instructions , extraction of toxin was done by using methanol solution and water . At first , injected extracts to the , Columns includes gells containing antibodies specifid for Aflatoxin B and G variants at the next step, washed by PBS solution that really the resumed B and G Aflotoxin attached to gell and other existing materials Were washed and to be separated or deleted then columns were cleaned With a special ethanol solution. applied as 2 step process added 125 ml of solution and in secondly 50 ml to the column and finally attached toxins been passed through columns with methanol and then collecting to be into particular vials .We entered mobile phase in which includes water , methanol , acetonitriede or mix of these and we pour it into device (plant)bottle then we turned on detector be justified 360 nm wave and switched on electronic record justified the current speed of mobile phase and device is placed in this current for 30 to 45 minutes without injection .After 45 minutes ,for absorption of the mobile phase, we played system suitability test and recorded the results. Results includes resolution factor, middle peaks, taling factor, number of sub –pages of column for measuring test material (theoretical plates), we calculated all of these and also copacity factor, the standard solution was injected 5 times and we obtained its scale value that this value was bigger than 98%, then we injected each test standards 3 times and last we injected reference standards, standard solution. Finally device sample the mean result average curved surface area compared to the standard curves and showed the effective material value on the base of ppd.

ELISA

The all samples prepared as mentioned above for an indirect Competition ELISA assay for fine quantitation of ochratoxin based on manufacturer instructions .for all samples and standards . of estimated and then corrected data's reflecting to standard curve obtained as ELISA reader

calibrated by 450 nm UV the light for comparing the density of sample sand standard OP and preparing final results.

RESULTS AND DISCUSSION

Study on the ochratoxin and aflatoxin G produced by *Aspergillus* species in north of Iran provinces and the culture medium grown in the laboratory conditions:

The greatest ochratoxin mean was realted to the sepcies A. *carbonarius* and also the greatest Aflatoxin G1 and total Aflatoxin mean was realted to the sepcies *A. carbonarius*. Aflatoxin G2 Mean is zero.

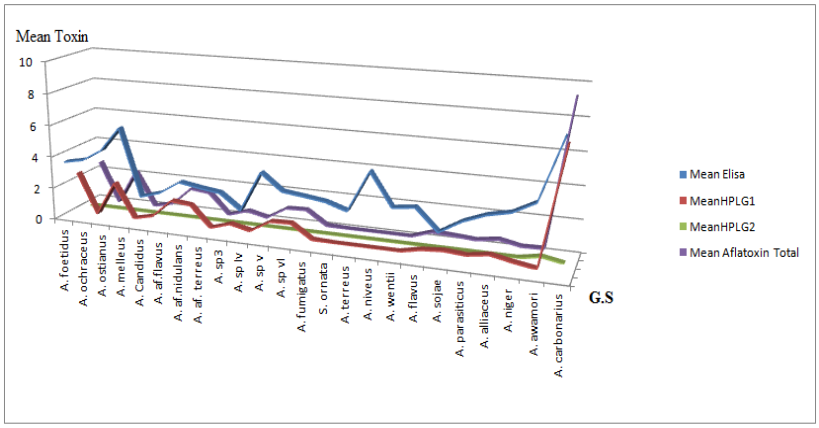

Figure. 1. Study on the ochratoxin and aflatoxin G produced by *Aspergillus* species.Comparing aflatoxin G_1, G_2 and ochratoxin using Wilcoxon Signed Rank test in the culture medium.

Comparing the mean ochratoxin and aflatoxin G indicated that there is significant relationship between them (z: -4.286 and sig: 0.00) beacase is sig<0.05.

Table 1. Significance or insignificance of numerical difference between aflatoxin G_1, G_2 and total aflatoxin to ochratoxin in the culture medium.

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Test Statistics[b]

	HPLCTOT ALAFLA - Elisaochra mean	HPLCG1 - Elisaochra mean	HPLCG2 - Elisaochra mean
Z	-4.200[a]	-4.286[a]	-4.286[a]
Asy mp. Sig. (2-tailed)	.000	.000	.000

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

Comparison of total aflatoxins and ochratoxin using Wilcoxon Signed Rank test in the culture medium

Comparing the mean ochratoxin and mean total aflatoxin indicates sig< 0.05 and there is significant relationship between them (z: -4.200 and sig: 0.00). They have nonlinear correlation so that by increase in ochratoxin, total aflatoxin rate will decrease. Given the total aflatoxin z (z: -4.200) against ochratoxin increase. Total aflartoxin decreases greatly compared to aflatoxin G (Z: -4.286).

Table 2. Significance or insignificance of numerical difference between aflatoxin G_1, G_2 and total aflatoxin to ochratoxin in the culture medium.

Test Statistics[c]

	HPLCG2 - HPLCG1	HPLCTOT ALAFLA - HPLCG1	HPLCTOT ALAFLA - HPLCG2
Z	-3.432[a]	-1.826[b]	-3.516[b]
Asy mp. Sig. (2-tailed)	.001	.068	.000

a. Based on positive ranks.

b. Based on negative ranks.

c. Wilcoxon Signed Ranks Test

In this comparsion it is obsrved that mean ochratoxin is in higher range comnapred to Aflatoxin G and total Aflatoxin. Mean Aflatoxin G_2 tends to zero. Also, the greatest toxin has been firslty produced ochratoxin and then by a short interal by Aflatoxin. *A. carbonarius* species had the largest mean toxin in ochratoxin, Aflatoxin G_1 and total Aflatoxin but in Aflatoxin G_2 its amount was zero .Amount of aflatoxin G and Total are lower than mean ochratoxin. Also, they have nonlinear correlation so that by increase in the ochratoxin, aflatoxin rate decrease.

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