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ORIGINAL ARTICLE

A comparative survey on Ochratoxin production spectra in culture medium of 24 different Asprergillus species from Iranian Northern states

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ABSTRACT
Mycotoxins are produced mainly by the mycelial structure of the filamentous fungi, commonly referred to as moulds. Fusarium species are plant pathogens commonly associated with cereals that, under favourable environmental conditions, can produce several secondary toxic metabolites.From the first May to the last October 2011, sampling was done according to"CBS" instructions for indoor and outdoor stations .In the study, minimum of Ochratoxin of 13 species (the greatest frequency) ranges 0-0.5 ppb(95.83%). 5 species toxin production ranges 2-2.5 ppb(4.17%) and 3 species toxin production ranges 1.5 -2 ppb (12.5 %). 2 species produce the toxin in the 1-1.5 ppb range of (8.33%) . 1 species produces the toxin in the range of 3-3.5 ppb. This comparison indicated that A.melleus produce Ochratoxin higher than standard limit both in the medium and in biomass (with more intensity in the biomass). In the biomass only one sample was greater than standard limit while in the medium, 3 species produced Ochratoxin higher than standard limit.
Keywords: Mycotoxin, Ochratoxin, Culture medium, Asprergillus, Northern Iran.

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INTRODUCTION

Mycotoxins produced by *Aspergillus, Fusarium* and *Penicillium spp*. are natural contaminants in foods [1]. Mycotoxins are well known to cause toxicities to humans and animals [2]. Occurrence of mycotoxin contamination in foods is moreprevalent in the tropical and subtropical countries resulting in acute and chronic mycotoxicoses in humans and animals [2]. Ochratoxin A (OTA), which is a potent nephrotoxin and nephrocarcinogenic mycotoxin, can occur in a wide range of unprocessed and processed food has been described widely in the literature and is receiving increasing attention.OchratoxinA is produced by Aspergillus species *(A.cretensis, A. flocculosus, A.melleus, A. ochraceus, A. ostianus, A.persii, A.petrakii, A.pesudoelegans ,A.roseoglobulosus. A. sclerotiorum, A. steynii, A.westerdijklae, A.sulphureusA.alliaceus, A.carbonarius, A.lacticoffeatus, A.niger ,A.sclerotioniger)* and *P.verrucosum and P. nordicum.* Several detailed risk assessments have been conducted for Ochratoxin A [3]). Given the known human exposure and the abundance of toxicological data from animal studies, the European Union Scientific Committee has recommended that Ochratoxin A levels be reduced to below 5 ng/kg of body weight per day [4,5]. In addition, several European countries have proposed individual regulations, with maximum tolerated concentrations varying greatly from country to country [6,*1*,7].

MATERIALS AND MTHODS

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. Six plates including Malt extract agar(MEA), Yeast extract agar(YEA), Czapek s agar(CZA) , Chapek s Yeast extract agar (CZYA), Saborud s dextrose 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 25±2 c 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 ,founding's and grown masses and also examining and micrometery was done by microphotometicestroscope and microscope, the samples with the help of slide culture prepared then were done by leicamicro analysis microscope hard and soft wares. The all samples prepared as mentioned above for an indirect Competition ELISA assay for fine quantization of total Aflatoxin 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 samples and standard OP and preparing final results .

RESULTS

Ochratoxin in the cutlre medium,mean and maximum using ELIZA:

In the study, minimum of Ochratoxin of 13 species (the greatest frequency) ranges 0-0.5 ppb (95.83%). 5 species toxin production ranges 2-2.5 ppb(4.17%) and 3 species toxin production ranges 1.5 -2 ppb (12.5 %) . 2 species produce the toxin in the 1-1.5 ppb range of (8.33%) . 1 species produces the toxin in the range of 3-3.5 ppb.In the study on the Ochratoxin means , the gretest number(9 species) produced the toxin in the range species(6 species) produced the toxin in the range of 2-3 ppb (25%). They produced the toxin with greater diffrence i.e., each with 3 samples in the range of 4-5 ppb and 1-2 ppb (each with 12.5 %) . In 6-7 ppb and 7-8 ppb ranges each one produced the toxin with 4.17 %. In the Ochratoxin maximums , the gratest toxin production ranges 2.5-5 ppb .

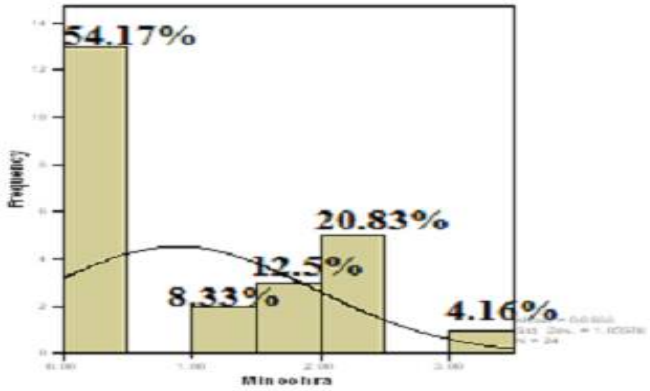

Figure 1- Distrubition of maximum Ochratoxin production by toxicogenes isolates in the culture medium .

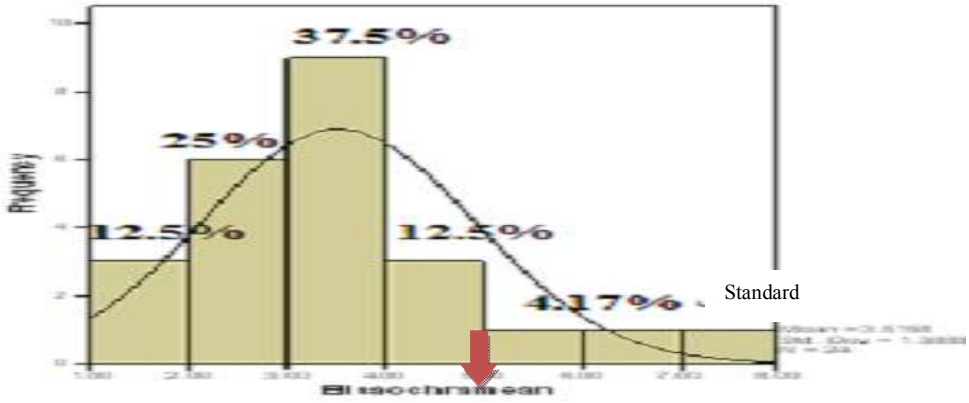

Figure2- Distrubition of mean Ochratoxin production by toxicogenes isolates in the culture medium .

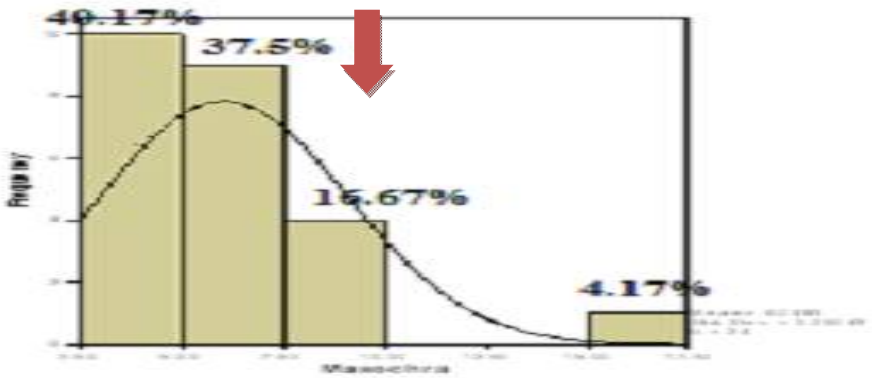

Figure 3- Distrubition of maximum Ochratoxin production by toxicogenes isolates in the culture medium

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Mean Ochratoxin in the studied species In present study , it is indicated that the greatest amount of Ochratoxin was related to *A. carbonarius* and then to *A. melleus* . The lowest amount of it was realted to *A. spIv*.

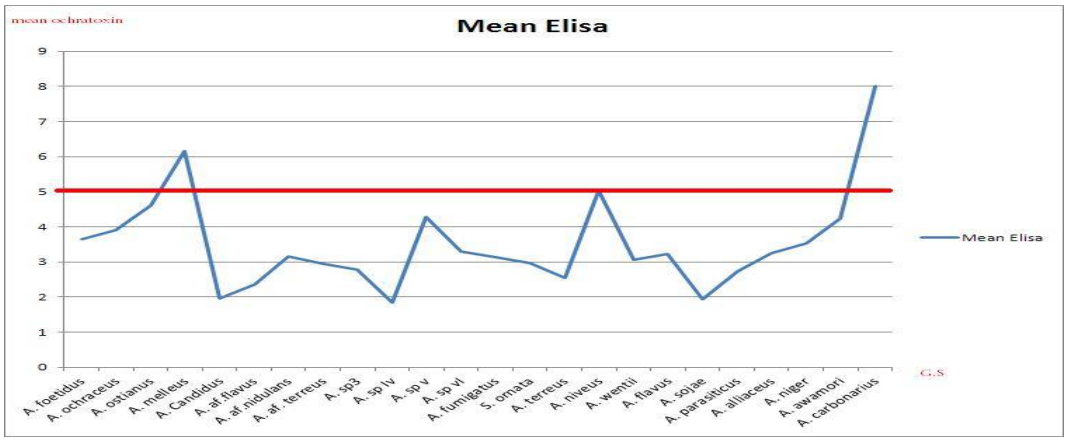

Figure 4- mean Ochratoxin in the studied species.

DISCUSSION

According to the results obtained, much samples isolated in Gilan province were species *A.af.flavus , A. flavus, A. nivenus.* Each with 12.5 %. In Mazandaran province, species *A.af.flavus, , A. flavus* had greatest frequency each with 16 % .In Golestan province, most species are *A.fumigates,A. af.flavus , A.flavus* each with 18.18 %.According to ELISA , *A. carbonarius* had the greatest amount of Ochratoxin (8.007pp). Standard rate of Ochratoxin is 5 ppb but this is higher than standard rate. Also the productivity of *A. niveus* (5.033 ppb), *A. melleus* (6.145 ppb) is higher than standard level. The lowest amount of Ochratoxin is related to *A. sp IV*. In the study, 87.5 % of Aspergillus samples have a toxin contamination to ochratoxin much lower than allowable limit defined in Iranian national standard (5 < ppb). Comparing the findings with allowable limit in Iranian standard indicated the contamination rate in 11.5 % of samples was seen relatively. This comparison indicated that *A. melleus* produce Ochratoxin higher than standard limit both in the medium and in biomass (with more intensity in the biomass). In the biomass only one sample was greater than standard limit while in the medium, 3 species produced Ochratoxin higher than standard limit.

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