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Comparative Study of Aflatoxin Production Pattern in Tea Samples from Northern Iran

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Abstract

Toxic metabolites of fungi or mycotoxins have been responsible for many epidemics in human and animal communities, especially in recent times. Samples were collected from cultivation and procreation centers in northern Iran and then isolated and recognized on five substrates CYA, YES, MEA, SYES and OA. The subspecies of Wentii, Flavi, Nigri, Circumdati,Candidi, and a number of unknown species were cultured on CGM / CM / DRYE triplicate media for motivation of toxicity and measurer. Therefore provisioned of theextract growth media and measured a amount of aflatoxin arrived to culture medium with CZ MEZ Eliza method. Most of the samples were from plantations and factories in eastern Guilan. In species segmentation, the highest number of species after unknown species was related to flavus species. There was a significant difference between the level of toxin was proportional to sector and species and also between aflatoxin levels measured at biomass and the culture medium ($P < 0.05$).Due to the fact that most of the samples were obtained from fields and factories in East Gilan, these areas are high risk in terms of aflatoxin isolate contamination.

Keywords: Aspergillus ,Aflatoxin,tea , northern

1. Introduction

Aflatoxins are mycotoxins produced by two types of molds called, Aspergillusflavus and AspergillusParasiticus. According to these studies, there are four main types of aflatoxins in nature, including B1, B2, G1, G2, and two types of metabolic products called M1 and M2. According to some official estimates, more than 25% of the world's grain production is exposed

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to fungal infections annually (WHO, FAO) [1]. It is believed that some species or groups of Aspergillus species Aflatoxins can be produced only under suitable and known conditions [2, 3 and 4]. Among these, fungal species, Aspergillusflavus and Parasiticus are the most important producers of these toxins. It has now been well established that toxic metabolites of fungi or mycotoxins have been responsible for many epidemics in human and animal communities, especially in recent times [5]. The discovery of these toxins caused the authorities to pay more attention to the harms caused by fungal toxins as a cause of food contamination and disease and even death in humans and animals. According to the Food and Agriculture Organization of the United Nations, millions of tons of food are lost every year due to mycotoxin contamination. In the 1990s, according to the US Centers for Disease Control, many food poisonings were caused by pathogenic fungi[6 ,7,8].

2. Material and Methods

2-1. Materials

From the first days of May to the end of October in Guilan and Mazandaran provinces sampling of closed and open positions (CBS firm) was done [9,10,1112, 13 , 14]. Since that time, no data and models of replication studies in other countries have been widely thought to have been accepted and data from previous studies have been available to identify the species and size of aerobic fungal species and groups at sampling sites. A sample group was sampled from each 50 hectares of farmland (110 crops) and from each tea processing plant (60 plants). 3 to 5 days after each rainfall at 9-15pm in sunny weather at 25± 3 ° C and when no air circulation (wind size less than 30 m / s) is provided by placing the plates in the open at 110 ° C. 90 cm from the bottom of each sampling station was performed [14 , 15]. Six plates containing malt extract agar,Yeast extract agar,Czapeckextract agar, Sabourauddextroseagarand Potato dextrose agar were all mixed with 100 ppm chloramphenicol and 50 ppm tetracycline .Plates containing 15-25 cm3 of agar (10-12 cm in diameter) were removed after 60,30 and 90 min (451 plates in the fields) and 15, 30 and 60 min (441 plates in the plants), then After insertion and labeling, they were placed in perforated polyethylene bags and sent to the laboratory [12,13, 14]. All plates were incubated at 25±2 ° C and aerobic as one plate from each culture medium was kept in the dark, another in the light and one pair in the light-dark period. For up to 15 days at intervals of 3,7,15 days, all plates were always examined to identify any markings that were visible or visible with the aid of a stereoscope, identified and marked with sterile glass needles and removed on plates. Pre-cultured. In slab agar plates and tubes from growth media of Malt Extract Agar, Fast Extract Agar, Potato dextrose Agar, Corn Mill Agar, Sabour Dextrose Agar, Prosthetic Agar, and Dox Agar Protein, all of the new mold samples were re-cultivated and cultured with the previous application. Incubation and any macroscopic and microscopic features were monitored and recorded in 5,10,15 days intervals. For up to 25 days, all viable and normal plates were continued to be examined, isolated and re-cultured as they were, and new findings were recorded. At the end of 300 Aspergillus coli (out of 600 mold isolates), 150 were selected. Plates were cultured on Macro and Microscopic Morphological Investigations at CzapeckDox agar, CzapeckYeastextract

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agar(with and without 20% sucrose), Malt Extract Agar, and CzapeckDox Agar (with and without 20% sucrose) at 25 ± 2 ° C. grown and examined after 3, 7 or 14 and 25 and sometimes 30 days and simultaneously slide calibration of each sample onto CzapeckDox Agar and CzapeckYeast extract The 20% sucrose rocket was provided with the growth pattern of the previous model and incubated at 37 Ċ to identify and classify according to ICPA guidelines [15,16].

2-2. Morphological studies

Macro and microscopic studies of morphology and photography were performed on one and two-week-old colonies and on the back of the colonies, respectively (in black aspergillus colonies of two to four weeks). Colony Width Measurement of coloration of front and back of colony, pigments, extroliths and imaging of parasites, cells and grown masses, spines, stipe, conidial crest and micrometrium of conidiophores, conidia vesicles, and hardness and micrometriumThe ascites or ascites were examined with a stereoscope. In all specimens, with the aid of slides, anterior and posterior slides of the conidiophore (steppe vesicles, coronal conidia, phyllides, metaulas, conidia or ascites and their inflorescences, micrometers or imaging with a Leica Microscope.

2-3. Provide extract of growth medium

A 10-lobed full lobe of PBS mixture and the conidia of each strain were grown on an extract agar plate and reconstituted into a 50 ml falcon tube containing a 2% Duplex Broth liquid substrate containing 2% malt extract agar. They were incubated at 200 round per minute at25±3°C in the dark-light period and inspected daily .After 7 days of floating mass or sedimentation in the liquid, the same as infant and small filaments. Germ germs were mildew fungi were harvested by centrifugation at 3000 rpm for 15 min and the inhibitor fluid culture medium . To match the protein size of each blend obtained from each Aspergillus isolate, measurements were performed using Bradford method and the diluted samples were diluted to 0.5 mg / ml. The diluted samples were centrifuged at 5 ° C for 1 to 3 days with cold 5-fold acetate and one times for centrifuge and then centrifuged at -20 ° C for 20 min at -20 ° C. Concentrated dilution and diluted samples were diluted in the same manner to ensure that all samples contained 0.5 mg / ml of protein.

4. Results and Discussion

In this study, samples obtained from fields and processing workshops in northern Iran (Gilan and Mazandaran) were cultured to standard substrates for toxin motivation and measurement. Then, growth medium extract was prepared and the amount of aflatoxin introduced into the medium was prepared. Culture was measured by MEZ CZ ELISA. Then the amount of toxin introduced into the culture medium was analyzed and the results of the toxin in biomass were measured using the R AgraQuant kit. Then extracts of growth medium were prepared and the amount of aflatoxin introduced into the medium Culture was measured by MEZ CZ ELISA. Then the amount of toxin entered into the culture medium was analyzed and the results of the toxin in the

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biomass were measured using the AgraQuant kit. From the genus Circumdati with seven species no species was found from Cremei and Sparsi and one species from Wentii, five species from Flavi, four species from Nigri, three species from Circumdati and one species from Candidi. Only one species of Aspergillusvenetti (A. wentii) was found from Wentii. Four species were found from the Flavi section and A. oryzae was also found. Aspergillusflavus was isolated from 19 sites, Aspergillus subject (A. sojae) from 8 locations, Aspergillusparasiticus from 7 locations, Aspergillusaliasceus from 4 locations. Four species were found from the niger section. Aspergillusniger from 7 locations, Aspergillusavamori from 5 locations, Aspergilluscarbonarius from 6 locations, Aspergillusfetidus from 4 locations Three species were found from Circumdati. Aspergillusochraceus from 5 locations, Aspergillusostianus from 5 locations, Aspergillusmelleus from 5 locations.Candidi only One species of Aspergilluscandidus (A. candidus) was found from 5 locations. Aflatoxinjaponicus swimming Were for. In this study, a sample of 53 cases was selected, one of which was a toxin in isolates obtained from two locations (farm air and processing plants) and in three northern regions of Iran (west of Mazandaran, east of Gilan and west of Gilan). From this sample, 41.5% (22 cases) were isolated from field air and 58.5% (31 cases). AspergillusspV / AspergillusspIV/AspergillusspIareAspergillusafnidulans.

Table 1. Cross table between the number of samples and the toxin content range.

Total	Between 40 and 50 ppb	Between 30 and 40 ppb	Between 20 and 30 ppb	Between 10 and 20ppb	Between 0 and 10 ppb	Less than 0 ppb	Range of toxin production / kit	
53	0	1	11	16	25	0	number of samples	KITAgraQuent
100 %	0.00%	1.9%	20.8%	30.2%	47.2%	0.00 %	Total percentage	

Most of the plantations belong to the East of Gilan and to the factories of the East of Gilan. But most of the samples are from the East of Gilan (Table 1). At two locations, the plant and the plant are approximately equal. Among the fields, East Guilan and East Guilan plants were separated by more species. However, most of the samples were obtained from the eastern Guilan factories, indicating that the risk of toxin production by Aspergillus diffuses to the areas under study. (Table 1) while ,obtained the same result. Most isolates produced toxin in the range of 0–10 ppb and the number of isolates isolated from East Guilan and East Guilan plants respectively. In this area they have been toxin-producing more than other areas and Aspergillusflavus has been toxin-producing after unknown isolates in this area. This indicates that the amount of toxin produced by flavus after unknown species is the highest and Not only have most of the flavus species produced toxin, but most of the toxin has been in the range of 0-10 ppb (more The most toxicogenic range is also related to the flavus species (after unknown isolates) ,whereas in the results of Mehdikar et al (2010),In this study, there was a significant difference between the amount of toxin and sector and the amount of toxin and species and the average amount of toxin produced in the culture and biomass, but there was no significant difference between the amount

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of toxin and the location. There was no significant difference between species and amount of toxin and location and only significant difference between toxin level and fraction. There was a significant difference between the mean of toxin produced in culture and biomass because some fungal species. They pump more of the toxin into the environment, and the rest of the toxin into the environment, and more into the bio. Q remains. In this study, the required surveys were carried out in different locations (plant and factory) and in different geographical areas (Gilan and Mazandaran), which showed that the contamination rate was highest in the factories and in the eastern part of Guilan. Also, inaccuracies in the process of harvesting and storing products have occurred.Because heat processes have no effect on reducing aflatoxin levels, products produced in crops and factories will be reduced if contaminated areas are produced. Aflatoxin accumulation in the human body will reduce adverse effects such as cancer. This is why the European Union and the developed countries are preventing the import of low-dose food products into their country, but in our country, the use of aflatoxins can easily enter the diet of consumers. Determining the comparative pattern of toxinogenicity in endemic Aspergillus in northern Iran can be used to rearrange and revise known species and to suggest new species, in anthropological classification, and to be used in establishing an ongoing health inspection order. The most valuable achievements of this study are the first reports of chemical isolation and identification of Aspergillus toxins in northern Iran. In this study, Iranian biomass and especially Aspergillus growth bed, which changes their morphological characteristics and physiological processes in particular , is believed to be a global research on Aspergillus and other fungi. North of Iran, which contains one of the world's oldest plant habitats (Hyrcanian forests), is carried out to recognize the characteristics of native and guest species. The results of this study indicate that Aspergillus fungi of northern Iran is very valuable and diverse. You can also find new species in that eye.

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Refrence

1. MyahyA.IsolatedAspergillus and aflatoxin determination in fish meal,corn.soybeanvkngalh.journal of nectar Chamran University ,No.17(1386);pp.95-105.
2. Inetrnational crops research Institute for the semi ariedtropics,aflatoxins in food.www.aflatoxin.info.asp.(2004).
3. LanyasunyaTP,WamaeLW,MusaHH,OlowofesoO,LokwaleputIK.The risk of mycotoxins contamination of dairy feed and milk on smallholder ,Journal of nutrition ,4,3 (2005);162-169
4. Schweitzer SH,QuistCR,GrimesGL,FosterDL,Aflatoxin Levels in corn available as wild turkey feed in Georgia.Journal of Wildlife Disease,37,3.(2001);657-659.
5. PlasenciaJAflatoxin in maize :A Mexican perspective . Journal of Toxicology,23.(2004);155-177.
6. Alborzi,s.,Pourabbas,Rashidi,M.,&Astaneh,B.Aflatoxin M1contamination in pasteurized milk in shiraz(south of Iran). Food control,17(7) .(2006),582-584
7. Johri,T,S.Exogenoustoxicants.Central Avian Research Institute,2005.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 7, 2021, Pages. 1189 - 1194
Received 05 May 2021; Accepted 01 June 2021.

8. SmelaME,CurierS.S.The chemistry and biology of aflatoxin B1,carcinogenesis .Journal of Wildlife Diseases,22,(2001);535-545.
9. Bhat,RV.,VasanthiS.Mycotoxins food safty risk in developing countries,Food.Agriculture and Environment, (2003).1-2.
10. Thorpe W,MuriukiHG,OmoreA,steal S. Dairy development in Kenya ;The past ,The present and the future .Paper presented at the annual symposium of The animal Production Society of Kenya ,Nairobi, (2000);22-23.
11. Kenya Ministry of Health (KMOH) ,Outbreak of aflatoxin poisoning in Eastern and central provinces,Kenia,Nairobi,(2005) ;1-5.
12. Klich,M.AIdentification Of Common AspergillusSpecies.C.B.S,Utrecht.Netherlands. .(2002a) .1-116pp.
13. Kozakiewicz,Z.Aspergillus species on stored products.Mycological Papers.161.(1989):1-188pp
14. Samson,R.A.,Houbraken,j.,Summerbell,R.C.,Flannigen,B,and Miller, J.D.Common and important species of fungi and actinomycetes in indoor environments.In: Microorganismam in Home and Indoor work Environments (eds. B. Flannigen,R.A.Samson and J.D.Miller). Taylor and Francis,New York. (2001).p:287-292.
15. Pitt, J.I.and Hocking, A.DFungi and food spoilage. Blackie Academic&Professional,London.(2nd ed). (1997).1-593 pp.
16. Klich,M.A. Biogeography of Aspergillus species in soil and litter. Mycologia.94.(2002b):18-3.