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Mycotoxin Production by Fungi Isolated from Rice and Stored Grains in Riyadh, KSA

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SUMMARY

We collected 30 samples of cereal crops (Wheat, millet, barley, corn, and rice) stored and offered for sale in Riyadh area markets. It was found that most of these samples were contaminated with fungus *Aspergillus sp*.(73.2%), and that the corn crop was highest to be hit by fungus *Aspergillus sp*. (23.3%) compared to the rest of the crops (wheat 16.6 %), (barley 13.3%), (millet 10%), and (rice 10%). It appeared in this study that most isolated species have the ability to produce aflatoxin. We studied the effect of temperature on the secretion of aflatoxin in isolated fungi; and the results are illustrated in the temperature table.

INTRODUCTION

One of the most important effects of post-harvest decays in fruits and vegetables, especially of seed and feed deterioration by fungi, is the induction of mycotoxicoses. This is a disease of animals and humans following consumption of seeds and foods invaded by fungi that produce toxic substances called mycotoxins (Amadi and Adeniyi, 2009). The food pollution-producing fungal toxins is one important problem of the present day. Food and Agriculture Organization reports indicated that at least 25% of the world's food contaminated with mycotoxins (Saleh, 2009). Occupies fungi *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* lead to the pollution of rice and wheat, corn, bread, seed cotton, peanuts, nuts, citrus fruits, olives, and other dairy products (Alhaji Sanusi, 2015). Mycotoxins also are transferred directly or indirectly to humans through animal and plant, causing them many diseases such as cirrhosis of the liver and fertility and lack of inhibition of protein synthesis and liver cancer, where man is unable to demolished them (Shaaban, 2004).

Maize is the third most important cereal used as human food and animal feed worldwide. However, maize is also a favored host for the aflatoxin producing fungi *Aspergillus flavus* (Chauhan, 2015). Maize, one of the main cereals, is as source of food, forage, and processed products for industry. It is widely cultivated throughout the world, and a greater amount of maize is produced each year than any other grain (Malíř, 2014). The colonization of maize and peanut by the fungal pathogen Aspergillus flavus results in the contamination of kernels with carcinogenic mycotoxins known as aflatoxins leading to economic losses and potential health threats to humans. The regulation of aflatoxin biosynthesis in various Aspergillus spp (Khera, 2015).

Wheat and Barley are the most production and consumption grains in the world. The necrotrophic Fusarium spp is pathogen caused many diseases on plants, the major two disease caused by Fusarium on wheat is Fusarium Crown rot (FCR) and head blight (FHB), also known as scab (Matny, 2015).

Rice is the second level of cereal staples consumed food worldwide after wheat, and it consists the major part of the diets for the half of the world. It is also reported that the rice is composed of 27% of the global diet and 20% of dietary protein intake in the developing countries (Alamer, 2014). Rice is one of the most cultivated food crops globally. About 593 million tons (Mt) of rice are produced annually (FAO, 2002). It is used in variety of food products. Cooked rice, breakfast cereals, desserts, and rice flour are some important food products of rice. It is also used to prepare local beer, while rice hull is used as fuel, fertilizer, packing, and insulation (Gupta, 2012). Rice bran is also used as a suitable substrate in mushroom cultivation. Straw from the leaves and stems is used as bedding for animals. Rice floor is used to prepare many traditional foods on some special occasions (Giddel and Jivani, 2007). Toxigenic fungi can attack rice in the field and during storage resulting in increased mycotoxin levels in this commodity. *Aspergillus*, *Fusarium*, and *Penicillium* are the predominant fungal genera associated with food grains during storage (Makun, 2011).

The main fungi, *Aspergillus flavus,* produce these mycotoxins thrive under favorable conditions on a wide range of foods and feed such as maize and peanuts, and are a world-wide problem (Sanusi, 2015). Toxigenic fungi in crops have been historically divided into two more or less distinct groups, the first includes those which invade and produce their toxins before harvest which are often rather loosely called "field fungi", the second group which becomes a problem after harvest is known as storage fungi. Invasion by fungi before harvest is governed primarily by plant host-fungus and other biological interactions while growth by fungi postharvest is governed by crop physical and biotic factors (Miller, 2000).

Mycotoxins can produce at field pre-harvest, harvest, transportation, and storage stages while the conditions are favorable for growing the fungus resulting in the mycotoxin production. The risk of contamination by mycotoxins is an important food safety concern for grains and other field crops. Mycotoxins affecting cereals is considered to be of greater significance world over for human beings (Parvathi, 2014).

MATERIALS AND METHODS

Isolation: 30 samples of maize seed, rice, wheat, barley, and millet were collected from locations stored in the agricultural fields, and the seeds were put in plastic bags and brought to the laboratory. These samples submerged in Clorox solution concentration of 10% for 1-2 minutes to purify the outer surface of the seeds of pollution, and then the seeds were taken out and put the nomination to get rid of the excess solution papers. The seeds were washed with sterile distilled water and put on the surface of the medium. We used Sabourauds Dextrose Agar (SDA) medium by the way (Emmon *et al*., 1974). Samples were incubated at a temperature of 25 and the dishes were checked after 7 days of incubation in order to isolate and diagnose fungus and for pure isolated farm species.

The percentage was determined to appear or occur following law:

The proportion

$$= \frac{\text{The emergence of a number of one type of appearing}}{\text{The total number of samples}} \times 100$$

Study The Feasibility of Species on The Secretion of Aflatoxin:

We studied the viability of the species isolated in this study on the production of aflatoxin using the two mediums Potato Dextrose Agar (PDA) by the way (Emmon *et al*., 1974) and Yeast Extract Sucrose (YES)

by the way (Davis *et al*.,1966) in order to determine the impact of the type of medium to produce aflatoxin. As it has been part of the colony fungal pure transfer to the center of the dishes containing the two mediums (PDA, YES) and dishes incubated at a temperature of 25 for a period of 7 days for the purpose of obtaining full colony. For the detection of aflatoxin, ammonia solution with concentration of 25% was used by adding 0.2 ml of this solution in the middle of the glass dish and cover overturned dishes (Saito and Machida, 1999) and incubated at a temperature of 25. Check dishes control after the second day of cuddling to note the color change of the rules of the colonies. If colonial rule changed to red color or pink or yellow orange, it indicates that the fungus is capable of producing aflatoxin and otherwise the fungus is unable to produce aflatoxin (Saito and Machida 1999, Lin and Dianese 1976).

Study The Effect of Temperature on The Secretion of Aflatoxin:

To study the effect of different temperatures on the viability of the species isolated during the study on the secretion of Aflatoxin, the following temperatures were selected: (20, 25, 30, 35, and 40). Fungus grown on the two mediums (PDA, YES) was due to varying temperatures mentioned above. After a week of portability, it showed cuddling fungi on the secretion of aflatoxin in each temperature test using ammonia as mentioned above.

Mycotoxin production

The medium used for this study was potato dextrose broth (PDB.). The medium was prepared routinely and sterilized. Twenty-five millilitres (25 ml) of the broth were dispensed into sterile conical flasks. The flasks were inoculated separately with 5 ml of any of the test fungi. The inoculated flasks were incubated on a shaker at a room temperature of 25 ± 1oC for 12 days. The filtrates of the test isolates were obtained and assayed for the presence of mycotoxins.

RESULTS

The results of this study indicated that the injury or contamination of crop seeds in the Riyadh region was at high rates, it He found that there are 22 sample was infected with one type or more types of *Aspergillus* of 30 samples collected from corn, rice, wheat, barley, millet an increase of 73.2% fungal infection. It appeared during the study that the corn crop is more fungal infection of crops compared to other crops where infection in 7 samples showed an increase of 70%, followed by wheat crop because of injury in any 6 samples appeared by 60% while the rice crop injury is the least compared to other crops, It was noted that the two samples were infected and by (20%). Was isolated and diagnosis of 5 types of *Aspergillus* during this study, was the type (1 A.) visible most of the other species where he appeared in (7) samples by (23.3%), followed by type (2 A.) where he appeared in (5) samples by (16.6%), two of which showed the emergence of similar proportions 10%), (Table 1) (Fig. 1).

Table 1: Organisms isolated from stored grains.

The proportion of appearing	The type of crop					Fungal species isolated
	rice	**Millet**	**Barley**	**wheat**	**corn**	
23.3	-	1	1	2	3	***Aspergillus sp.*** (1)
16.6	1	1	1	-	2	***Aspergillus sp.*** (2)
13.3	-	1	1	2	-	***Aspergillus sp.*** (3)
10	1	-	-	1	1	***Aspergillus sp.***(4)
10	-	-	1	1	1	***Aspergillus sp.***(5)
73.2	2	3	4	6	7	The total number of isolates

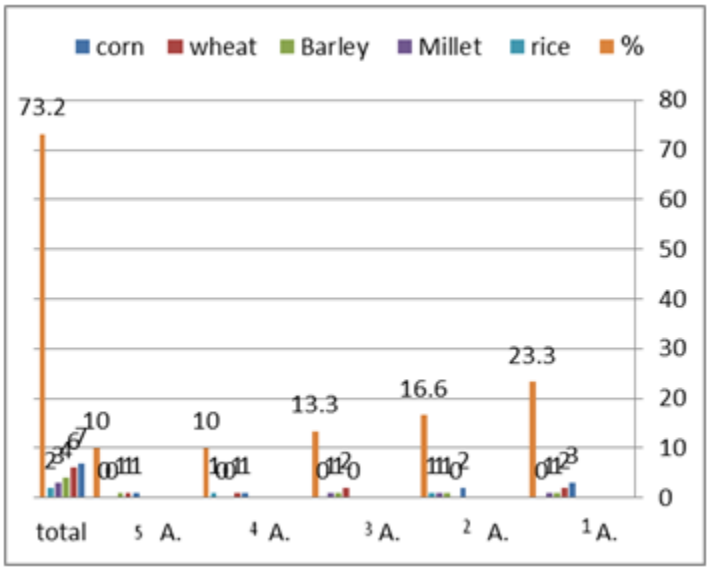

Fig. 1: Organisms isolated from stored grains

Upon detection of the ability of this species on the secretion of aflatoxin, it appeared during the study that all types of fungi isolated its ability to produce aflatoxin growth at the medium (PDA). Through color change, the rules of fungal colonies were in red-pink or yellow-orange using the ammonia solution concentration 25%. But when using the medium (YES), it was observed that all species showed the ability to secrete aflatoxin except three types of *Aspergillus* did not demonstrate any ability to secrete aflatoxin at this medium (Tables 2&3). (Figs. 2&3).

Table 2: Detection of toxic activity (aflatoxin) of isolated fungi using the two mediums PDA, YES 25C°

medium		Fungal species
YES	**PDA**	
+	+	***Aspergillus sp.*** (1)
-	+	***Aspergillus sp.*** (2)
-	+	***Aspergillus sp.*** (3)
-	+	***Aspergillus sp.*** (4)
+	+	***Aspergillus sp.*** (5)

Table 3: The effect of ammonia solution on isolated fungi.

medium		Fungal species
YES	**PDA**	
+++	+	***A.*** (1)
-	+	***A.*** (2)
-	+	***A.*** (3)
-	+	***A.*** (4)
++	+	***A.*** (5)

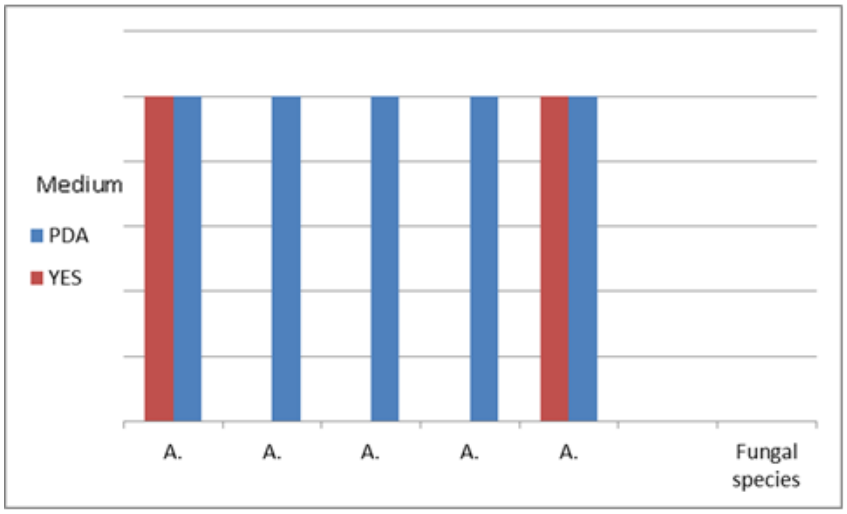

Fig. 2: Detection of toxic activity (aflatoxin) of isolated fungi using the two mediums PDA, YES 25C°

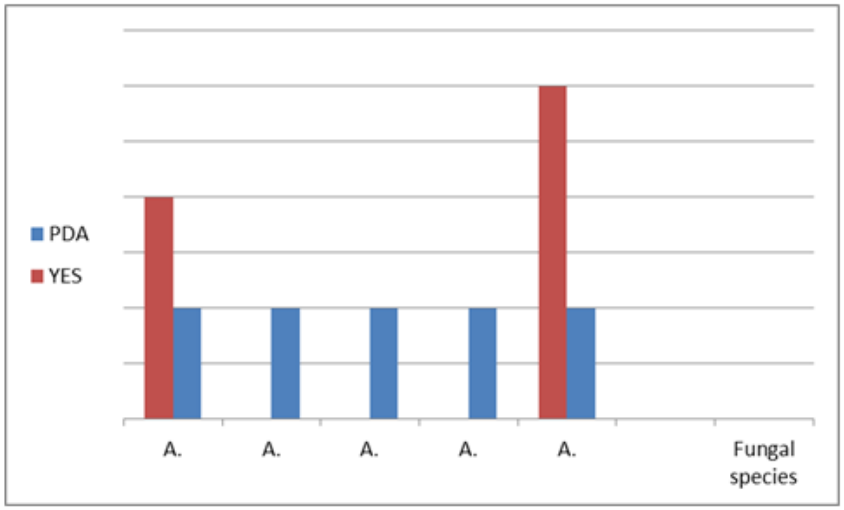

Fig. 3 A: The effect of ammonia solution on isolated fungi

--- + +++ ++

+: Means change color to red-pink or yellow-orange

++: Means a slight change in color towards the gloom

+++: Means that the color became bleak

---: Means that the rules did not change the color of the colonies

Fig. 3 B: The effect of ammonia solution on isolated fungi

Note that in (Table 4) (Fig. 4) the temperature has a clear impact on the production of aflatoxin in both the two mediums. It showed fungi isolated and developed on medium (PDA) and the ability to produce aflatoxin compared with the growth on medium (YES). This was noted during the study variation in susceptibility fungus aflatoxin production in different temperatures. So it is clear that the ability to produce aflatoxin fungus when temperatures (20, 25, and 30) were down, while this increased susceptibility at a temperature of 35 and the fungus showed the highest ability

to produce aflatoxin in temperature 40. It was observed that *Aspergillus sp*. gave a high affinity to produce aflatoxin in temperatures (35 and 40). The *Aspergiulls* sp.(2) shows no ability to produce aflatoxin in temperatures (30 and 25) when growth on the medium (YES). Also types (3 and 4) of *Aspergillus sp*. show no ability to produce aflatoxin in temperature 25 when growth on the medium (YES).

Table 4: Effect of temperature on the production of aflatoxin in isolated fungi

medium										Fungal species
YES					PDA					
Temperature					Temperature					
40	**35**	**30**	**25**	**20**	**40**	**35**	**30**	**25**	**20**	
+++	+	+	+	+	+++	++	+	+	+	***A.*** (1)
+++	++	-	-	+	+++	+	+	+	+	***A.*** (2)
+++	++	+	-	+	+++	++	+	+	+	***A.*** (3)
++++	+	+	-	+	++++	+	+	+	+	***A.*** (4)
+++	++	+	+	+	+++	++	+	+	+	***A.*** (5)

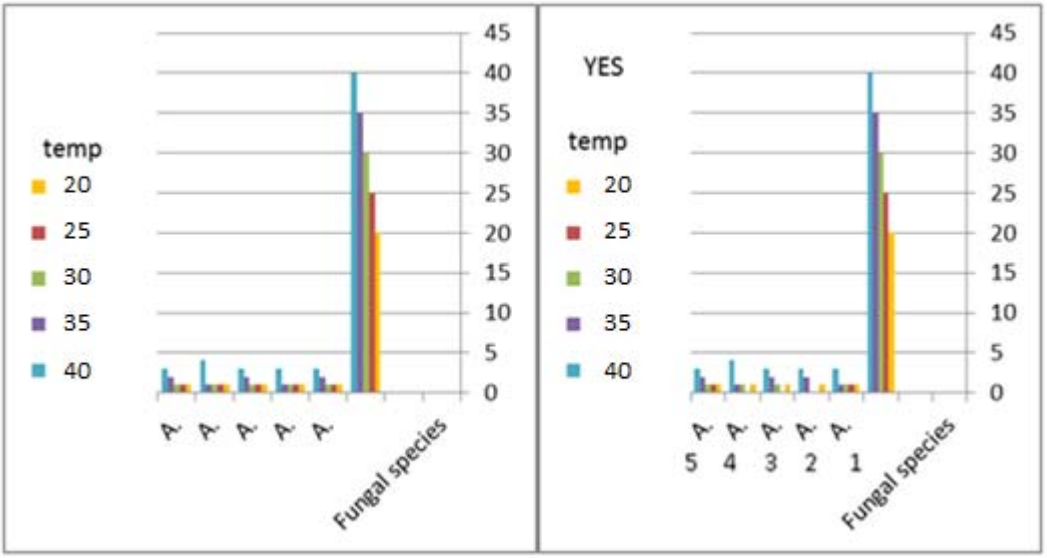

Fig. 4: Effect of temperature on the production of aflatoxin in isolated fungi

DISCUSSION

The study reviewed contamination of crop seeds that have been studied in this research, and that all fungi gave an account of the positive use of the medium (PDA) and this may be due to the nature of the central components of the fungus, which gave an account of the negative using the medium (YES) that does not mean they are unable to produce aflatoxin but perhaps due to the nature center components or cuddling degree heat. It is believed that the cause of seed infection with fungi is the presence of factors that help increase such incidence of insects and spiders and the quality of the crop and mixing with other materials contaminated during harvesting and storage. Toxic fungus *Aspergillus flavus* is responsible for the loss of 25% of the grain in the world through pollution (Alhaji Sanusi, 2015). Crops and nuts injury poisons aflatoxin is one of the problems that affect food and feed on a global level. Mold product of this poison can grow and produce the toxin when the conditions of its heat and moisture are available, and with ill-handling and storage operations for those products, it may be that those toxins formed in the products inside warehouses and stores (Ibrahim, 2014).

One of the most important ubiquitous fungal species in tropical environments is *Aspergillus flavus* which can be found in soil and other substrates. *A. flavus* is associated with many diseases of humans, most severe

of which is invasive aspergillosis. It can also cause diseases in insects as well as in crops (Chowdhury, 2015). Aflatoxins can contaminate corn, cereals, sorghum, peanuts, and other oil-seed crops. Thus, food contamination by this group of mycotoxins has been implicated in both animal and human aflatoxicosis. Aflatoxins often occur in crops in the field prior to harvest. Postharvest contamination can occur if crop drying is delayed and during storage of the crop if water is allowed to exceed critical values for the mold growth (Arapcheska, 2015). Aflatoxins are secondary metabolites produced by *Aspergillus* section flavi group and fungi, these Aflatoxins are potent carcinogenic, teratogenic, mutagenic, hepatotoxic, and immunosuppressive agents that cause significant damage to human and animal health (Srilakshmi, 2015). Aflatoxins are natural contaminants of cereals and other commodities throughout the world. Chronic dietary exposure even to low doses of aflatoxins is a known risk factor for liver cancer and effect protein metabolism and immunity (Hina, 2014). *Aspergillus flavus* produces the secondary metabolites aflatoxins B1 and B2 and other mycotoxins such as cyclopiazonic acid. A. flavus is the predominant species [1,2] responsible for aflatoxin contamination of crops prior to harvest or during storage (Whitelaw, 2004).The global impact of aflatoxins on trade and health was featured in a presentation which demonstrated a synergistic effect between aflatoxin and hepatitis B virus to cause liver cancer in humans (Bowman, 2015). Under unfavorable field conditions stressful to the crop, some fungi produce mycotoxins, secondary metabolites that may contaminate various agricultural commodities in the field and in storage (Hruska, 2015). Mycotoxins are low molecular weight secondary metabolites produced by filamentous fungi that are commonly resistant to a wide spectrum of environmental factors and, therefore, undergo slow degradation. They are stabile at high temperatures and at low pH values typical of the gastric juice of animals (Pfliegler and Pusztahelyi and Pócsi, 2015).

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REFERENCES

Alhaji Sanusi, farmer Zaria, Moffat Matolo, farmer (2015). Makueni county-Aflatoxin Control in Maize and Peanuts Project -

Ahmed Ahmed al sarawi – mycotoxins (2009). Arab scientific community organization

Ajay Kumar Gautam, Heena Gupta and Yamini Soni (2012). International Journal of Theoretical & Applied Sciences

Amadi, J. E. and Adeniyi, D. O. (2009). Department of Plant Biology, University of Ilorin.

Amin O. Elzupir[1,2], Abdulaziz S. Alamer[1], and Michael F. Dutton[3] (2014). Informa health care -

A. Nisa*, N. Zahra and S. Hina (2014). J. Sci. Ind. Res. 49(3):189-194.

Eileen Herrera and John E. Bowman - Aflatoxins in maize and other crops-World Mycotoxin Journal - 2015.

Giddel and Jivani, (2007). International Journal of Theoretical & Applied Science -

H. sudini, P. srilakshmi, k. vijay Krishna kumar, Samuel M.C. (2015). Njoroge , moses osiru , anitha seetha and f. waliyar

Hussaini Anthony Makun[1,2,4], Michael Francis Dutton[2], Patrick Berka Njobeh[2], Judith Zanele Phoku[2] and Clarence Suh Yah-2011.

H. Yao[1*], Z. Hruska[1] and J. Diana Di Mavungu[2] (2015). World Mycotoxin J., 8(2): 181-191 -

Jake C. Fountaina, Pawan Kheraa,b,c, Liming Yanga,c,d, Spurthi N. Nayakb, Brian T. Scullye,Robert D. Leef, Zhi-

Yuan Cheng, Robert C. Kemeraita, Rajeev K. Varshneyb, Baozhu Guoc, - a Department of Plant Pathology,- 2015.

J.D. Miller-Proceedings of the 6th international working conference on stored product protection - 2000.

Jiujiang Yu a,*, Catherine A. Whitelaw b, William C. Nierman b,c, Deepak Bhatnagar a, Thomas E. Cleveland a - FEMS Microbiology Letters 237, 2004.

K.Parvathi , V.Uma , M.S Begum and E.G. Wesely - Parvathi K et al. / Journal of Science / Vol 4 / Issue 11 / 2014.

Md Fakruddin, Abhijit Chowdhury, Md Nur Hossain and Monzur Morshed Ahmed*- Fakruddin et al. Springer Plus - 2015.

Mila Arapcheska1., Vangelica Jovanovska1., Zivko Jankuloski 1, Zehra Hajruali

Musliu2, Rise Uzunov2 - IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2 Issue 2, February - 2015.

Mohammed Ibrahim (2014). al zawiah University.

Munir Mohammed Shaban - Faculty of Agriculture - Kafr El Sheikh Branch - Tanta University --2004.

Oadi N Matny* - Department of Plant Protection, University of Baghdad, Iraq - Volume 2 Issue 1 - 2015.

Talal H. Saleh Ali A. Qasim Ghassan M. Daghir - Biology Department - Education College (Misan) - Basrah University- 2009.

Tamer Tawfiq Mohammed Abdul located Sisi - Ain Shams - the Institute of Environmental Studies and Research - Agricultural Sciences- 2006.

Vladimír Ostrý[1,2], František Malíř[2] Annie Pfohl-Leszkowicz[3] (2015). ACTA VET. BRNO

Walter P. Pfliegler , Tünde Pusztahelyi4 and István Pócsi - Mycotoxins – prevention and decontamination by yeasts - 2015.

Yashvir Chauhana,*, Jeff Tatnella, Stephen Kroscha, James Karanjab, Benoit Gnonlonfinc,Immaculate Wanjukic, James Wainainac, Jagger Harvey - Field Crops Research 178 - 2015.

ARABIC SUMMARY

إنتاج السموم الفطرية بواسطة الفطريات المعزولة من الأرز والحبوب المخزنة في الرياض، المملكة العربية السعودية

منيرة القحطاني
جامعة الأميرة نورة بنت عبد الرحمن، الرياض، المملكة العربية السعودية
المملكة العربية السعودية ص.ب ١٠٢٢٧٥ الرياض ١١٦٧٥
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تم جمع ٣٠ عينة من محاصيل الحبوب (القمح والدخن والشعير والذرة والأرز) المخزنة والمعروضة للبيع في أسواق منطقة الرياض. وقد تبين أن معظم هذه العينات كانت ملوثة بفطر الاسبرجلس بنسبه (٧٣.٢٪)وجد ان محصول الذرة أعلى المحاصيل تلوث بفطر الاسبرجلس بنسبه (٢٣.٣٪) مقارنة مع بقية المحاصيل والتي وجد فيها فطر الاسبرجلس كالاتي (القمح بنسبه ١٦.٦٪)، (الشعير بنسبه١٣.٣٪)، (الدخن ١٠ بنسبه ٪)، و (الأرز بنسبه ١٠٪).من خلال استخدام اختبار محلول الامونيا في هذه الدراسة وجد أن اكثر العزلات لديها القدرة على إنتاج الأفلاتوكسين. كما درس تأثير درجة الحرارة على إفراز الأفلاتوكسين في الفطريات المعزولة ويتضح من النتائج في جدول درجة الحرار.