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Fungal contamination and Aflatoxigenic potential - producing of *Aspergillus* Strains Isolated from grain Rice samples in Duhok Province / Iraq

ASIA A. M. SAADULLAH

Department of Biology /College of Sciences / University of Duhok,

Abstract

A total of 50 grain rice (*Oryzae sativa*) samples were tested to establish their mycological contamination and their aflatoxigenic potential. Rice is the most extensively consumed cereal grain by a substantial portion of the world's society, and in Asia predominantly. Under certain conditions, a variety of fungi may develop within rice grains; some of which have the capacity to synthesize mycotoxins. Thus, rice consumers are considered to be a high-risk population specially since this toxin has been linked to health problems and is also highly associated with liver cancer today. When compared to non-local samples, samples from Iraqi markets (of various origins), particularly imported ones, exhibited high quantities of fungus. From samples, three species of *Aspergillus* section *Flavi* (*A. flavus, A. parasiticus,* and *A. tamarii*) have been isolated and identified. Culture-based and ELISA approaches were used to detect aflatoxigenic *A.*strains. Fluorescence in response to UV long-wavelength (365 nm) light and pigment synthesis in response to ammonium hydroxide were used. By both methods, the ratio of aflatoxigenic *A. flavus* isolates to non-aflatoxigenic strains was higher. All the tested strains of *A. parasiticus* showed aflatoxigenic potential. Aflatoxigenic potential of selected strains by ELISA technique for *A.parasiticus* isolates ranged from 181.0 to 360 ppb, whilest, levels of aflatoxins production for *A.flavus* isolates ranged from 183 to 300 ppb. 9p

KEY WORDS: Rice, fungi, contamination, aflatoxins, Duhok.

Introduction:

Grains are a significant source of nutrition for the world's rising population. The most widely grown cereal grains include rice, wheat, sorghum, maize, barley, rye, oats, and millet. Rice (*Oryza sativa L.*) is one of the world's most cultivated food crops. Rice is bent in Iraq at a rate of 593 million tons (Mt) a year [1]. It is one of the most important grains crops for Iraqi food consumers, who are concerned about food quality and safety. In the developing world, food security, defined as having enough food to provide appropriate nourishment for a healthy life, is a crucial concern. Rice is essential for the life of roughly three Billion peoples, or nearly half of the world's population. Rice is consumed at every meal by a large portion of the Asian population, and rice accounts for more than 70% of human calorie intake in certain nations [2]

Rice is a monocotyledonous angiosperm that grows from the seed of the grass species *Oryza sativa L*. Only two species of the genus *Oryza* are considered cultivated rice: *O. sativa*, which is grown in Southeast Asian countries and Japan, and *O. glaberrima*, which is grown in West Africa. The third highest worldwide production of agricultural commodity in 2016 (741.5 million tons) was rice, after sugarcane (1.9 billion tons) and maize (1.0 billion tons).

For the past 50 years, global cereal output and yield have been significantly boosted to satisfy the needs of the growing global population [2]. Although rice crops exhibit less mycotoxin contamination than in many other agricultural harvest, its yet appear as an expressing part of the main crop contaminated.

Mycotoxins are toxic secondary metabolites naturally produced by a certain of fungal species (such as *Fusarium, Aspergillus and Penicillium* genera). The contamination of food and feed by certain mycotoxins is greatly harmful to human and animal well-being, as they can have a variety of negative impacts; such impacts may include: Cancer induction, estrogenic, gastrointestinal, and kidney problems, as well as mutagenicity [3].

Th particular act of the contamination of this food crop may happen at any of the different stages during the culturing, growth, harvesting or even one of many other agricultural processes such as processing, handling and shipping. In general, members of the *Pseudomonadaendospore*-forming bacteria, yeast and molds are the most common rice microflora [4].

Twenty-seven species of fungi, originating from sixteen genera were found to be associated with rice samples. Of which, *Bipolaris oryzae was* the most predominant one, it was associated with a high percentage reaching 82.08% of seed samples. This number was followed by *Alternaria padwickii* (63.36%), *Curvularia lunata* (46.08%), *Pyricularia oryzae* (44.64%), *Alternaria alternata* (34.56%), *Fusarium moniliforme* (27.36%) and *Curvularia pallescens* (21.6%). *Aspergillus flavus* and *Curvularia oryzae* had an occurrence of (15.84%) [5]. Furthermore, sixteen fungal species comprising 11 genera were associated with the 5 rice varieties. These major fungi were: *Fusarium moniliforme, F. oxysporum, Aspergillus flavus, Curvularia lunata, Bipolaris oryzae and Rhizopus* spp [6].

AFB1 and AFG1 are two of the most important groups of mycotoxins found in food .The ingestion of mycotoxin-containing foods has been linked to a variety of mycotoxicoses in people and animals. [7]. *A. flavus* is the most commonly isolated toxigenic fungus and is known for producing aflatoxins, which are carcinogenic compounds. There are numerous reports available to demonstrate the toxigenic potential of *A. flavus* isolated from various crops or foods. The purpose of this study was to determine the level of fungal contamination in various rice samples taken from both local and non-local sources in Duhok province and study their aflatoxigenic potential by cultural method and ELISA technique.

MATERIALS AND METHODS

Samples collection:

A total of 50 stored rice samples were acquired at a local market in Dohuk's main market. The samples were labeled, wrapped in polyethylene bags, transported to the laboratory, and stored at 5° C until analysis.

Mycological analyses

Upon 1 minute of surface disinfection with a 2% sodium hypochlorite, the rice grains were rinsed with sterile distilled water. Each Petri plate included ten pieces, containing Dichloran Rose Bengal Chloramphenicol (DRBC) agar media (Fluka- Germany). They were monitored on daily basis with a stereomicroscope for both fungal growth and sporulation. Each different sample was grown on six petri plates, furthermore, Pure fungal colonies were cultured on media selected specifically for their identification. Based on characteristic physical and cultural traits, the majority of observed taxa were identified to species level. The manual of [8] was used to identify fungi other than *Aspergillus* and *Penicillium*.

Pure colonies were grown in Four different conditions in accordance with the work of [9] for the identification of species in the two genera *Aspergillus* and *Penicillium* (2000). The following media were the selected ones: Czapek yeast extract agar, which was incubated at 25°C and 37°C (CYA25) for seven

days. Czapeck yeast extract agar with 20 percent sucrose was incubated for seven days at 37°C (CYA37). [9], [8] all mention the ingredients and processing of the above five media.

To inhibit bacterial growth, media were treated with 250 mg/L chloramphenicol (SDI). For each culture, two plates of CYA and one plate each of CY20S and MEA were used. Plates were inoculated individually in the center and incubated in a dark area for seven days. At 37°C, one CYA was incubated. The remaining cells were incubated at 25°C.All species were identified using the keys and descriptions supplied by [9], [10].

Determination of Aflatoxigenic potential of fungi in culture media: -

The aflatoxigenic potential of all known *Aspergillus* strains belonging to section *Flavi* was investigated. As detailed by [11], fast methods for identifying aflatoxigenic strains of *Aspergillus* section *Flav*i based on color change after exposure to ammonia vapor were used. Section *Flavi* strains were cultivated in Petri plates using coconut cream agar for testing. [12], were used to prepare the medium. In 9-cm Petri dishes, each strain was inoculated in the center of solidified coconut cream agar medium and incubated at 27°C in the dark. To witness the colony's color change in reverse after 4 days of incubation, the Petri dishes were turned upside down and a drop of ammonia solution was placed in the lid. The aflatoxin-producing colony's backside turned pink [11]. When aflatoxigenic strains were exposed to UV light (365nm), a bright blue or blue-green fluorescent zone appeared around the colonies.

Aflatoxin analysis by ELISA technique

The Enzym e linke d Immuno sorbent Assay (ELISA) was used to do a quantitative study of aflatoxin. The aflatoxins assay was carried out according to the manufacturer's instructions (Veratox Aflatoxin Quantitative Test, Neogen Corporation, USA). The amount of aflatoxins generated by isolates was determined using a standard curve derived from standardized aflatoxins quantities and expressed in parts per billion (ppb).

Results and Discussion

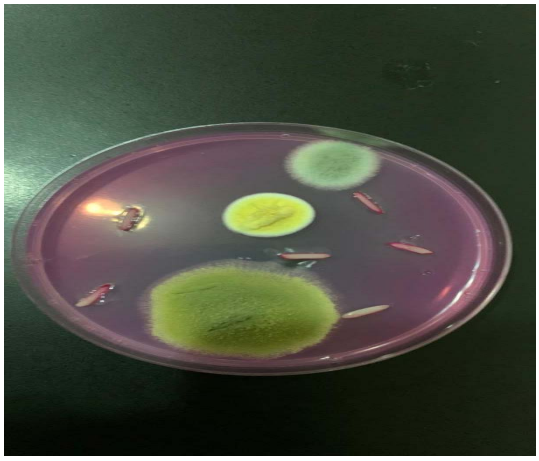
The Results of Fungal infection of fifty samples of rice are shown in table (1) from some local markets of Duhok province, Iraq. Sampling difference in contaminating the types of fungi isolated, the percentage of their presence within the samples and percentage of frequency in samples were examined. The data presented isolation and identification of*: Cladosporium spp., Alternaria, A. niger A. flavus, A. parasiticus A.ochraceous , A. tamarii, Fusarium spp Penicillium citrinum, P.glabrum, Mucor spp., Trichoderma sp., Rhizopus sp. , A. alternaria and Ulocladium sp.*, another studies that isolation similar fungi from rice samples like *Aspergillus, Penicillium ,Fusarium, Phoma, Curvularia, Helminthosporium, Cladosporium, Arthrinium* , *Alternaria*. *Trichoderma* and *Chaetomium* [13], [14]. Fungal species isolated from rice samples were found in the instruction of *Rhizopus* spp. (76%) *Aspergillus* spp (67%) *A. flavus* (42%) *Mucor* spp. (64%) and *Penicillium spp*. (31%) [15], [16]. The expansion of fungi, particularly *Aspergillus*, *Fusarium* and *Penicillium* species, is a difficult in storage They are accountable for quantitative and qualitative fatalities and below convinced conditions these species can progress toxic metabolites [17].

Table 1. Frequency Percentage of isolated fungi from rice grains.

No.	Fungi	Frequency %
1	*Alternaria alternate*	10.0
2	*Aspergillus carbonarius*	53.0
3	*A.flavus*	76.0
4	*A.feotidus*	51.0
5	*A.fumigatus*	40.0
6	*A.niger*	71.0
7	*A.ochraceus*	36.0
8	*A.tamarii*	30.0
9	*A.parasiticus*	70.0
10	*Cladosprium*	24.0
11	*Curvularia*	8.8
12	*Eurotium amstelodami*	3.3%
13	*Fusarium*	50.0
14	*Geotrichum*	9.5
15	*Penicillium glabrum*	60.0
16	*P. citrinum*	55.0
17	*Rhizomucor pusillus*	5.2
18	*Trichoderma*	20.0
19	*Ulocladium*	12.0
20	Yeasts non identified	7.0

A, B

C



Picture 1: growth of each of: *Aspergillus flavus* on rice A- PDA medium, B- *Fusarium* growth on rice

MEA medium C- *A. niger* and *A.flavus* DRBC medium

Table 1 shows a list of the isolated fungi from rice along with their frequency percentage. The rice was collected from markets and stores in Duhok Province. The genera found to be contaminating rice in order of decreasing predominance were; *Aspergillus, Penicillium, Fusarium, Cladosporium Trichoderma, ulocladium, Alternaria, Geotrichum Curvularia RhizoMucor, Eurotium* and non -identified yeasts.

The identified fungi above was distributed into *Aspergillus* section *Flavi* (*A.Flavus*, *A.Parasiticus* and *A.tamarii*) with percentage frequency of 76,70 and 30% respectively .*Aspergillus* section *Nigeri* represent by *A.niger* ,*A.carbonarius* ,*A.foetidus* with percentages of 71,53,and 51 %respectively. Thermotolerant fungus *A.fumigatus* and *A.ochraceous* isolated with percentages of 40 and 36 respectively. *Penicillium glabrum and P.citrinum* were recorded percentage of frequency of 60 and 55
while lowest percentage of frequency recorded was 24% and 20% 12%, 10%, 9.5% 8.8 % and 5.3% in the funguses *Cladosporium, Trichoderma, Ulocladium, A. alternarnata Geotrichum, Curvularia and Rhizomucor pusillus* respectively.
Teleomorphic ascomycetes, namely *Eurotium amstelodami*_were identified with the percentage frequency 3.3%.
This study's findings on the preponderance of *Aspergillus* section *Nigri* isolates on grin rice are consistent with those of [18] in India, [19].

Many challenges face developing countries around the world that are dealing with a severe problem of mycotoxigenic fungus contamination of food and feed. These include the high cost of analytical equipment as well as a shortage of analytical expertise. As a result, efforts have been made to apply low-cost alternatives to culture-based approaches to detect aflatoxigenic strains in large quantities of food [20], as well as [21].

Based on the methodologies of [11], table 2 demonstrated the results of screening *Aspergillus* section *Flavi* strains for aflatoxigenic production abilities. Three of the five *A.flavus* strains were positive (55.5 percent). A recent study [22] found that out of 24 and 18 *Aspergillus flavus* strains, 15 strains (62.5 percent) from maize grains and 10 strains (55.5 percent) from sunflower seeds both demonstrated positive aflatoxigeic ability.

It is generally known that not all *A.flavus* strains produce aflatoxin, and the ratio of non-flatoxgenic to aflatoxigenic generating strains varies depending on the source and region.

[23]; [21] .

. Table 2 further revealed that all *A.tamarii* strains were non-producers, whereas all *A.parasiticus* strains had (100%) good findings. This finding is consistent with prior research on the aflatoxigenic capability of different strains of *Aspergillus niger*.

Both species were isolated from seeds and medicinal plants in [22]

Table 2: The ability of some *Aspergillus* section *Flavi* strains isolated from rice grains to produce Aflatoxins *in - vitro*.

***Aspergillus* isolate**	**Number of strains**	**Positive strains**	**%Positive strains**
A. flavus	5	3	55.5 %
A. parasiticus	3	3	100 %
A. tamarii	1	-	0

Table two showed R.esult of all isolates screned for the particular identification of A.*flavues* (5 isolates) and A. *parasitecus* (3 isolates) and A. *tamareii* (1 isolate)from rice for their aflatoxigenic capacities in culture media as detected by ELISA technique. Three strains of *A. flavus,* and all isolates of *A. parasitius* were positive for their ability to produce aflatoxins, whereas no isolates of *A.tamarii* showed such results. The tested isolates showed marked variation in their aflatoxin potential. This is in line with several other studies that indicate variability in aflatoxin production abilities for *Aspergillius* section *Flavi* strains [24], [25].

Several studies indicated that not all strains of A. *flavus* have aflatoxigenic potential and the ratio of the non-aflatoxigenic isolates to the aflatoxigenic ones varied depending on the source and location of the isolates; [23], [22].

Table 3. *Invitro* quantitative analysis of Aflatoxins by strains of *Aspergillus* section *Flavii* isolated from Rice grains using ELISA method.

Fungal isolate	Aflatoxin (ppb)
Aspergillus flavus (strain 1)	300.0
A.flavus (strain 2)	100.0
A.flavus (strain 3)	183.0
A.flavus (strain 3)	N.D
A.flavus (strain 3)	N.D
Aspergillus parasiticus (strain 1)	360.0
A.parasiticus (strain 2)	310.0
A.parasiticus (strain 3)	181.0
A.tamarii (strain 1)	N.D

N. D ... Not detected (negative)

Conclusion

In spite of the fact that this study was limited in both the places numbers where samples were collected and number of samples, results pointed out that, the contamination of rice grains with Aflatoxins was systematically pay attention. Also, inadvisable handling of the rice such as drying process or improper storage conditions are observed in both local and non-local samples.

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Article highlight

This research highlighted the contamination of samples of rice both local and non local sorts by different kind of fungi and many of these fungi have ability to produce aflatoxins which is very serious and interest subject .

REFERENCES

1. FAO Food and Nutrition paper 81 Worldwide Regulations for Mycotoxins in Food and Feed in 2002. Rome, Italy. DOI: 10.1007/978-1-4615-0629-4_27
2. Park, H.; Oh, N.; Jang, J.; Yoon, H. R. and Cho, M. (2016). Study on Importance-Performance Analysis Regarding Selection Attributes of Rice-Convenience Foods. *Journal of the Korean Society of Food Science and Nutrition*. 45: 593-601. https://doi.org/10.3746/jkfn.2016.45.4.593
3. Marin, S., Ramos, A. J., Cano-Sancho, G. and Sanchis, V. "Mycotoxins occurrence, toxicology, and exposure assessment," Food and Chemical Toxicology,2013. (60), 218–237. https://doi.org/10.1037/a0033537
4. Haque, A. and Russell, N.J. Phenotypic and genotypic characterisation of Bacillus cereus isolates from Bangladeshi rice. *International Journal of Food Microbiology*. 2005. 98: 23-34. https://doi.org/10.1016/j.ijfoodmicro.2004.04.025
5. Archana, B. and Prakash, H. S. Survey of seed-borne fungi associated with rice seeds in India. *International Journal of Research in Pure and Applied Microbiology.* . 2013. 3: 25-29. http://dx.doi.org/10.20546/ijcmas.2016.512.018
6. Atta, K.A. Mycoflora associated with seeds of five rice (*Oryza sativa*) varieties in Ghana. *Greener Journal of Plant Breeding and Crop Science,* 2015. 3:014-019. http://hdl.handle.net/123456789/1311
7. Peraica, M. and Domijan, A. M. Mycotoxins in food and human health Arh. Hig. Rada Toksikol. 2001 .52:23-35. https://doi.org/10.3109/15569541003598553
8. Pitt, J.I. and Hocking, A.D. (1997) Fungi and food spoilage 2nd edition London, Blackie Academic & professional. DOI: 10.4236/aim.2015.54027
9. Klich, M.A. Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures.The Netherlands.2002. pp 116. https://doi.org/10.1017/S0269915X03243123
10. Abarca, M.L., Accensii, F., Bragulat, M.R and Cabanes, F.J. Current importance of ochratoxin A-producing Aspergillus Spp. J. Food Prot. 2001 64:903-906. **https://doi.org/10.4315/0362-028X-64.6.903**
11. Saito, M. and Machida, S. (1999). A rapid identification method for aflatoxin-producing trans of *Aspergillus flavus* and *A. parasiticus* by ammonia vapor. Mycoscience 40:205-208. https://doi.org/10.1007/BF02464300
12. Dyer, S.K.and McCammon, S.. Detection of aflatoxigenic isolates of *Aspergillus flavus* and related species on coconut cream agar. J. Appl. Bacteriol. 1994 .76:75-78. https://doi.org/10.1111/j.1365-2672.1994.tb04418.x
13. Uraguchi, K. and Yamazaki, M. Toxicology, Biochemistry and Pathology of Mycotoxins. Halsted Press, John Wiley and Sons: New York, 1987. 1-106.
14. Rama Devi IP, Subbranyam K, Krishn RV, Chiranjeevi, V (1988): Effects of certain plant extracts on rice grain mycoflora. Bull. Grain Technol. 26(1):55-58
    residues in infusions Microbiol. Alim. Nutr.10:267-271.
15. Tahir, M.A., Aziz, T., Farooq, M.,and Sarwar, G. (2012). Silicon-induced changes in growth, ionic composition water relations, chlorophyll contents and membrane permeability in two salt-stressed wheat genotypes https://doi.org/10.1080/03650340.2010.518959
16. Sundaram K., Didolkar, A., Thau, R., Chaudhuri, M.,and Schmidt, F. Antagonists of luteinizing hormone releasing hormone bind to rat mast cells and induce histamine release. Agents Actions.1988. (25)307–313. https://doi.org/10.1007/BF01965036
17. Makun, H. A, Gbodi, T.A., Akanya, H. O, Salako, A.E., and Ogbadu, H.G Fungi and some mycotoxins. 2007
18. Archana, B. and Prakash, H. S. Survey of seed-borne fungi associated with rice seeds in India. *International Journal of Research in Pure and Applied Microbiology.* . 2013. 3: 25-29. **DOI:** 10.4314/sa.v7i2.46011

19. Aqeel M. Ali1, Amina N.a AL-Thwani1, Shatha A. Yousif. . Comparing the fungi contamination of rice samples collected from local and non-local markets. *Iraqi Journal of Biotechnology, December* 2018, Vol. 17, No. 3, 85-91
20. Davis, N.D., Iyer,S.K.and Diener, U.L. Improved method of screening for aflatoxin with a coconut agar medium. Appl.Environ.Microbiol. .1987 .53:1593-1595. https://doi.org/10.1128/aem.53.7.1593-1595.1987 .
21. Abbas, H.k., Sheir, W.T., Horn, B.W. and Weaver, M.A. Cultural methods for aflatoxin detection. J. Toxicol. 2004. 23:295-315 https://doi.org/10.1081/TXR-200027854
22. Abdullah, S.K.and Al-Mousawi, K.A. Incidence of Aspergillus species in seeds of corn and sunflower cultivars grown in Iraq and aflatoxin-producing potential of Aspergillus section Flavi. Proc. 1 st Scient. Conf. Biol. Sci. Mousul University, 22-23 April, 2009. p 299-307.
23. Abdel-Malek, A.Y., El-Maraghy,S.S and Hassan, .H.A.A. Mycotoxin-producing potential of some Aspergillus, penicillium and Fusarium isolates found on corn grains and sunflower seeds in Egypt. J.Islamic Acad.Sci. 1993.6:189-192.
24. Vuamonde, G., A., Fernandez pinto, V,Comerio , R and Deqrossi , C. Variability of aflatoxin and cyclopiazonic acid by Aspergillus section Flavi from different substrates in Argentina. Int.J. Food Microbiol. 2003 .88:79-84. https://doi.org/10.1016/S0168-1605(03)00101-6
25. Embaby, E. M., Hagagg, L. F., & Abdel-Galil, M. M. (2012). Decay of some fresh and dry fruit quality contaminated by some mold fungi. *Journal of Applied Sciences Research*, (June), 3083-3091.