The Efficiency of *Aloe vera* Gel Extract in Inhibiting the Growth of *Aspergillus flavus* Fungus Associated with Imported and Domestic Rice Grains in Iraq and its Ability to Reduce Aflatoxin B1 Production

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Abstract

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The study aimed to evaluate the efficiency of different concentrations of *Aloe vera* gel extract in inhibiting the growth of *A. flavus* on potato dextrose agar (PDA) culture medium, as well as evaluating the efficiency of the extract in inhibiting the growth of *A. flavus* and reducing Aflatoxin B1 (AFB1) production in rice grains. The results obtained showed that the concentrations 1, 2, 3, and 4% of *Aloe vera* gel extract caused variable inhibition rates of *A. flavus* growth on PDA culture medium; of 97, 100, 5.88 and 17.64%, respectively. The best concentration from the laboratory experiment, (2%) was used, to evaluate its efficiency in inhibiting the growth of *A. flavus* on rice grains during storage and reduced AFB1 production by 86.5%, from 21.2 ppb in the control to 2.86 ppb in the treatment using HPLC high- performance liquid chromatography. The same treatment reduced AFB1 concentration in rice grains contaminated with AFB1 by 74.7%, from 22.88 ppb in control to 5.78 ppb in the extract treatment.

Keywords: *A. flavus*, mycotoxins, aflatoxin B1, *Aloe vera*, food, feed, toxicity.

Introduction

Rice (*Oryza sativa* L.) is considered one of the important foods on a global scale, as it is the major food for more than half of the world's population, especially in Asia (FAO, 2003). Additionally, it is one of the important staple crops in the world, along with wheat, maize, and barley. In 2019, the global rice production reached 498.95 million tons, and the global cultivated area was 163.43 million hectares. China ranked first in rice production for 2019, with a total yield of 146 million tons (USDA, 2019).

A. flavus is one of the most important pollutants of food and feed, and it is one of the most important fungi producing mycotoxins, especially AFB1, and most of the human exposure to mycotoxins comes from contaminated grains such as corn, peanuts, and rice (Horn, 2003; Hussein, 2008).

Mycotoxins are secondary metabolites produced by some fungi naturally, and these fungi can grow on many food crops before and during harvesting and storage; most of these toxins are stable and cannot be destroyed by heat, food processing, and traditional cooking processes. Furthermore, most mycotoxins have a low molecular weight of 97-710 Dalton, and due to their low molecular weight, they are resistant to harsh environmental conditions and also cannot stimulate the human immune system (Ismail, 2014). The low doses of mycotoxins cause persistent shivering in animals (Pitt, 2000).

Aflatoxin is produced in nature by *A. flavus*, *A. parasiticus*, and *A. nomius* fungi, and *A. flavus* is the most common foodborne fungus (Klich & Pitt, 1988). There are many types of Aflatoxins in nature, but the most dangerous ones are B1, B2, G1, and G2, which are toxic to humans and animals as they are found in all major food crops (WHO, 2018).

Aloe vera belongs to the family Asphodelaceae (Liliaceae). It is a succulent plant with a light green color. Moreover, the plant consists of thick leaves with edges containing thorns (serrated). The leaves consist of three layers, the inner is a pure gel consisting of 99% water, 1% glucomannans, glycosides, amino acids, fats, sterols, and vitamins, the middle layer that contains latex is a yellow juice made up of anthraquinones and glycosides, whereas the third layer is the thick outer layer called the cortex and has two functions: protection and formation of carbohydrates and proteins, as well as the transport vessels inside it (Surjushe *et al*., 2008).

Interestingly, reported that *Aloe vera* is a short-stemmed succulent medicinal plant, as the gel contains more than 75 active compounds such as aloin, elmodin, aluminan, saponins, sterols, and other compounds, and levels of these substances are vary according to the plant's growth conditions (Shireen *et al.*, 2015).

Materials and methods

Preparation of *Aloe vera* gel extract

The fresh leaves of *Aloe vera* were washed with running water for 5 minutes, then rinsed with sterile deionized water, and the side spines and green outer cortex layer were removed with a clean knife; after that, the colorless gel was homogenized well in blender, and then filtered through a Whatman No.1 filter paper, then the filtrate was centrifuged

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at 500 rpm for 5 minutes, and the supernatant was used as extract (Rasouli *et al*., 2019).

Evaluation of the efficiency of different concentrations of *Aloe vera* gel extract in inhibiting *Aspergillus flavus* growth on PDA culture medium

PDA was prepared and cooled to 45°C, then the antibiotic tetracycline at a concentration of 250 mg/liter was added to prevent bacterial growth. The medium was then poured into five 250 ml flasks (100 ml per flask). The *Aloe vera* gel extract at concentrations 1, 2, 3, and 4% (ml/100 ml) was added to each flask and one of the flasks was left without gel extract as a control, all flasks were mixed well and poured into Petri dishes of 90 mm diameter and left to solidify. Subsequently, the Petri dishes were inoculated with a suspension of *Aspergillus flavus* spores isolate Asf04 which was molecularly diagnosed and deposited in the NCBI with the accession number MN944453 (Al-Hamiri, 2020). The spores suspension of the fungus isolate was prepared by adding 10 ml of sterile deionized water to a 10 mm diameter disc of 7 days old *A. flavus* colony. A drop of the fungus spore suspension was taken using the isolation needle and spread into the center of a Petri dish and incubated at 25±2°C. The diameters of the developing colonies were measured every two days until the control dishes were full with fungal colonies, and the inhibition rate was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{Average diameter of colony in control - average diameter of colony in the treatment}}{\text{Average diameter of colony in control}} \times 100$$

Efficacy of *Aloe vera* extract in inhibiting *Aspergillus flavus* growth and AFB1 production in rice grains during storage

The best concentration of *Aloe vera* gel extract was determined in the laboratory experiment to inhibit the growth of *A. flavus* and prevent it from producing AFB1 toxin on rice grains. The experiment was conducted in a randomized complete block design RCBD. Rice grains were soaked in water for 20 minutes, then filtered and distributed in large (20 cm in diameter and 5 cm high) glass dishes at a rate of 150 g -dish, then 100 ml of deionized water was added to each dish, sterilized in the autoclave at 121°C and a pressure of 1.5 kg/cm^2 for 20 minutes. Moreover, it was re-sterilized after 24 hours to ensure the complete killing of the microbial content. Subsequently, it was inoculated with *A. flavus* isolate with 2 discs of 10 mm diameter for each dish using a cork borer. The dishes were then shaken well to ensure a homogeneous distribution of *A. flavus* spores (Shotwell *et al*., 1966).

Six dishes were divided into two groups of three dishes each; to the first group *Aloe vera* extract was added at concentration of 2 ml/100 g of rice grains, whereas the second group was left without extract addition, as a control group. The dishes were then stored at room temperature for 30-days and were dried in the oven at a temperature of 50°C overnight, whereas 25g was grinded from each replicate for each treatment as well as for the control group to estimate the concentration of AFB1 and its reduction ratio using HPLC technique.

Efficacy of the of *Aloe vera* gel extract in reducing AFB1 production in rice grains

The best concentration of *Aloe vera* gel extract to reduce AFB1 production from rice grains was determined. Rice grains were soaked in water for 20 minutes, then filtered and distributed in large glass dishes with a diameter of 20cm and a height of 5cm at a rate of 150g/dish, then a 100 ml of deionized water was added to each dish, and sterilized in an autoclave at 121°C and a pressure of 1.5 kg/cm^2 for 20 minutes, and it was re-sterilized after 24 hours to ensure complete sterilization glass dishes were the inoculated with *A. flavus* isolate, using two 10-mm diameter disc from a PDA culture for each glass dish. The dishes were then shaken well to ensure a homogeneous distribution of- *A. flavus* spores, and were incubated at 25±2°C for 21 days with shaking and stirring daily for 5-days to ensure the spread of fungal spores and their growth on the rice grains (Shotwell *et al*., 1966).

At the end of the incubation period and after the rice grains became contaminated with AFB1, all dishes were autoclaved at 121°C and a pressure of 1.5 kg/cm^2 for 20 minutes. Subsequently, the dishes were divided into two groups; 2% *Aloe vera* gel extract was added to the first group, whereas the second group was left without any extract addition as a control.

The dishes were stored at laboratory temperature for a month and then dried in the oven at a temperature of 50°C overnight. A 25 g was grinded from each replicate for each treatment as well as for the control group to extract AFB1 and estimate its concentration in the samples using the HPLC technique.

Extraction of AflatoxinB1 from the solid medium (rice grain)

AFB1 was extracted from rice grains prepared as mentioned above according (AOAC,2005).

After extracting AFB1 from the solid medium (rice grains), it was quantified using an HPLC device according to the following equation (Liu *et al.*, 2012).

$$\text{AFBI concentration} = \frac{\text{AFB1 standard concentration x area of sample curve}}{\text{Area of the AFB1 standard curve}} \times \frac{\text{Dilution factor}}{\text{Sample volume}}$$

Evaluation of the effect of *Aloe vera* gel extract on Quail (*Coturnix japonica*) weight and mortality

The chicks of Quail birds were brought at the age of 5 days for bioassay for 40 days; a special air-conditioned room for breeding was prepared and sterilized with formalin, and 15 cages were prepared which were divided into five groups, each group had three replicates, and each replicate had three birds, as follows: (i) the first group was fed with mixed fodder with rice contaminated with *A. flavus* which was treated with *Aloe vera* extract in a ratio of 1:1, (ii) the second group was fed with mixed fodder with rice contaminated with AFB1 and treated with *Aloe vera* extract in a ratio of 1:1, (iii) the third group was fed with mixed fodders with rice

contaminated with *A. flavus* only in a ratio of 1:1, (iv) the fourth group was fed with fodder mixed with rice contaminated with AFB1 only in a ratio of 1-1, (v) the fifth group was fed with fodder without any contamination as a control.

The dimensions of the cages used for each replicate were 40×35×35 cm. Cages were sterilized with formalin and marked with the type of the treatment and replicate. Additionally, their floors were covered with sawdust with chicken water fountains and a feeder placed in each cage.

The weight of chicks was recorded every 5 days and until the end of the experimental period. Chicks weight and death rate was recorded. Unlike chickens, quail birds are characterized by rapid growth, short sexual maturity, and strong immunity and resistance against diseases. Nevertheless, quail birds were vaccinated with the Newcastle vaccine at the age of 20 days only to ensure its protection.

Results and discussion

Efficiency of different concentrations of *Aloe vera* gel extract in inhibiting *Aspergillus flavus* growth on PDA culture medium

The results of the laboratory experiment showed that the use of concentrations 1, 2, 3, and 4% of *Aloe vera* gel extract in the PDA culture medium inhibited the growth of *A. flavus* by 97, 100, 5.88, and 17.64%, respectively (Table 1). The best concentration was 2ml of extract/100ml PDA, where the fungus was inhibited completely 100%. It was noticeable that the higher concentration of the extract was associated with less ability to inhibit the fungus.

Table 1. The effect of different concentrations of *Aloe vera* gel extract on the inhibition rat of *A. flavus* growth on PDA.

Treatment	Concentrations (%)	Average colony diameters (mm)	Inhibition %
Aloe Vera	1	0.25	97.05
Gel Extract	2	0.00	100.00
	3	8.00	5.88
	4	7.00	17.64
Control	0	8.50	0.00
LSD at P=0.05		0.5871	

The effect of *Aloe vera* gel extract in inhibiting *Aspergillus flavus* growth and AFB1 production in stored rice grains

The results revealed significant differences between the grains contaminated with *A. flavus* only and grains contaminated with the fungus treated with *Aloe vera* gel extract.

The 2% concentration of *Aloe vera* gel extract inhibited *A. flavus* growth and inhibited production of AFB1 in grains by 86.5%. AFB1 concentration decreased from 21.2 ppb in the control treatment to 2.86 ppb in the group treated with gel extract (Table 2).

Furthermore, 2% concentration of *Aloe vera* extract reduced AFB1 in rice grains inoculated artificially with AFB1 by 74.34%, where AFB1 was reduced from a concentration of 22.88 ppb in the control treatment to 5.87 ppb in the grains treated with the extract (Table 2).

Evaluation of the effect of *aloe vera* gel extract on the weight and mortality rate of Quail birds (*Coturnix japonica*).

The results obtained showed that feeding birds with fodders contaminated with the *A. flavus* caused a significant reduction in their weights from the age of 10 days to the age of 45 days. The 45 days old bird's weight was 204.33 g compared to 216.66 g for birds fed on fodders without any contamination (control). Birds fed with fodders contaminated with *A. flavus* and treated previously with *Aloe vera* extract significantly increased bird weights, as the average weight of 45 days old birds was 225.5 g which is significantly higher than the control group (Table 3).

The results obtained showed that the fodders contaminated with AFB1 only caused a significant reduction in the weights of birds, from the age of 10 days until the age of 45 days, as the average weight of the birds was 200.66 g. Whereas feeding on fodder contaminated with AFB1 and treated with *Aloe vera* extract caused a significant increase in the weight of birds, as the average weight of 45 days old birds was 227.6 g compared to 216.66 g for birds fed on fodders without any contamination (control) (Table 4).

The findings of this study confirmed the high efficiency of *Aloe vera* gel extract in inhibiting the growth of *A. flavus*, as it inhibited 100% the growth in vitro when used at 2% concentration, as well as its efficiency in reducing AFB1production.

Table 2. The efficiency of *Aloe vera* gel extract in inhibiting the growth of *Aspergillus flavus* and inhibition AFB1 production.

Treatment	Extract concentration/ 100 g Rice	The concentration of AFB1 (ppb)	Inhibition ratio%	Reduction ratio%
Rice contaminated with *A. flavus* and treated with *Aloe vera* extract	2 ml	2.86	86.50	-
Rice contaminated with *A. flavus* only (control)	-	21.20	0.00	-
Rice contaminated with AFB1 and treated with *Aloe vera* extract	2 ml	5.87	-	74.34
Rice contaminated with AFB1 only (control)	-	22.88	-	0.00
LSD at P=0.05		1.493**		

Table 3. Effect of *Aspergillus flavus* inhibition and reduction AFB1 production by treatment with *Aloe vera* extract on the weights of quail birds of different ages.

Treatment	Average bird weight* (g)									Mortality rate (%)
	5 days	10 days	15 days	20 days	25 days	30 days	35 days	40 days	45 days	
Fodders contaminated with *A. flavus*	50.00	53.40	74.06	92.90	133.13	150.53	170.80	197.5	204.33	22.22
Fodders contaminated with *A. flavus* and treated with *Aloe vera* extract	42.90	52.50	90.83	108.33	158.33	198.33	206.60	220.00	225.50	0.00
Fodders only (control)	35.33	58.53	95.00	109.90	156.63	175.00	201.36	216.66	216.66	0.00
LSD at P= 0.05									8.94	

* Each value represents an average weight of three replicates, each of 3 birds.

Table 4. The weights of quail birds of different ages after being fed with fodder contaminated with AFB1 reduction and treated with *Aloe vera* extract.

Treatment	Average bird weight* (g)									Mortality rate (%)
	5 days	10 days	15 days	20 days	25 days	30 days	35 days	40 days	45 days	
Fodders contaminated with AFB1	41.50	48.60	68.30	86.66	133.26	143.66	175.00	200.00	200.66	11.11
Fodders contaminated with *AFB1* treated with *Aloe vera* extract	43.54	56.66	79.16	108.30	170.80	185.00	210.00	220.00	227.66	0
Fodders only (control)	35.33	58.53	95.00	109.90	156.63	175.00	201.36	216.66	216.66	0
LSD at P= 0.05									6.436	

* Each value represents an average weight of three replicates, each of 3 birds**.**

الملخص

الحميري، كمال عبد الكريم عباس وحليمة زغير حسين. 2022. كفاءة مستخلص هلام *Aloe vera* في تثبيط نمو الفطر *Aspergillus flavus* المرافق لحبوب الأرزّ المحلي والمستورد في العراق وقدرته على تقليل إنتاج الأفلاتوكسين ب 1. مجلة وقاية النبات العربية، 40(2): 164−168.
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هدفت الدراسة إلى تقييم كفاءة تراكيز مختلفة من مستخلص هلام صبار الألوفيرا (*Aloe vera*) في تثبيط نمو فطر *A. flavus* على مُستنبت آغار ديكستروز البطاطا (PDA)، وكذلك تقييم كفاءة المستخلص في تثبيط نمو الفطر *A. flavus* واختزال إنتاجه لسُمّ الأفلاتوكسين B1 (AFB1) على حبوب الأرزّ. أثبتت النتائج أن التراكيز 1، 2، 3 و 4% لمستخلص هلام الألوفيرا قد حقّقت تثبيطاً متبايناً لنمو فطر *A. flavus* على الوسط الزرعي PDA وفق النسب 97، 100، 5.88 و 17.64%، على التوالي. وفي تجربة الخزن، اختير أفضل تركيز (2%) للمستخلص حسب التجربة المختبرية لاختبار كفاءته في تثبيط نمو الفطر *A. flavus* على حبوب الأرزّ والحدّ من إنتاج AFB1، وكذلك كفاءته في اختزال AFB1 على حبوب الأرز الملوثة بالسمّ AFB1. حقّق التركيز المستخدم تثبيطاً لنمو الفطر وحدّ من انتاجه للسمّ بنسبة 86.5%، حيث اختزل تركيز AFB1 من 21.2 جزء في البليون في معاملة المقارنة إلى حدود 2.86 جزء في البليون في معاملة المستخلص حسب قراءات جهاز الكروماتوغرافيا السائل عالي الأداء (HPLC)، كما حقّق التركيز نفسه للمستخلص اختزالاً بنسبة 74.7% لتركيز AFB1 في حبوب الأرزّ الملوّثة به، إذ تمّ خفضه من التركيز 22.88 جزء في البليون في معاملة المقارنة إلى 5.78 جزء في البليون في معاملة المستخلص.

كلمات مفتاحية: *A. flavus*، السموم الفطرية، الأفلاتوكسين B1، الألوفيرا، أغذية، أعلاف.

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