Controlling of aflatoxin B_1 using Oyster *fungus* (*Pleurotus ostreatus*) in Basil plant (*Ocimum basilicum*)

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Abstract

The study was carried out at Faculty of Agriculture, University of Kufa from March to August 2018 to detect the ability of *Aspergillus flavus* isolates to produce aflatoxin B1 and to determine the effect of *Pleurotus ostreatus* in reducing of Aflatoxin B_1 produced by *A. flavus*. The results showed presente of three species of fungi including *A. flavus*, *Aspergillus niger* and *Penicillium* spp. It was found that there was a significant domunion of *A. flavus* followed by *A.niger* and *Penicillium* spp. Their frequency rates were 63.88%, 22.22% and 13.88%, respectively. However, appearance rates of these isolates were 70%, 40% and 20%, respectively. Ammonia detection test showed that there were 15 out of 23 isolates of *A. flavus* have ability to produce Aflatoxin B1, with a rate of 65.21%. However, layer chromatography (TLC) test showed only 10 out of 23 isolates of *A. flavus* were able to produce Aflatoxin B_1, with a rate of 43.47%. The present results also showed that adding of *P. ostreatus* significantly affected the logarithmic number of *A. flavus* spores which was 5.74 (5.6 x 10^5spore / g) in the treatment of 5 ml *A. flavus* + 15 g *P. ostreatus*. In addition, *P. ostreatus* has capacity to reduce the Aflatoxin B1 produced by *A. flavus*. Spectrophotometer results showed that the treatments of 5 ml *A. flavus* + 15 g *P.ostreatus*, 10 ml *A. flavus* + 15 g *P.ostreatus*and 15 ml *A. flavus* + 15 g *P.ostreatus* significantly reduced the percentage of produced Aflatoxin B_1 (84.5%, 84.4%, 88.3%), compared to the treatment of *A. flavus* + 10 g *P.ostreatus* which gave about 69.8% reduction rate.

Keywords: Basil plant, *Pleurotus ostreatus*, *Aspergillus flavus*, Aflatoxin B_1

السيطرة على سم الافلاتوكسين B_1 باستخدام الفطر المحاري *Pleurotus ostreatus* في الريحان

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المستخلص

احريت هذه الدراسة للكشف عن قابلية بعض عزلات الفطر *Aspergillus flavus* على انتاج السموم الفطرية افلاتوكسين B1 من الريحان والتعرف على تاثير الفطر المحاري والحد من السموم الفطرية المنتجة بواسطة الفطر *A. flavus* . أظهرت نتائج عزل الفطريات من الريحان وجود 3 أنواع من الفطريات هي *Aspergillus flavus* و *Aspergillus. niger* و *Penicillium* . واتضح إن هناك سيادة للفطر *A. flavus* يليه الفطر *A. niger* ثم الفطر *Penicillium*. إذ بلغت نسبة ترددها 63.88 , 22.22 , 13.88 % على التوالي. ولكن نسب ظهور هذه الفطريات الثلاثة بلغت 70 , 40 , 20 % على التوالي .وبين الكشف الكيميائي عن سموم الافلاتوكسينات بطريقة أختبار الامونيا لعزلات الفطر *A. flavus* والمعزولة من الريحان أن 15 عزلة من مجموع 23 عزلة من الفطر *A. flavus* قادرة على انتاج الافلاتوكسين B_1 من خلال تغير لون قاعدة وسط جوز الهند المنمى عليها عزلات الفطر وبنسبة 65.21 % . وعند أستعمال طريقة كروماتوكرافيا الطبقة الرقيقة (TLC) Thin Layer Chromatgraphy وجد ان 10 عزلة قادرة على انتاج الافلاتوكسين B_1 من اصل 23 عزلة من الفطر *A. flavus* وبنسبة 43.47 % . أظهرت نتائج اضافة الفطر المحاري *Pleurotus ostreatus* وجود إختلافات احصائية معنوية في معدل لوغارتم اعداد ابواغ الفطر *A. flavus* إذ بلغ معدلها في معاملة 5 مل *A.flavus*+ 15 غم *Pleurotus ostreatus* 5.74(5.6 × 10^5 بوغ /غم) ,علاوة على الفطر المحاري له القدرة على تقليل السموم الفطرية (افلاتوكسينB1)حيث كانت نسبة الاختزال والتي قدرت بواسطة بجهاز Spectrophotometer قد اظهرت بان المعاملات 5 مل *A.flavus*+ 15 غم *Pleurotus ostreatus* , 10 مل *A.flavus*+ 15 غم *Pleurotus ostreatus* , 15 مل *A.flavus*+ 15 غم *Pleurotus ostreatus* قد اثرت تاثيرا معنويا في نسبة انتاج الافلاتوكسين B1 (88.3, 84.4, 84.5 %) بالمقارنة مع معاملة 5مل *A.flavus*+ 10 غم *Pleurotus ostreatus* والتي اعطت نسبة اختزال قدرت بحوالي 69.8 %.

الكلمات المفتاحية (الفطر المحاري *Pleurotus ostreatus* ,الريحان ,السموم الفطرية ,*A.flavus*)

Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi. The mycotoxin-producing fungi are of the genera *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium* species (25). These mycotoxins may be produced in a number of food products and can lead to acute and chronic toxicity, mutagenicity and teratogenicity in human and animal health (8). Aflatoxins are a group of acutely toxic compounds which produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The main types of aflatoxins found in agricultural products are B_1, B_2, G_1 and G_2 (21). The International Organization for Cancer Research (IRC) was identified and placed Aflatoxin B_1 in the first group of chemical compounds carcinogen which is one of the liver cancer tumors causes (17).

Several methods have been used to prevent infection and contamination of different types of fruits with these fungal species, including physical methods by treating fruits with hot water, as well as usingsome preservatives. Microorganisms, especially bacteria, were also used in the treatment of fruits by dipping those fruits with some bacterial species have antagonistic activity towards fungi produced mycotoxins (19). More recently, *Pleurotus ostreatus* (Agaricales: Pleurotaceae) was used as a biocontrol agent to reduce of Ochratoxins produced by *A. niger* species (6). It is ften consumed as a food because it contains many substances include dietary fiber and several other polysaccharides (a class of carbohydrates found to affect immune function). It also has a distinct effect in reducing the cholesterol level in human blood, its obvious effect as an anti-tumor of cancer and its effect in stimulating cellular immunity (14). Furthermore, this fungus contains antibody substances act as antimicrobial agent against viruses, bacteria and fungi and their mycotoxins (22) such as a1-octen-3-ol compound which is antibacterial that found in the fruit body of the fungus and c 4-Methoxy benzaldehyde compound, which is found in the fungal mycelium (26). It also plays an important role in the destruction of toxic substances in the medium when this fungus growsthrough the enzymes that it produced (23 and 20).

The most common use of basil is for cooking, such as in tomato sauce, pesto, or vinegars. But it also can be sprinkled over salads and sliced tomatoes, either whole or chopped (1). There are a little information about isolating of *A. flavus* species in basil and detect their capacity to produce aflatoxin B1. Also, there is no information about the efficacy of *P. ostreatus* in reducing of Aflatoxin B_1 produced by *A. flavus*. The study objectives, therefore, were to (1) isolate and diagnosis of fungi associated with basil; (2) detect the ability of *A. flavus* isolates to produce aflatoxin B1 using both ammonia and thin layer chromatography (TLC) methods and (3) determine the effect of *P. ostreatus* in reducing of Aflatoxin B_1 produced by *A. flavus*.

Materials and Methods

The pure culture of *P. ostreatus*was obtained from the laboratory of Fungi, Faculty of Agriculture, University of Kufa.

Potato Sucrose Agar (P.S.A.)

200 g of potato tubers, were cut into small pieces and boiled with 500 mlof distilled water for 20-30 minutes in a glass beaker. After the boiling period, then filtered content by a piece of gauze. 10 g of sucrose and 17 g of agar were dissolved in 500 ml of distilled water and then the potato leach and full size to a liter. The media was distributed in flasks according to its use, and their vents were sealed with cotton bolts and sterilized with the sprinkler device at 121°C and 15 lb/kg for 20 minutes. After that, the flasks were left to cool and then placed in the refrigerator until use. This media was used to isolate and grow the fungi.

Coconut Extract Agar

The media was prepared by mixing 100 g of coconut powder with 300 ml of distilled water and then the mixture was heated for 20 min. After the heating period, the mixture was filtered using a piece of gauze and then 1.5 g of agar was added and full size to 300 ml. the media was sterilized using the same procedure described above.(12)

Isolation and diagnosis of fungi associated with basil

Samples were collected from basil (10 samples from the fields of basil in Najaf city and 10 samples from Babylon) and isolated using dilutions method on P.S.A until the fourth dilution. The Petri dishes were incubated at 25^{o}C for 3 days. the temperature of 25 m for three days. After the of incubation period, growing isolates were purified. and identified according to the taxonomic characteristics cited by both (24, 16).

The percentage of appearance and frequency of fungi were calculated according to the following equations:

$$\text{Percentage of appearance (\%)} = \frac{\text{No. of occurent samples of each fungal isolate}}{\text{Total number of samples}} \text{ x } 1$$

$$\text{Percentage of Frequency (\%)} = \frac{\text{No. of samples for each fungal species}}{\text{Total number of all fungal isolates}} \text{ x } 100$$

Detection of the ability of *A.flavus* isolates to produce aflatoxin B_1

Detection of aflatoxins using ammonia treatment**:**

The ability of *A. flavus* strains that isolated from basil on producing of Aflatoxin B1 was tested using Dianese and Lin (12) method. The coconut media was used to cultivar the fungal strains using 9-cm Petri dishes by placing 5 mm tablet from each fungal strain which already grows PDA. Where each fungal strain was replicated three times.The dishes were sealed by a parafilm and incubated at 25°C for a week. Afterward, the ability of *A. flavus*strains to produce aflatoxins was detected using ammonia solution 20% by placing filter papers saturated with ammonia solution in the lid of the dish containing fungal strain grew on the coconut media. The plates have incubated upside down at 25°C for 2 days. Changing the color of fungal strain from a transparent color to pink or red indicates that this strain was able to produce Aflatoxins.

Detection of Aflatoxins using thin layer chromatography (TLC)

The isolates of *A. flavus* were developed on P.S. D. media by placing tablets with 5 mm diameter (oneweek old) of each isotate at the center of each dish where it was repeated three times. Then, the dishes were incubated at 30 ° C ± 2 ° C for a week. After that, one dish of each isolate was selected and cuttted by a sterile knife to small pieces. Then, they were transfered by a sterile needle to an electric mixture containing 20 mL of chloroform for 10 minutes. The mixture was filtered with filter paper and then taken into a clean, sterile flask and placed in an electric oven at a temperature of 40 °C to dry. And then , the mixture was dissolved in 1 ml of chloroform. To detect the presence of Aflatoxin, B1 using TLC (20-20 cm), where the plates were activated in the oven at 105 ° C for one hour before using (18).

A light straight line was made on a TLC plate 1.5 cm from the base of the plate with 15 microliters were taken by a capillary tube of standard AFB1 ()and placed on the line 2 cm from the left edge of the plate and at 2 cm from the spot of the standard toxin, *A.flavus* at the same distance and quantity equal to the amount of standard poison, then left the srtains to dry and then placed in the separation basin containing the separation system composed of chloroform and methanol mixture and the size of 2:98 size.-1 size was observed until the solution reaches a distance of about 2 cm from the upper end of the plate. The plates were removed and dried under the conditions of the seal T for a period of 5 minutes and then examined under UV radiation and a wavelength of 365 nm was detected aflatoxin B1 matching deportation Rf coefficient and sparkle color standard for color with antivenom and deportation the extract of *A.flavus* samples (27).

Effect of *Pleurotusostreatus* on the percentage of aflatoxin B1 in the basil plant

This experiment was carried out to investigate the effect of *P.ostreatus* on the reduction of Aflatoxin B1, produced by *A.flavus* as follow:

1- Preparation of the *A. flavus* by growing the fungus strains on PSA for a week at 30 ± 2°C. Then, the fungal spores were harvested by adding 10 ml of sterilized distilled water for

each petri dish and gently scraping the surface of the cultures with a sterile inoculating loop to dislodge the spores from the surface of the agar plates. The number of spores was determined using a haemocytometer. Spore concentration of all fungal strains was determined to be 10^7 conidia ml^{-1}.

2- Preparation and growth of *P.ostreatus* on the treatment of wheat straws (hay)that obtained from wheat fields around the Faculty of Agriculture, University of Kufa. They were cut into small pieces (3-4) cm and then moistened up to 50% and without sterilization and placed in 30 x 51 cm plastic bags with 1 kg of hay per bag. Fungal suspension was added as a 5% of the mean weight (22) in multiple layers between residues (7) and then slot of the bags was sealed tightly. The bags were then transferred to the incubation chamber, 4 x 3 m in size with a heating and cooling device (Split), at 25 ± 2°C, leaving the fungus to grow on that treatment of wheat straws for 21 days.(15)

Plastic pots each with 25g of compost was prepared and the experimental treatments were as following:

1- Adding 5ml of spore concentration of *A. flavus* per pot.
2- Adding 5ml of spore concentration of *A. flavus*+ 5 g of *P. ostreatus* per pot.
3- Adding 5ml of spore concentration of *A. flavus*+ 10 g of *P. ostreatus* per pot.
4- Adding 5ml of spore concentration of *A. flavus*+ 15 g of *P. ostreatus* per pot.
5- Adding 10ml of spore concentration of *A. flavus* per pot.
6- Adding 10ml of spore concentration of *A. flavus*+ 5 g of *P. ostreatus* per pot.
7- Adding 10ml of spore concentration of *A. flavus*+ 10 g of *P. ostreatus* per pot.
8- Adding 10ml of spore concentration of *A. flavus*+ 15 g of *P. ostreatus* per pot.
9- Adding 15ml of spore concentration of *A. flavus* per pot.
10- Adding 15ml of spore concentration of *A. flavus*+ 5 g of *P. ostreatus* per pot.
11- Adding 15ml of spore concentration of *A. flavus*+ 10 g of *P. ostreatus* per pot.
12- Adding 15ml of spore concentration of *A. flavus*+ 15 g of *P. ostreatus* per pot.

Basil seeds were planted (15 seeds per pot) and after seed germination, the number of seedlings per pot was reduced to 10 seedlings. All plants were maintained in a growth chamber at 23 ± 2°C for 21 days. The percentage of contamination was calculated in each treatment by taking 10 g of each replicate of all treatments and mixing them individually in an electric mixer with 100 ml of distilled water for 5 minutes. Then, a series of dilutions till 10^5conidia ml^{-1} and 1 ml of last dilution was cultured on P.D.S. Each treatment was replicated three times. All plates were incubated at 25 ± 2°C for 4 days, and then the number of colonies was recorded on each plate. The number of active spore of each fungal strain was calculated according to the following equation

Number of active spores = average number of spores per replicate x inverted dilution (10). Aflatoxin B1 was measured by spectrophotometer after extracting the fungal toxins from the samples of the experimental treatments. Aflatoxin B1 was extracted by taking 10 g of shoot fresh weight from each treatment and then transferred to an electric mixer containing 100 ml of chloroform and mixed for 10 minutes. The mixture was filtered by filtration paper and placed in a 250 ml Separatory Funnel. The mixture was gently shaken for 30 seconds, with accumulated gases being expelled as needed (at least twice). It was left for a min to separate the two layers, where the upper layer was neglected, and the bottom layer was taken. The mixture was put in a clean and sterile flask and placed in an oven at 40°C to dry, and then dissolved in 5 ml of chloroform. Afterward, the concentration of Aflatoxin B1 was measured usingspectrophotometer. This technique is based on spectrophotometric, which depends on the properties of the compound to absorb light in the field of ultraviolet or infrared radiation. Where there is a direct correlation between the absorption time and the concentration of poison, during a standard curve between light absorption and concentration of poison and extract the concentration value corresponding to the reading given by the anonymous sample. The readings were then compared with standard

poison concentrations where the wavelength was 365 nm and the reduction ratios were calculated by the following equation:

Statistical analysis

Statistical analyses werecarried out using complete random design (CRD) as a single-factor experiment. Mean comparisons were performed usingLSDtest at 5% level of significance ($P< .05$) (2).

Isolation and diagnosis of fungi associated with basil

The results showed that there were three isolates of fungi belong to two genus which includes *Aspergillus* and *Penicillium*. In addition, there was a for *A. flavus*, followed by *A. niger* and *Penicillium* spp., where the frequency rates were 63.88, 22.22, and 13.88% respectively, while the rates of appearance were 70, 40 and 20%, respectively (Table 1). The most important reason for the appearance of *Aspergillus* spp. in basil may be related to its widespread in the environment as a result of its ability to produce a large number of spores that resistant to adverse environmental conditions. These spores are minor (less than 15 nm), thus they can reach many places through the windows and other openings, as well as they can grow in wide ranges of temperature and humidity, as some species of *Aspergillus* species can grow in a temperature ranged between 5 and 45°C (16).

Table (1) Percentage of frequency and appearance of fungal spesies isolated from Basil

Fungal species	Frequency(%)	Appearance (%)
A. flavus	63.88	70
A. niger	22.22	40
R. stolonifer	13.88	20

Detection of the ability of *A. flavus* isolates to produce aflatoxin B_1 Ammonium test

The results of this test showed the ability of 15 isolates out of 23 isolates of *A. flavus* to produce aflatoxin B1 by changing the color of coconut media that they were cultured on, while 8 isolates gave negative test results. Furthermore, the producing isolates were differed in producing aflatoxin B1 depending on the intensity of colony color change. The most important isolates in producing Aflatoxin were AF11, AF15 and AF18. While AF3, AF8 and AF13 showed a moderate capacity for aflatoxin B1 production and other isolates were weak in their production of aflatoxin B1 (Table 2). Similarly, Ali (5) found that 23 isolates out of 38 isolates of *A. flavus*were able to produce aflatoxin B1. In addition, Davis and Diener (11) reported that 86% of *A. flavus*s isolates were able to infect the field pistachios and contaminate them with aflatoxins. Al-Adil et al. (3) reported that 59% of *A. flavus*isolates which isolated from some foods in Baghdad city are capable to produce aflatoxins. Ali (4) reported that 17 isolates out of 22 isolates of *A. flavus*from dates were able to produce aflatoxin B1. Differences in the ability of aflatoxin B1 isolates to produce aflatoxin B1 may be relatedto their genetic differences, which can explain the gradient in red color. Where isolates with dark red indicate their ability to produce large amounts of aflatoxins.

Detection of Aflatoxins using thin layer chromatography (TLC)

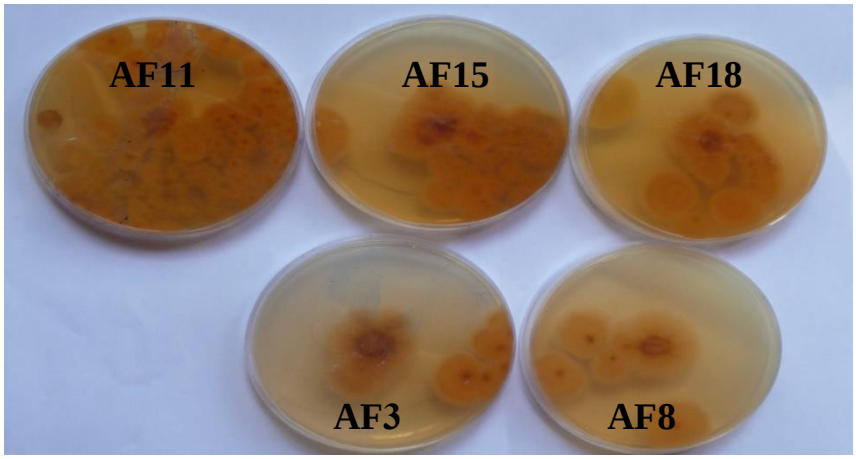
The results of this tests showed the capacity of ten isolates out of 23 (43.47%) isolates of *A. flavus* on the production of aflatoxin B1 (Table 3). However, these isolates were varied in their production of aflatoxin B1. Chemical analysis of TLC showed that the isolates AF18 and AF11 were the most productive of aflatoxin B1 based on their intensity. The results obtained in the present study are consistent with those of Gherbawy et al (13) who indicated that 38.88% of *A. flavus* isolates were able to produce aflatoxin B1. Ali (4) found that 10 isolates out of 22 (45.45%) isolates of *A. flavus* which isolated from dates were able to produce aflatoxin B1 and 45.45%. Differences in the ability of aflatoxin B1 isolates to produce aflatoxin B1 may be related to their genetic differences (21). It was also noted that the number of isolates which were able to produce Aflatoxin B1, using this technique was less than the number of isolates that have been detected to produceAflatoxin B1 using

Ammonium test. Therefore, this method (TLC) can be more accurate in the identification of isolates that able to produce fungal toxins in general, including Aflatoxin B1.

Table (2): Ammonium test to detect the ability of *A. flavus* isolates to produce aflatoxin B_1

Fungal isolate	ability of aflatoxin B_1 production	Fungal isolate	ability of aflatoxin B_1 production
AF1	-	AF13	++
AF2	+	AF14	+
AF3	++	AF15	+++
AF4	-	AF16	-
AF5	+	AF17	+
AF6	-	AF18	+++
AF7	+	AF19	-
AF8	++	AF20	+
AF9	-	AF21	+
AF10	+	AF22	-
AF11	+++	AF23	+
AF12	-		

(+) = changing the convers colour of media to pink ; (-) No changing of the convers colour of media



Figur (1) Ammonium test to detect the ability of *A. flavus* isolates to produce aflatoxin B_1

Table (3): Testing the susceptibility of isolates *A. flavus* on production of Aflatoxin B_1isolated from Basil Plant using thin Layer chromatography (TLC).

Fungal isolatie	ability of aflatoxin B_1 production	Fungal isolatie	ability of aflatoxin B_1 production
AF1	-	AF13	+
AF2	-	AF14	-
AF3	++	AF15	++
AF4	-	AF16	-
AF5	+	AF17	+
AF6	-	AF18	+++
AF7	-	AF19	-
AF8	+	AF20	-
AF9	-	AF21	+
AF10	-	AF22	-
AF11	+++	AF23	+
AF12	-		

Effect of *P. ostreatus*in the growth and development of *A. flavus*in a basil plant

The results of this experiment showed a significant effect on the mean number of spores of *A. flavus*, where the mean logarithm number of spores in the treatment of 5 ml of *A. flavus*+ 10 g of *P. ostreatus* and 5 ml *A. flavus*+ 15 g *P. ostreatus* were 5.85 (7.23 × 10^5spores/g), 5.74 (5.6 x 10^5spores/g), respectively. While treatment of 5 ml of *A. flavus* only showed about 6.25 (18.33 × 10^5spores/g). In the treatment of 10 ml of *A. flavus*+ 10 g *P.ostreatus*,and 10 ml of *A. flavus*+ 15 g *P.ostreatus*,the rate of logarithm of spores were 5.94 (9.16 × 10^5 spores/g), 5.79 (6.33 × 10^5 spores/g), compared to 6.35 (22.66 x 10^5 spores/g) at a treatment of 10 ml of *A. flavus* (Table 4).

The treatments of 15 ml *A. flavus*+ 10 g of *P. ostreatus* and 15 ml *A. flavus*+ 15 g of *P.ostreatus* have been given the lowest logarithmic number of fungal spores 5.87 (7.93 × 10^5spores/g), 5.84 (7.1 x 10^5 spores/g) respectively, compared with 6.44 (28.16 x 10^5 spore / g) at treatment 15 ml of *A. flavus* only. The reason may be related to *P. Ostreatus* which contains effective extracts or compounds and antibody substances work as antimicrobial of fungi, bacteria and their mycotoxins (22). For example, the 1-octen-3-ol compound, which presents in the sporangiaand the 4-Methoxy benzaldehyde, which presents in the mycelium. These two compounds play an important role in the destruction of their mycotoxinsin the media where *P.ostreatus* is grown because of the number the enzymes that its produced (23, 20). As well as its ability to work as an antibody to parasites, including nematodes by removing theirsecondary metabolic toxins and attacks those nematodes (9).

Table (4) Effect of *P. ostreatus*in the growth and development of *A. flavus*in a basil plant

Treatment	logarithmic number of fungal spores	mean number of spores of *A. flavus* (10^5× spores/g)
5 ml of *A. flavus* only	6.25	18.33
5 ml of *A. flavus*+ 5 g of *P.ostreatus*	5.97	9.50
5 ml of *A. flavus*+ 10 g of *P.ostreatus*	5.85	7.23
5 ml of *A. flavus*+ 15 g of *P.ostreatus*	5.74	5.60
10 ml of *A. flavus* only	6.35	22.66
10 ml of *A. flavus*+ 5 g *P.ostreatus*	6.12	16.66
10 ml of *A. flavus*+ 10 g *P.ostreatus*	5.94	9.16
10 ml of *A. flavus*+ 15 g *P.ostreatus*	5.79	6.33
15 ml of *A. flavus* only	6.44	28.16
15 ml of *A. flavus*+ 5 g of *P.ostreatus*	5.92	9.00
15 ml of *A. flavus*+ 10 g of *P.ostreatus*	5.87	7.93
15 ml of *A. flavus*+ 15 g of *P.ostreatus*	5.84	7.10
L.S.D.(0.05)	0.1467	

Effect of *P. ostreatus* in reducing of Aflatoxin B1 produced by *A. flavus* using spectrophotometer

The results presented in Table 5 showed the rates of Aflatoxin B1 absorptive by plant and their transfer through the food chain and the capacity of *P. ostreatus* in reducing of Aflatoxin B1 produced by *A. flavus*. The reduction rate which measured by spectrophotometer showed that the treatments of 5 ml *A. flavus*+ 15 g *P. ostreatus*, 10 ml *A. flavus* + 15 g *P.ostreatus* and 15 ml *A. flavus* + 15 g *P.ostreatus* significantly reduced the percentage of produced Aflatoxin B1 (84.5%, 84.4%, 88.3%), compared to the treatment of *A. flavus*+ 10 g *P.ostreatus* which gave about 69.8% reduction rate. *Pleurotus ostreatus* may be due to the presence of effective extracts or compounds and is characterized by containing antimicrobial, fungus and toxin substances (22). It contains antiviral, bacteriological and fungicidal substances such as the 1-octen-3-ol

antifungal compound In the body and 4-Methoxy benzaldehyde, which is found in the fungal spinning, which is a volatile vehicle Shu-25) and also has a role in the destruction of toxic substances in the center where the growth of this fungus by the enzymes produced (23, 20). As well as its ability to antifungal parasites, including worm worms as it removes metabolic toxins and attacks those worms (9).

Table (5) **Effect of *P. ostreatus* in reducing of Aflatoxin B1 produced by *A. flavus***

Treatment	Amount of Aflatoxin B_1 Microgram / 100 ml	Percentage of Reduction %
5 ml of *A. flavus* only	43.00	0.0
5 ml of *A. flavus*+ 5 g of *P.ostreatus*	21.67	49.6
5 ml of *A. flavus*+ 10 g of *P.ostreatus*	13.00	69.8
5 ml of *A. flavus*+ 15 g of *P.ostreatus*	6.67	84.5
10 ml of *A. flavus* only	54.33	0.0
10 ml of *A. flavus*+ 5 g *P.ostreatus*	32.00	41.1
10 ml of *A. flavus*+ 10 g *P.ostreatus*	16.00	70.5
10 ml of *A. flavus*+ 15 g *P.ostreatus*	8.33	84.6
15 ml of *A. flavus* only	68.33	0.0
15 ml of *A. flavus*+ 5 g of *P.ostreatus*	28.00	59.0
15 ml of *A. flavus*+ 10 g of *P.ostreatus*	17.33	74.6
15 ml of *A. flavus*+ 15 g of *P.ostreatus*	8.00	88.3
L.S.D.(0.05)	6.211	10.82

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