Measuring Toxicity of Aspergillus niger isolated from different sources and effect of Agaricus bisporus filtrate and sodium bicarbonate on their toxin

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Abstract

The present study aimed at performing of fungi isolate from (soil from streets, dried fruit). Where the fungi accompaining the local and imported dried fruits were isolated and diagnosed, and the fungal isolates that have the ability to produce toxins, specifically Ochratoxin A, were selected. The following fungal species were isolated:

(Aspergillus niger, A.parasiticus, A.flavus, Rhizopus stolanifer, Pencillium natatum) the relative frequency of each type was calculated, and it was found that there were significant differences, and the highest frequency rate belonged to the fungus A.niger. The HPLC test was conducted for a group of fungal isolates to investigate the presence of mycotoxins, and the results showed that the highest concentration of ochratoxin A, which reached (41.89 ppb), which resulted from the fungus A.niger. where the Agaricus bisporus fungus was used as a bio-resistance agent, and when conducting an antibody test, it was proved that the fungus Agaricus bisporus has an antidote ability against the poisonous fungus A.niger.

The results are showed its ability to significantly reduce dry weight when testing the effect of Agaricus bisporus filtrate on A.niger fungus in liquid medium (PDB) by increasing the concentration of filtrate, and the effect of sodium bicarbonate was tested on both radial growth in solid media (PDA) and dry weight. For A.niger growing in a liquid medium (PDB), where the results demonstrated the high ability of sodium bicarbonate to inhibit radial growth and reduce the dry weight of the fungus, as the results proved that the higher the concentration of sodium bicarbonate and the fungus filtrate Agaricus bisporus, the greater the rate of inhibition of the poisonous fungus.

Keywords: *A.niger, Agaricus bisporus, Sodium bicarbonate, Ochratoxin A.*

INTRODUCTION

Aspergillus niger is one of the black filamentous fungi which grows very fast and capable of tolerating low pH environment. Dried fruits are of high nutritional value in the human diet, but despite this they provide a suitable medium for the growth of mold and fungi that produce toxins, and the presence of these mycotoxins in food products poses a serious threat to human health, so investigating their presence in the diet is of great importance (Heshmati et al.2017)

More than 400 types of mycotoxins have been identified. The majority of people eat small amounts of mycotoxins in their food and do not show clear pathological effects, but when eating food that contains high concentrations of these toxins and eating them frequently and for a long period of time, it can cause serious health problems (Bhat and Vasanthi, 2003). Fungi are

important pathogens, as grains are infected during harvest, transportation or storage by many fungal species that are naturally present in agricultural soils, the most important of which are Aspergillus, Alternaria, Ascochyta, , Fusarium, Penicillium, Pythium, Rhizoctonia, Rhizopus, Sclerotium, Uromyces and other fungus causing cereal rots. Emergence and rotting of the roots and bases of the stems (Al-Jubouri, Iyad Waleed Abdullah, 2011). The search has also increased to find new alternatives such as A.niger, which has achieved impressive results as a natural source of antibiotics, as the secretions cellular extra of the mycelium of A.nigers are effective and very resistant to bacteria and fungi (Hess et al., 2003), including Agaricus bisporus (The white button fungi) is a staple in the economy and the beneficial effects of this type of A.nigers have been known for a long time, improving immune function (Solano-Aguilar et al., 2015).

Materials and Methods:

Samples of dried fruits (raisins and apricots) were collected from the markets and shops in the center of the city of Diwaniyah, and samples from road soil in the governorate to investigate the presence of contaminated fungi. its outer surface and left at room temperature to dry.

Filtrates preparation

Fungal filtrates (A.niger and Agaricus bisporus) were prepared using PDB food medium in glass beakers with an amount of 100 ml of medium for each beaker and sterilized by autoclave at a temperature of 121°C. and a pressure of 15 pounds / ing2 for 15 minutes and after cooling the antibiotic Chloramphenicol 250 Mg/L was added to the medium and the beakers were inoculated. Two tablets (5 mm) in diameter of 7 days old A.nigers were placed in each flask and the flasks were incubated at 25 °C for 3 weeks with continuous shaking every two days. Filters with a diameter of 0.22 microns and keep the filter until use at a temperature of 4 C. Detection of mycotoxins using HPLC technique.

Detection of the toxic fungal filtrate

The analysis was carried out in the laboratories of the Department of Environment and Water of the Ministry of Science and Technology, as the toxins produced by some types of fungi were detected using the HPLC device model Skyam, and using a vector phase consisting of (Mobile phase = D.W: 5% Formic acid: methanol (20: 5: 75) and a C18-ODS (25 cm 4.6 mm) separation column using Fluorescence detection and the mobile phase. (Flow rate = 1ml/min) and after conducting the analysis of the standard substance that was prepared by taking 0.1 g of the standard poison and dissolving it in a volume of 250 ml in a volumetric vial of 250 ml capacity, if the initial concentration was 40 µg/ml or 40 ppm, a concentration of 10 ppm was prepared by taking 0.4 ml from the initial concentration and complete the volume to 10 ml in a volumetric vial of 10 ml. 100 µl was taken from the last concentration and injected into a HPLC device under the same conditions (Tolgyesi et al., 2015), and using the following equation described by (Akiyma and Chen, 1999), The concentration of toxins was calculated in the fungus filtrate samples by the standard formula is C = m/V, where C is the concentration, m is the mass of the solute dissolved, and V is the total volume of the solution.

Effect of A.bisporus filtrate on radial growth of the toxic fungus Aspergillus niger on PDA medium

Determining the effectiveness of A.bisporus tested in the radial growth of the A. niger followed the (Dixit et al.,1976) method

Poisoned Food Technique if three concentrations of A.bisporus filtrare, 10.20.30% of the sterile food medium Potatos Dextrose broth , move the medium a spiritual movement and then leave the dishes to harden and then by the mortal drill a piece of toxic fungus growth was made, Then a piece measuring (5) mm from the end of the radial growth of the A.niger and at the age of 7 days was transferred to the center of the dish and three repeaters per concentration and placed in the incubator for (7 days) at a temperature of 25 °C but the comparison treatment included dishes without adding the A.bisporus filtrate . After the completion of the incubation phase, the growth rate of fungus in the transactions of different compositions was measured 30,20,10% taking the growth rate of laboratory fungus in dishes using the ruler and after the fungal yarn reached the edge of the dish in the comparison treatment, radial growth was calculated by taking the growth rate of perpendicular diameters of developing colonies and then calculating the percentage of inhibition (percent inhibition of radial growth).

Effect of sodium bicarbonate concentrations on radial growth of toxic fungus A.niger to determine the effectiveness of sodium citrate tested in the radial growth of fungus A.niger followed (Dixit et al.1976), poisoned food technique, if three concentrations of sodium bicarbonate salt were prepared: (30.20.10 mg/ml), the concentration is prepared 10% by taking (1g) of salt and dissolving it in 100 ml of sterile implant medium (PDA) Potatos Dextrose Agar, To prepare the concentration 20% by taking (2g) of salt is taken and dissolved in 100ml of sterile implant medium and so on for a concentration of 30% and followed the the same previous steps.

The effect of Agaricus bisporus, Sodium bicarbonate and their interaction treatments on the radial growth of the fungus A.niger

Followed the way of Poisoned Food Technique (Dixit et al., 1976) ,where Agaricus bisporus filtrate was used as a biological resistance agent with different concentrations and sodium bicarbonate as a chemical control agent (NaHCO3). With the same concentrations, an interaction was made for the biological and chemical factors and for the same concentrations to control the fungus contaminated with soil and dried fruit where different concentrations of both factors were added and overlapped to the PDA culture medium and the medium was poured into Petri dishes (7 mm) with three replicates for each concentration, while the control dishes were left without any In addition, after the solidification of the medium, a hole was made in the middle and a disk of contaminated A.nigers (5 mm) was placed in it. The dishes were incubated at a temperature of 25 0C. After 7 days, the growth of the fungal colonies was observed and their growth rate was calculated by taking the growth rate of two perpendicular diameters of the developing colonies, and then the percentage was calculated to inhibit.

Results and Discussions:

The results showed the use of high-performance liquid chromatography (HPLC) to detect fungus toxic, A.niger filtrate .The value of the detention time is (3.37) minutes as it represents the standard area of the toxins, and these values indicate the presence of toxic fungus filtrate toxins in the examined isolates based on matching the detention time of the standard substance with the time of appearance of these values for the same conditions in which the analysis of the standard substance was conducted. The results showed that the toxic

fungi had the ability to secrete mycotoxins in all samples, as the highest concentration of the toxin produced from the filter of the selected fungus isolate A.niger for the sample of dried fruit was 41.89 ppb, and the concentration recorded in the sample of soil road 30.58 ppb. The results shown in Table (1) showed the inhibition ability of Agaricus bisporus fungus filtrate on the growth of A.niger fungus on PDB liquid medium, where a significant decrease in dry weight rates was observed compared with the control treatment. As the average dry weight of the fungus ranged in a concentration of 30% of the PDB medium prepared in advance about (0.07gm) and with an inhibition rate of (75%), while in a concentration of (20%), the weight of fungal growth was (0.12gm) with an inhibition rate of (75%) and at a concentration of (10%) the weight of fungal growth was with a percentage of Inhibition (35%) compared with the control treatment in which the average dry weight was (0.28±0.01) and this means that the greater the concentration of the fungal filterate in the liquid media, the greater the percentage of inhibition in the effect of the fungal filterate of Agaricus bisporus on the growth of the poisonous fungus A.niger.

As for the effect of sodium bicarbonate, Table (1) shows the presence of some significant differences in the treatment of sodium bicarbonate, as the highest inhibition rate for A.niger fungus was at a concentration of 30% of bicarbonate, as the average weight of the fungal colonies was (0.05±0.01 gm), and the inhibition rate was (82%) and, at a concentration of (20%). The average weight of the colonies (0.13±0.01 gm) and the rate of inhibition (53%) while we notice the lowest rate of inhibition in the concentration was (10%), as the average weight of the colonies was (0.20±0.01) and the percentage of inhibition (28%) compared with the average diameter of the colonies in the control treatment (7.5 cm).

This indicates the existence of a direct relationship between the concentration of bicarbonate and its inhibitory ability, and this is consistent with what was stated by (Rasheed, 2021), in which it was shown that sodium bicarbonate had significant effects on the dry weights of the tested fungi, and it was noted that there were significant differences between the concentrations used, as the inhibitory effect increased by increasing focus. As for the effect of the interaction between sodium bicarbonate and Agaricus bisporus fungus filtrate on the dry weight of A.niger fungus in the liquid medium, the results showed that the effect of the interaction between them was more effective in effect compared to the effect of each of the bicarbonate and fungus filtrate individually. The highest percentage of inhibition was (85%) at a concentration of (30%), where the average dry weight of the poisonous fungus was (0.04 gm), as shown in Table (1).

Table(1) Effect of A.bisporus fungus infiltrate and sodium bicarbonate and their interaction on the dry weight of A.niger fungus in PDB liquid medium

Concentration mg/ml and percentage of fungal filterate	Treatment *A.bisporus*		Treatment sodium bicarbonate		Interference treatment between sodium bicarbonate and *A.bisporus*		Concentration inhibition rate ±standard deviation
	Weight (gm)±SD	Inhibition %	Weight (gm) ±SD	Inhibition %	Weight (gm) ±SD	Inhibition %	
10	0.18±0.02	35	0.20±0.01	28	0.11±0.02	60	
20	0.12±0.01	57	0.13±0.01	53	0.08±0.01	71	
30	0.07±0.01	75	0.05±0.01	82	0.04±0.01	89	
Control	0.28±0.01		0.28±0.01		0.28±0.01		
Inhibition rate coefficients ± deviation							
LSD value	0.03		0.03		0.01		

As for the effect of sodium bicarbonate on the radial growth of A.niger fungus in PDA solid medium, as mentioned in Table (2), it shows the effectiveness of sodium bicarbonate with its different concentrations and its significant effect on the growth of A.niger fungus compared to the control treatment, and its effect on the growth of the pathogenic fungus increases with increasing concentration. Where the highest percentage of inhibition of the poisonous fungus was reached in the PDA food medium containing a concentration of 30% of sodium bicarbonate, where the average diameter of the colonies was (1.13±0.01 cm) and as shown in Table (2)

As for the effect of A.bisporus fungus filtrate on the radial growth of A.niger fungus on PDA medium, A.bisporus filtrate with its different concentrations gave significant differences, as the highest inhibition rate for A.niger fungus was reached in PDA medium that contained a concentration of 30% of the fungus filtrate. Shown in Table (2).

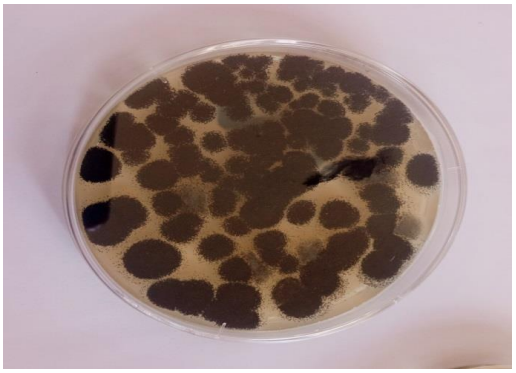
Also, the results of the effect of the interference of A.bisporus fungus infiltrate were tested. and sodium bicarbonate in the radial growth of the fungus A.niger, it was found that there were significant differences between the different concentrations of the fungus A.bisporus and sodium bicarbonate, which greatly affected the growth of the fungus, and the results showed that the diameters of the poisonous fungus were inversely proportional to the concentration of both the filterate and the bicarbonate and as Shown in Table (2).

Table(2) The effect of interaction between sodium bicarbonate and A.bisporus fungus filtrate on the radial growth of A.niger fungus in solid media.

Concentration mg/ml and percentage of fungal filterate	Treatment *A.bisporus*		Treatment sodium bicarbonate		Interference treatment between sodium bicarbonate and *A.bisporus*		Concentration inhibition rate ±standard deviation
	Diameter (cm) ±SD	Inhibition %	Diameter (cm) ±SD	Inhibition %	Diameter (cm) ±SD	Inhibition %	
10	3.5±0.2	53	3.31±0.01	55	2.9±0.01	61	
20	2.6±0.1	65	2.11±0.01	71	2.42±0.02	67	
30	1.25±0.01	83	1.12±0.01	85	1.13±0.01	84	
Control	7.5±0.01		7.5±0.01		7.5±0.01		
Inhibition rate coefficients ± deviation							
LSD value	0.29		0.26		0.27		

The effect of the interaction between sodium bicarbonate and A.bisporus fungus filtrate on the radial growth of A.niger fungus on solid media. Fig-1 shows the growth of the fungus A.niger in the control dish. Fig-1 shows the growth of the fungus in the dish treated with a concentration of 30% of sodium bicarbonate and Agaricus bisporus fungus filtrate.

Fig-1 (A) The growth of A.niger in control dish (B) Effect of interaction of sodium bicarbonate and filter of A.bisporus fungus at a concentration of 30%



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B

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