Effect of Oyster mushrooms (*Pleurotus ostreatus*) on adiet poultry contaminated with fungus exudate toxins *Aspergillus flavus* and the possibility of inhibition growth and break down toxins producers from it

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ABSTRACT: This study was conducted in the laboratory of Advanced fungi at the Department of Biology/ College of Education for girls / University of Kufa for the period from 03/01 - 15/05/2016 in collaboration with the laboratory of the veterinary clinic to the Iraqi Ministry of Agriculture, so as to detect the ability of oyster mushroom (Pleurotus ostreatus) to protect the contamination of diet poultry by fungi and the possibility of breaking down the Aflatoxin and Ochratoxins which produced by Aspergillus flavus. Tested way to extract the poison, using an organic solvent is Acetonitrail, results showed for the detection of the toxin using organic solvent in the recovery of the venom of the contaminated samples using several standard poison under study and using the ELISA device showed the ability of p.ostreatus to break down toxins contaminated with aflatoxin to diet in the treatment of treatment mushroom oyster duration of fermentation 28 days where the highest percentage smash hit 1.0 mcg / kg, followed by treatment T4 treatment also mushroom oyster duration of fermentation 21 days Reached at 1.5 mcg / kg compared to the treatment of T2 untreated mushroom oyster which showed the highest contamination rate was 165.3 mcg / kg either treatment control were free of contamination and mycotoxin which gave 0.0 mcg / kg. way to extract the poison, using an organic solvent is Acetonitrail, results showed for the detection of the toxin using organic solvent in the recovery of the toxin of the contaminated samples using several standard poison under study and using the ELISA device showed the ability of p.ostreatus to break down toxins contaminated with aflatoxin to diet in the treatment of T4 treatment mushroom oyster duration of fermentation 28 days where the highest percentage smash hit 1.0 mcg / kg, followed by treatment T3 treatment also mushroom oyster duration of fermentation 21 days reached at 1.5 mcg / kg compared to the treatment of T2 untreated mushroom oyster which showed the highest contamination rate was 165.3 mcg/ kg either treatment control were free of contamination and mycotoxin which gave 0.0 mcg / kg.

Key ward : ELISA technique , Pleurotus ostreatus, Aspergillus flavus toxin,diet poultry

INTRODUCTION

in breaking down toxic substances in the medium in which it grows the fungus through the enzymes secreted by the fungus (Lacina et al, 2003).

For the mushroom oyster significant impact in the treatment of cancer and prevent the growth of tumors (Wolff *et al*, 2008) characterized by belonging to the mushroom species oyster *Pleurotus*

The mushroom oyster *(Pleurotus ostreatus*) food fungi that have medicinal benefits because it contains extracts and effective compounds (Vamanu, 2012), and is marked by anti-fungal substances and toxins (Oei,2005) has the effect of privileged as anti-cancer tumors and in stimulating cellular immunity (Hatvani et al ,2008), also it has a distinct role

requirements for food security (Billard, 2004), and this specification increased the food, health and administrative requirements (Nagy, 2006). As well as the presence of mycotoxins in feed materials used in diets and one of the most important problems facing poultry in developing countries. This is due to the lack of these countries for scientific foundations, It followed in modern agricultural operations, including the seeds and stored drying technology. Popcorn is the first and the longer this problem being a silo strategy in diets of poultry, they accounted for 50-60% of components and increase their exposure to pollution that poisons especially cultivated in the loop autumn, as it marks the date harvested nearthe winter months, solutions and thus will drag on for drying and then increase the likelihood of mycotoxin contamination.

We have many countries the maximum allowable limits proceeded to the availability of mycotoxins in the human diet and the diets of animals with 20 mcg / kg (Adler, 2002), and that means that the majority of maize marketed grain stores in Iraq unfit for human consumption or feed materials included in the industry diets, according to the results of recent studies in country (Hussain, 2000; Mughallis 2004; Hadithi, 2005). place among the mycotoxins of importance, being the most natural substances dangerous in the complex health effects in low pollution and spread in the thermal ranges wide levels until Pat contamination of feed crops and diets poisons(IFLA)thing difficult to avoid in spite of all the modalities modern and means used in high viability of colonialism and the speed of growth for spinning mildew on among different agricultural media and environmental conditions (Kong, 2004 Ahmed, (2015) indicated that the fungus highly competitive against a number of contaminated diet fungi. and currently occupies the mushroom oyster ranked second after agricultural mushroom white (*Agaricus bisporus*) and by 25% of the world's production of food fungi(Zhang *et al* ,2014) and has a high nutritional value because it is rich in protein, which constitutes 20-40 % dry weight (Ahmed *et al*, 2009).

The poultry of topics that are notable for the importance of their contribution to the provision of a major food source for humans to meet the needs of the animal protein and because of the rapid development that took place in the production of commercial hybrids for broiler chickens characterized by fast growth and high ability in feed efficiency. As well as the poultry confronted with some problems, including a key determines the availability of feed materials used in the diets of the developing broiler and then higher prices (US Grains Council, 2003). The availability of suitable forage qualities at a reasonable price is the key to success in the production process for broiler chickens, and so that there would be competition with the domestic market, it is important to go to the feed use of non-conventional and entered into the diets of broiler chickens combination so as to reduce production costs, (Basak *et al*,2002).

The chicken meat industry, global corporations started to produce global strains characterized by high productivity specifications to provide minimum

study was conducted to remove the negative effects of fungal toxins from diets.

production (Gratz et al, 2004). Free fodder crops has become media of mycotoxins of the priorities that must be introduced to the success of the poultry projects, so this

The important themes of this study are:

1- Test the inhibition ability of the oyster mushroom(*Pleurotus ostreatus)* against a number of contaminated fungi of poultry diet.

2 -The use of oyster mushroom (*Pleurotus ostreatus*) in the remediation of contaminated diets industrially certain level of poison aflatoxin toxin secreted by the *Aspergillus flavus* fungus.

3. The important aim to use oyster mushroom *(Pleurotus ostreatus)* to reduce the Aflatoxin and Ochratoxinin poultry diet by biodegradation by oyster mushroom .

Materials and methods

This study included conducting laboratory experiments It was obtained pure isolation from oyster mushroom (*Pleurotus ostreatus*) mushrooms and producer of mycotoxins *Aspergillus flavus* fungi in the laboratory of the Plant Protection Department - Faculty of Agriculture, University of Kufa.The medium which are used to isolate and the Potato Dextrose Ager are used to growth and all testing of fungi . It prepared by dissolving 39 g per liter of distilled water, according to the manufacturer instructions and 250 mg antibiotic Chloramphenicol was added 1L. The medium autoclaveed at 121 °C and pressure 15 pounds / in 2 for 20 minutes,The medium powred in plastic dishes (9 cm diam.and using according to the aim of study us the growth of oyster mushroom ,isolation and classification of some fungi from diet poultry .

The inhibition test of oyster mushroom against the isolated fungi from poutry diet a number of fungi contaminated poultry diet.

The double culture technique in Petri dishes was used the dish is divided into two equal parts and Disc with 0.5 cm diameter placed on the center of the first part .while growth the isolated fungus from poultry diet was placed on the center of second part . The treatment of each fungus repeated three times . All the dishes were incubated at a temperature 25 ± 2 °C, The inhibition ability of mushroom oyster was measured after 7 days according to Bell *et al*, (1982) .

Isolation and diagnosis of fungi associated with poultry diet by Dilutions Plate method

In order to know the fungi in the diet poultry have been taking random samples from different parts of the diet bag comparison was mixed uniformly and

then taken 10 g of which was added to a beaker containing 90 ml sterile distilled water and mixing brokered an electric mixer to break up its parts and uniformly, then taking one ml of it was added to the test tube containing 9 ml sterile distilled water to get The concentration of 10^{-2} . Then it worked dilution series until reaching dilutiong 10^{-4} and 10^{-5} One ml of each of the two dilutions put in asterile petri dish diameter of 9 cm and then added a 20 ml of medium with chloramphenicol 250 mg/L medium . Repeated three times for each dilution and stirred dishes capstan movement, and left the dishes in order to become solid ,after that incubated in incubator upside down at a temperature of 25 ± 2 ° C for four days. The high frequency fungi were isolated and then purified individually on P.D.A. medium Depending on the importance of fungi , they classified by using the taxonomic keys and with the help of Prof. Dr. Majeed M . Dewan it was then estimate the frequency of isolated fungi ratios according to the following equation :

$$\text{The percentage of frequency (\%)} = \frac{\text{The number of colonies of fungus}}{\text{The total number of colonies}} \times 100$$

(Booth et al., 1988).

Fermentation processes of the diet poultry,by mushroom ostereatus :

The following operations conducted respectively, in the laboratory of Advanced fungi in the Department of Biological Sciences / College of Education / University of Kufa for the period from 01/03- 10/06/2016.The creation of diet poultry growth stage (15-35 day) (Titus and Fritz, 1971 and Ibrahim, 1987 and Fayad and Nagy, 1989), has been taken into account in these diets that adequacy the bird needs of crude protein and energy metabolic ratio energy: protein at every stage and according to the (Ibrahim, 1987), as well as adequacy the bird need of essential amino acids was according to the (NRC, 1994), minerals, vitamins and fatty acid linoleic it according to the (NRC, 1984), it was the amount of metabolic energy calculated to equal the diets exactly as crude protein and energy ratios calculated: protein are equal to a very large extent.

Used method of (Fermentation solid state) in the fermentation of the diet , the process according to the method cited by (Semeniuk et al, 1970), with added water to the diet by 60% to get the moisture required for the development of mushroom oyster *Pleurotus ostreatus* and fungus product poison aflatoxin *Aspergillus flavus* were fill in diet in transparent plastic bags with dimensions 30 × 51 cm where to put 1 kg of diet on the basis of wet weight in each bag closed bags in court and were sterilized by (Autoclave) at a temperature of 121 °C and pressure of 15 pounds / Ang 2 for 20 minutes and after that cooled the bags have been added The vaccine Fungal for both two fungi 5% of the weight culture (Oei, 2005) and in multiple layers (Balakrishnan and Nair, 1995) and then was shut down the barrel of the bag floss tightly. Repeated three times, and then transferred the bags inoculated into the incubator has been installed temperature at 25 ± 2 °C leaving the fungus to grow on a diet for 21,28 days where control mycelium growth speed and biodegradable materials in the diet with a shake sacks process

from time to time to ensure oyster mushrooms distributed uniformly, conducted a chemical analysis of the diet before and after the development of the fungus as it took a diet, and mixed well and the use of electric grinding mill to conduct analyzes on them. **Transactions were as follows:**

T1: control treatment diet sterile

T2: treatment fermentation of the diet to add fungus *Aspergillus flavus* for 21 days

T3: treatment fermentation T2 by oyster mushroom *Pleurotus ostreatus* for 21 days

T4: treatment fermentation T2 by oyster mushroom *Pleurotus ostreatus* for 28 days

Detection of the toxin Aflatoxin and Ochratoxin using the ELISA test

Was added to each sample 1 mL of methanol alcohol and then mix well samples by Vibrating Vortex. It used the standard kit of Neogen Corporation Analytical System for detecting toxin Aflatoxin and Ochratoxin . Developed the kit standard Filed under temperature of 4°C in the laboratory for one hour in order to become a degree lab temperature by the company producing the standard for several instructions have been grinding model are soft which is a diet poultry relay growth by electric grinder and took him 5 grams, was then preparation 70% of alcohol Methanol and took him to 25 ml, were mixing 5 grams of the bush to be estimating the toxins in with 25 ml of alcohol are well were mixed for three minutes to a rocking after which he was nominated by nomination watman type .no.1 to get rid of impurities and to obtain clear solution Paper then take him to 5 ml and then softened with distilled water and 1: 1 ratio is considered at this stage is ready for testing, then add 100 Microlietter of enzyme linked (enzyme

Conjugate) in thickly glass dish and then followed by the addition of 50 microlitter of samples T1, T2, T3, T4 to the canyons and added the same amount of standard solution poison the concentrations 0,5, 15 and 50 mg / l in the side grooves of the

dish and shake the dish helicopter movement of the sample in order to mix with enzyme connectivity solution. Add 100 microlitter antibodies Solution to the canyons and mixed ingredients helicopter movement of the dish and then Vaccinate of the dish for 2 minutes at the laboratory temperature. Neglected components of the dish and then washed gullies using 4- 5 times washing solution and dry the dish so light means the tissue paper to get rid of any trace of the washing solution. Add 100 microlitter initiator of Substrate for each gully solution and incubated for 3 minutes degree laboratory temperature . Add 1 Ayari HCL solution to stop the reaction and then read the results Bakarye ELISA on the wavelength of 650 nanometers and applied the same as the previous steps for the detection of ochratoxin A Ochratoxin cm except that it was used five standard concentrations of the poison is 25,10,5,2,0, as well as the weight of 10 grams of sample to be examined poison her instead of 5 grams and alcohol were prepared 50% instead of 70% and take him 40 ml instead of 25 ml either periods of cuddling is used in the case of monoclonal antibodies and the solution of the initiator 10 minutes, according to the following equation can concentrate the toxin in the sample account tested using a standard curve.

$$\text{Absorption\%} = \frac{\text{Absorption standard solution containing the poison or sample}}{\text{Absorption standard solution of non-Hawi on poison}} \times 100$$

statistical analysis :

The percentage of the appearance and frequency of fungi isolated,toxic and pathogenicity experiments using a randomized complete design data analysis (Completely Randomized Design) according to international experience, two factors, and the averages were compared by the way less significant difference average L.S.D. At the level of probability of 0.05 (the narrator and Khalaf Allah, 2000).

Results and Discussion

The frequency Percentages fungal species isolated from poultry adiet and the ability of oyster mushroom(*P. ostreatus*) on antagonism with it: -

It was isolated and diagnosed the following fungi nine types of fungal samples from poultry diet as showing in table (1) The fungus *Aspergillus flavus* was more frequency,The ratio reached to 22.3%which a higher than all other isolated fungi, and this gives indicator of stronger of the ability of fungus *A. flavus* the presence successfully in such environments, stability and growth and to the face a lot of other microorganisms and estimated the percentage of high frequencies of other species *A. niger* , *Penicillium. sp* ,*A. parasiticus* and *A.terreus* it showed at 15.8 , 16.6 ,11.2 and 9.4%, respectively. It also notes

the presence of some fungi percentage and a low-frequency, showed to 7.7 , 6.9 , 5.8 and 4.3 of the other fungi like *Rhizopus sp* , *Alternaria* , *Fusarium* and *Mucor*. respectively.

Also the results showed the *P. ostreatus* has a high study of the study high the ability to grow against contaminated fungi, ?It covered the full-size of Dish space without allowing the other fungi grow, except the fungus *Fusarium sp* it covered one-third of the dish and mushrooms *P. ostreatus* covered two-thirds of the dish area . These results are in agreement with Kong, (2004) that belonging to the mushroom oyster species *Pleurotus sp* characterized by high viability of colonialism and speed mycellium growth among the different agricultural material and different environmental conditions, and is also consistent with referred to Ahmed, (2015) that the fungus is highly competitive against a number of contaminated mushrooms in a diet .

Table (1) shows the percentage frequency fungi isolated from poultry diets and the degree of antagonism with oyster mushrooms *pleurotus ostreatus*

isolated fungi	% frequency	Antagonism degree
Aspergillus niger	15.8	1
A.terreus	9.4	1
A.flavus	22.3	1
penicillium sp	16.6	1
Aspergillus parasiticus	11.2	1
Mucor sp	4.3	1
Rhizopus sp	7.7	1
Alternaria sp	6.9	1
fusarium sp	5.8	2
L.S.D 0.05	2.53	0.3

The effect of oyster mushroom on diet poultry contaminated sorter fungus toxins *Aspergillus flavus* and recognize the effect of mushrooms to break down Aflatoxin Productive .

showed table (2) susceptibility oyster mushrooms *p.ostreatus* to break down toxins contaminated with aflatoxin to diet in the treatment of T3 where oyster mushrooms used the duration of

fermentation 28 days where the highest percentage bresking down 1.0 mcg/ kg, followed by treatment T4 wher of oyster mushrooms the duration of fermentation 21 days amounted to 1.5 mcg / kg in comparison with untreated T2 oyster mushrooms, which showed the highest contamination rate was 165.3 mcg / kg either the control treatment were free of contamination and mycotoxin which gave 0.0 mcg / kg.

Table (2) Test the effect of oyster mushroomson diet poultry contaminated sorter fungus toxins *Aspergillus flavus* and recognize the effect of mushrooms to break down Aflatoxin Productive

Sample	Description	Optical density	Preliminary Results	Dilution Factor	Final Result
1	0ppb	1.825	0.0	–	–
2	5ppb	1.260	4.7	–	–
3	15ppb	0.712	16.9	–	–
4	50ppb	0.345	47.3	–	–
5	T1	1.595	0.0	1.0	0.0
6	T2	0.117	165.3	1.0	165.3
7	T3	1.664	1.0	1.0	1.0
8	T4	2.023	1.5	1.0	1.5
L.S.D	0.05				1.537

Method :Direct Cometitive , Units :ppb , Corr Coeff :0.996 , Slope :-2.253

Test the effect of oyster mushrooms contaminated feed sorter mushroom toxins *Aspergillus flavus* and recognize the effect of mushrooms to break down ochratoxin Productive

The toxin ochratoxin showed the highest rate of contamination in the treatment of T2, which amounted to 3.0 mcg / kg while she was treating fermentation mushroom oyster T3 and T4 have made clear significant difference of 2.1 mcg / kg and no show significant differences among themselves. As the control treatment T1 achieved less pollution accounted for 1.1 mcg / kg.

Table (3) **Test the effect of oyster mushrooms contaminated feed sorter mushroom toxins *Aspergillus flavus* and recognize the effect of mushrooms to break down ochratoxin Productive**

Sample	Description	Optical density	Preliminary Results	Dilution Factor	Final Result
1	0ppb	2.000	0.0	–	–
2	2ppb	1.383	2.1	–	–
3	5ppb	1.038	5.0	–	–
4	10ppb	0.800	8.9	–	–
5	25ppb	0.421	26.9	–	–
6	T1	1.384	1.1	1.0	1.1
7	T2	1.248	3.0	1.0	3.0
8	T3	1.374	2.1	1.0	2.1
9	T4	1.587	2.1	1.0	2.1
L.S.D 0.05					0.326

Method :Direct Cometitive , Units :ppb , Corr Coeff :0.997 , Slope :-1.917

The contaminated feed and fungal toxins very important problem in the process of raising poultry, where this influence may extend to the threat of production as well as the consumer. And make up to get rid of the fungus found in contaminated feed process extremely difficult obstacle.The protective effect of the fungus *P. ostreatus* against contaminated fungi, and thus control over it and their toxins, in addition to the highly competitive mentioned fungicide against many of the contaminated mushrooms in a diet and as many of the studies the effectiveness of fungus *P. ostreatus* showed the adsorption of many of mycotoxins where he found (Mtunai and Ani 2009) that is very effective mushrooms in shorthand T-2 toxin, one of the most dangerous toxins belonging to the group trichothecens by 68%. The results consistent with (Motomura et al ,2003) to the ability of oyster mushrooms to break down the poison AFB1 It was concluded enzyme from mushrooms poison him smashing the said property.

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