

## Using of Plant Extracts for Cinnamon, Syzygium and Thyme to Degradation of Aflatoxin B1

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### Abstract

This study had done in post graduate studies lab in Dep. OF Biology- College of Science – Diyala University, plant extracts of Cinnamon (*Cinnamomum zylanicum*), Syzygium (*Syzygium aromaticum*) and Thyme (*Thymus vulgaris*) were used with four different extraction methods to show their activities in degradation of aflatoxin B1. The results proved that plant extracts were effecting on AFB1, and showed that all extraction methods were in the same activity on AFB1 at 24 µg/kg concentration. Hexanic extract for Thyme at 250 µg/ml concentration gave's the highest degradation percentage of AFB1 98.7%, followed by alcoholic extract for Syzygium at 500 µg/ml concentration with degradation percentage for toxin 97.5%, cold water extract for Cinnamon at 1200 µg/ml concentration with degradation percentage for toxin 96.9%, alcoholic extract for Thyme at 1000 µg/ml concentration gave's degradation percentage for toxin 93.7%, cold water extract for Thyme at 850 µg/ml concentration with degradation percentage for toxin 84.3%, and the other extracts like hot water extract for Thyme at 1350 µg/ml, alcoholic extract for Cinnamon at 180 µg/ml, cold water extract for Cinnamon at 1250 µg/ml, hot water extract for Syzygium at 1000 µg/ml and hexanic extract for Cinnamon at 110 µg/ml all of them gave degradation percentage 78.7% on AFB1 concentration. So hot water extract for Syzygium at 1000 µg/ml and hexanic extract for Cinnamon at 110 µg/ml concentrations lowered the percentage of AFB1 in 78.1%, and hot water extracts for Thymus at 1250 µg/ml and Syzygium at 1100 µg/ml concentrations lead to lower AFB1 concentration with 77.5%, Hexanic extract for Syzygium at 200 µg/ml and cold water extract for it at 850 µg/ml concentrations led to destroy AFB1 with 75%, The cold water extract for Thymus at 1250 µg/ml and hexanic extract for Syzygium at 250 µg/ml concentrations led to AFB1 degradation with 72.5%. Then both extractions for Cinnamon the hexanic at 100 µg/ml and hot water at 1300 µg/ml concentrations led to AFB1 degradation with 62.5%, 53.75% on respectively, Less degradation AFB1 percentage 12.5% from alcoholic extract for Syzygium at 400 µg/ml. other plant extracts such as hexanic extract for Thymus at 200 µg/ml, cold water extract for Syzygium at 750 µg/ml, alcoholic extract for Cinnamon at 220 µg/ml concentration were not affected by AFB1 concentration.

**Keywords:** Plant extracts, *Aspergillus flavus* fungus and Aflatoxin B1.

### INTRODUCTION

The aflatoxins known as Secondary metabolism Fungal compounds produced by several fungal strains, mostly belonging to the fungus *Aspergillus flavus* and *Aspergillus parasiticus*, both grows on corn grain, Rice, barley, wheat, legumes, and vegetables and fruits (Al-Jenaby, 1998 and Hashim, 1994) and are four types B1, B2, G1 and G2 and the AFB1 considered the most toxic as mutagenic and carcinogenic to humans. medicinal plants considered of the most important strategic materials in the pharmaceutical industry and Al-Saidy et al 2012 and due to the prevalence of the use of these medicinal plants in the Arab world in folk medicine as well as food uses, Given that these plants are non-toxic and safe to use and has no harmful side effects, and economically feasible, So the researchers turned attention to the use of extracts of these plants as anti-pathogenic microorganisms, including fungi and toxins, and among those medicinal plants, medicinal herbs and spices cinnamon, cumin, black pepper, cloves and thymus and other. From the above This study aimed to: Evaluate the effectiveness of extracts of cinnamon, cloves and thymus in degradation AFB1

### MATERIALS AND METHODS

#### Plant extracts

Four types of extracts of cinnamon, thyme and syzygium, a hot and cold water and alcohol and acetone, were prepared and used in this study.

#### Cold water extracts

Following the method of Parekh and Chanda (2007) 10 g of the powdered samples in glass beaker with 100 ml of distilled water, put the flask in an incubator vibrators for 24 hours and at 37 m, then was nominated mix mediated medical gauze in glass tubes, have renounced quickly 5000 r/min. for 10 min., was nominated was nominated output mediated filter paper, the filtrate is evaporated in an oven at a temperature 40 °C for dry powder extract and save that in a dark glass bottle and sealed in frozen (- 20 °C) until use.

### **Hot water extract**

According to the method of El-fallal and El-kattan (1997) 10 g of the powder plant material were mixed with 100 ml of boiled distilled water, put glass beaker in the incubator vibrators in temperature of 28 °C for 30 min., was nominated mix the use of medical gauze, distributed filtrate in glass tubes and have renounced at 3000 r/min. for 10 min., collecting the filtrate in glass dishes (diameter 20 cm) of water and dry it in the oven at a temperature 40 °C until the water evaporates completely, to get a hot water extract powder, which saved the same way above.

### **Alcoholic extracts**

Following the method of Shtayeh and Abo-Shdeib (1999) 10 g of the powder plant materials were mixed with 100 ml of 70% ethanol, put the mixture in the incubator vibrating at a temperature of 35° C for 24 hours, then was nominated mix using the medical gauze, distributed filtrate in glass pipes and quickly have renounced 3000 r/min. for 10 minutes, collecting the filtrate in glass dishes (diameter 20 cm), dry alcohol in the oven at a temperature 40 °C, to get the alcoholic extract powder which saved the same way.

### **Acetone extracts**

Following the method above replacement with ethanol, acetone.

### **Preparation of concentrations of plant extracts**

The concentrations of water extracts of cinnamon, cloves and thyme were prepared by dissolving 10 g of powder plant extract in 30 ml of distilled water, and in 30 ml of Phosphate Buffer Saline (PBS) to hexanic and alcoholic extracts and using the law of general dilution  $C1V1 = C2V2$  and was sterilized by using filters minute (0.22 Micro Metter). The Stolk concentrations 50, 60, 70, 80, 90 and 100µg/ml were prepared To access the Minimum Fungicidal Concentration(MFC) and Minimum Inhibition Concentrations (MIC) and was sterilized by using filters minute (0.22 Micro Metter), and using vacuum pursuer to speed up the nomination process.

### **Susceptibility of *Aspergillus flavus* isolation to produce aflatoxin B1.**

Tested susceptibility the isolation of fungus *Aspergillus flavus* link ex Fires on the production of AFB1 using rice medium to production AFB1, by placing 150 g in a sterile glass dish (diameter 18 cm), the rice wet to 65% using distilled water, sterilized dishes in autoclave at a temperature of 121 °C and pressure of 1.5 kg/cm<sup>2</sup> for 20 min. and twice in two days. Inoculated dishes with disk (6 mm diameter) per dish from farm fungus and incubated at a temperature  $2 \pm 25^{\circ}\text{C}$  for 14 days with a hand-shake dishes for four days to ensure the homogeneity of the inoculum, Dried samples using a Electrothermal thermostatic box under the temperature of 50 °C, and grind in the electric grinder, and save in the frozen (-18°C) until use. AFB1 estimated quantitatively and qualitatively in High-Performance Liquid Chromatography (HPLC), by adding 10 g of powder sample to 30 ml of Acetonitrile . Shake the solution in a shaking ultrasound (Ultra sonic bath) for 30 min., and was nominated by using Whatman filtered paper 0.5 Micro metere to remove the material is not dissolved from the filtrate, then inject 20 micro liters of the filtrate in the column of HPLC for analysis under the terms of separation standard for AFB1, are:

1- separation standard solution, which consists of distilled water, methanol, Acetonitrile (10:40:50) units, respectively .

2 - examination under UV radiation at a wavelength of 365 nm.

3- temperature separation of 25 °C .

4 - Average deportations (Rate Flow) RF 1.0 ml / min.

### **Aflatoxin B1 production from the *Aspergillus flavus* isolation**

The liquid medium of Yeast Extract Sucrose (YES) were used as a good medium for fungal growth and toxin production (Davis et al, 1966).The medium were prepared and distributed in three glass flasks (250 ml) of 100 ml per flask, and sterilized in the autoclave, and flasks inoculated in disk (6 mm diameter) of the fungus farm *A.flavus* developing on the Sabouraud Sucrose Agar (SSA) medium at the age of 7 days. flasks were incubated in the incubator at a temperature  $25 \pm 2^{\circ}\text{C}$  for a period of 14 days, then the contents of the flasks had nominated by using Whatman filter paper (No. 1). Put the filtrate YES in sterile glass flasks 500 mL for the purpose of estimating the amount of AFB1.

### **Treatment the yeast extract sucrose medium contaminated with AFB1 in extracts of cinnamon, cloves and thyme**

Concentrations of plant extracts have been used to kill or inhibition of *A. flavus* fungus that produces AFB1, By putting 5 ml of the YES contaminated in AFB1 in a test tube (20 ml) and treated in concentrations of plant extracts lethal or inhibitory to the growth of fungus *A. flavus* and put the test tubes in the shaker incubator, at a temperature of 25 °C for one hour, dried sample in the dark at 40 °C for two days, then softened at 1:3 with sterilizer distilled water in order to knowing the extent of AFB1 degradation in the sample.

### **Using ELISA technique to estimate the percentage of AFB1 after treatment in extracts of cinnamon, cloves and thyme**

To assess the effectiveness of extracts of cinnamon, cloves and thyme in degradation of AFB1, special Kit were using to measuring the concentration of aflatoxin B1-called AFB1 ELISA Test Kit produced by Shenzhen

Lvshiyuan Biotechnology Co. , Led Chinese 2011 as follows:

1-put the contents of the Kit in the laboratory at a temperature 20-25 °C for 30 min. with shaking Kit containers regularly before use.

2-put a plate special of ELISA Kit in the refrigerator (2-8 °C).

3-saves buffer solution used to wash the Kit at room temperature before use.

4 - Signs were putting on the holes of special and normal plate of Kit ,provided that the signs were putting on the plates in the same place, then 100 µl were putting in signs holes of normal plates for each treatments.

5 - Kit Contains a 6 standard solutions representing a 6 concentrations of AFB1, 0.0, 0.1, 0.3, 0.9, 2.7 and 8.1 mg / kg (ppb), 0.0 ppb concentration represents a higher permeability to light a 100 at a wavelength of 450 nm in Spectrophotometer is a correction factor for the rest of the readings, (Treatments and other standard solutions), 8.1 ppb concentration representing a higher absorbency of light and low permeability of 50 on the 450 nm wavelength in a Spectrophotometer, and the concentration 8.1 ppb is the highest concentration of AFB1 can be read in Kit. Standard solutions used in the formulation of the standard curve, which are installed other readings for the treatments to know the concentration of AFB1. 50 µl from treatments and standard solutions were adding to parameter holes in plate of ELAISA Kit, Then add the solution containing antibodies manufactured specifically for AFB1 in quantity 50 µl per hole, blends the special plate of ELISA Kit manually by moving the plate well, and covered the hole tightly membrane and incubated in the dark at room temperature 25 °C for 30 min.

6 - The holes are washing with a little water after the lifting of the membrane gently then add a 300 µl of special buffer solution of Kit, and the holes were washed with a buffer solution fully 5 times in a row, then flips the plate and placed under it blotting paper in order to drying.

7 - 50 µL of special coloring A solution of Kit were adding for each hole, then added 50µl of coloring solution B of Kit for each hole, too, blends the plate manually and incubated in the dark at room temperature for 15-20 min.

8 - 50 µl of Kit measurement solution were added to each hole in the plate in order to measuring the absorbance in Spectrophotometer and then mixing well manually. Read the absorbance at 450 nm wavelength for determining optical density (OD) passers-by the hole, and is obtained by the concentration of AFB1(ppb) by comparing the absorbance of hole and the correction factor, where the corrected absorbance is measured by the equation:

$$\% \text{ absorbent value} = \frac{B}{B_0} \times 100$$

B: optical density (OD) of the pit samples or standard solutions (reading device).

Bo: optical density (OD) of a standard solution with a concentration 0.0 ppb.

Multiply the result by the inverted dilution of the samples.

#### **Qualitative detection for active compounds in the plant extracts**

##### **Marqus reagent**

Marqus reagent were prepared by Harborn (1984) method , by mixing 1 ml of formaldehyde with 10 mL of sulfuric acid. The positive result is the appearance of turbidity.

##### **Mayer reagent**

Mayer reagent were prepared by the Harborn (1984) method and include :

A- Add 1.36 g of HgCl<sub>2</sub> in 60 ml of distilled water .

B- dissolving 5 g of potassium iodide in 10 ml of distilled water .

Been mixing two solutions (A) and (B) and complete the volume to 100 ml with distilled water. The positive result is the appearance of sediment or turbidity.

##### **Detection of alkaloids**

Alkaloids were detected by using the Marqus and Mayer reagent, by the method of Harborn(1984).

##### **Detection of tannins**

##### **Detect ferric chloride**

Several drops of ferric chloride 1% were added to a test tube containing 0.5 ml of the plant extract. The appearance of a bluish green color were indicating to the presence of tannins (Adeday et al 2001).

##### **Detection of resins**

1 g of dry vegetable powdered were mixing with 10 ml of 95% ethanol , the solution were leaving for 1 min. in a water bath (100 °C), the solution was nominated and added a 10 ml of an aqueous solution of 4% hydrochloric acid , the appearance of turbidity indicates to the presence of resins (Shihata ,1951).

##### **Detection of flavonoids**

1 ml of plant extract were dissolving in 1 ml of sulfuric acid, the appearance of a dark yellow color was indicating to positive detection ( Al-Kazragi, 1991).

## Detection of saponins

### Detection of Mercuric chloride 1%

2 ml of plant extract were added to 2 ml of mercuric chloride 1% (HgCl<sub>2</sub>), the appearance of white sediment indicating to the presence of saponins (Al-Kazragi, 1991).

### Detection Triterpenoids

1ml of sulfuric acid were adding to 1 ml of chloroform, and then the resulting solution was added to 2 ml of the plant extract. The appearance of red or purple indicates to the existence of triterpenoids Harborn(1984).

### Detection of volatile oils

Following the method of Harborn (1984). Taking 10 ml of each extract of plant extracts used experimentally and then marking, the filter paper were satiated in 10 ml of filtrate extracts and exposing to the source of ultra violet light, the appearance of a bright pink color indicates to the volatile oils.

### Statistical Analysis

The data were analyzed by practical experiment using CRD and the significant differences LSD with levels 0.05 and the SPSS program was use to analyze data (Al-Rawi,1984).

## Result and Discussion

### Qualitative detection for active compounds in the plant extracts

The results of the qualitative detection showed active substances in extracts of cinnamon Table 1, the presence of alkaloids, resins, triterpenoids and volatile oils in all extracts of cinnamon, and didn't appear tannins in the hexanic extract of cinnamon and saponins in the hexanic and alcohol extract of cinnamon, while not shown flavonoids and flavonols in all extracts of cinnamon.

**Table 1. Qualitative detection for active compounds in cinnamon extracts**

Active compounds		Qualitative Detection	Extracts			
			Hexanic	Alcoholic	Cold water	Hot water
1	Alkaloids	Marqus reagent	+	+	+	+
		Mayer reagent	+	+	-	-
2	Tannins	Ferric chloride test	-	+	+	+
3	Saponins	Mercuric chloride test	-	-	+	+
4	Resin		+	+	+	+
5	Flavonoids and Flavonol		-	-	-	-
6	Triterpenoids		+	+	+	+
7	Volatile oils		+	+	+	+

The results in Table 2 showed the active substances in the cloves, and the presence of alkaloids, flavonoids, flavonols and triterpenoids in all extracts of cloves, and didn't appear tannins and resins in hot water extract for cloves, and didn't appear volatile oils as well as in the cold water extract cloves, didn't show saponins in all extracts of cloves .

**Table 2. Qualitative detection for active compounds in clove extracts**

Active compounds		Qualitative Detection	Extracts			
			Hexanic	Alcoholic	Cold water	Hot water
1	Alkaloids	Marqus reagent	+	+	+	+
		Mayer reagent	+	+	-	-
2	Tannins	Ferricchloride test	+	+	+	-
3	Sapnins	Mercuric chloride test	-	-	-	-
4	Resin		+	+	+	-
5	Flavonoids and Flavonol		+	+	+	+
6	Triterpenoids		+	+	+	+
7	Volatile oils		+	+	-	+

The extracts of Thyme Table 3 Showed the presence of tannins, flavonoids, flavonols and Triterpenoids in all extracts of thyme, while not shown alkaloids in the cold and hot water extract for thyme and saponins in hexanic extract for thyme, and alcoholic extract for thyme and resins in cold hot water extract and for thyme and volatile oils in the cold water extract of thyme.

**Table 3. Qualitative detection for active compounds in thyme extracts**

Active compounds		Qualitative Detection	Extracts			
			Hexanic	Alcoholic	Cold water	Hot water
1	Alkaloids	Marqus reagent	+	+	-	-
		Mayer reagent	-	-	-	-
2	Tannins	Ferric chloride test	+	+	+	+
3	Sapnins	Mercuric chloride test	-	-	+	+
4	Resin		+	+	-	-
5	Flavonoids and Flavonols		+	+	+	+
6	Triterpenoids		+	+	+	+
7	Volatile oils		+	+	-	+

The qualitative detection results of alkaloids are consistent with that of Farag et al (2006) and Rasooli et al (2009) and Jaber (2006), where is the alkaloids of compounds that don't dissolve in water or dissolve partially but dissolve in hexane and acetone and alcohols, the qualitative detection results of tannin has consistent with that of Denbos (2011) and Al-Sadik (2006), the presence of resins and tannins and saponins and flavonoids in thyme, the reason for the appearance of saponins in water extracts and not appearing in the hexanic extracts and alcoholic are attributable to the usability water solubility and the formation of foam, does not dissolve in hexane or alcohol, and this is consistent with that of Jaber (2006) that the saponins consist of a sugary portion, the part of the foundation in composition and often have sugar glucose, The detection results of flavonoid are consistent with that of Al-Saeed et al (2003) is considered where flavonoids (phenols), active substance that causes the inhibitory effectiveness of thyme and clove. As well as the detection results of resins are consistent with that of Jaber (2006) is considered as resins are oxidation products for different types of essential oils, The detection results for volatile oils are consistent with that of Hussain (1981), the presence of volatile oils in more than 2,000 plants and back to the 60 plant family nearly, where increasing volatile oils in the family Labiatae, including thyme (*Thymus vulgaris*) and the family Lauraceae, including cinnamon (*Cinnamum zeylanicum*) and other of plants, The reason for non-appearance in the cold water extracts, because it does not dissolve in water only a very small proportions (Chandler, 1989).

**The effect of thyme, cloves and cinnamon extracts in Aflatoxin B1**

The results in Table 4 showed that the extracts of thyme influential high degradation in AFB1 in concentration 24 µg / kg, where the hexane extract of thyme gave highest percentage of degradation 98.7% for AFB1 meaning it causes to reduce the quantity of AFB1 of 24 µg / kg to 0.3 µg/kg, Followed by alcoholic extract of thyme 1000 µg/mL, cold water extract of thyme 850 µg / ml, hot water extract of Thyme 1350 µg / mL, hot water extract of Thyme 1250 µg/mL, and the cold water extract of thyme 1250 µg/mL with degradation percentages 93.7, 84.3, 78.7, 77.5 and 72.5%, respectively. The hexanic extract of Thyme 200 µg/mL and alcoholic extract of thyme 500 µg / ml didn't appear any effect on AFB1.

**Table 4. Effect of thyme extracts in degradation AFB1.**

No.	Thyme extracts	Conc. (µg/ml)	AFB1 (µg/Kg)		AFB1 degradation %
1	hexanic extract	200	≤ 24	≤ 24	0.0
2	hexanic extract	250	≤ 24	0.3	98.7
3	alcoholic extract	500	≤ 24	≤ 24	0.0
4	cold water extract	850	≤ 24	3.75	84.3
5	alcoholic extract	1000	≤ 24	1.5	93.7
6	hot water extract	1250	≤ 24	5.4	77.5
7	cold water extract	1250	≤ 24	6.6	72.5
8	hot water extract	1350	≤ 24	5.4	78.7

The extracts of cloves table 5, Showed that the alcoholic extract of clove of 500 µg/ml was given the highest degradation percentage 97.5% meaning it caused a reduction in the quantity AFB1 from 24 µg/kg to 0.06 µg/kg, followed by hot water extract of cloves 1000 µg/mL, hot water extract of cloves 1100 µg/mL, cold water extract of cloves 850 µg/ml, hexanic extract of cloves 200 and 250 µg/ml with degradation percentages 78.5, 77.5, 75.0, 75.0, and 72.5%, respectively. The lowest degradation percentages of AFB1 was in alcoholic extract of cloves 400 µg/ml of 12.5%. cold water extract of cloves 750 µg/ml didn't appear any effect in the degradation of AFB1.



**Table 5. Effect of Syzygium extracts in degradation AFB1.**

No.	Syzygium extracts	Conc. (µg/ml)	AFB1 (µg/Kg)		AFB1 degradation %
1	hexanic extract	200	≤ 24	6.0	75.0
2	hexanic extract	250	≤ 24	6.6	72.5
3	alcoholic extract	400	≤ 24	21.0	12.5
4	alcoholic extract	500	≤ 24	0.6	97.5
5	cold water extract	750	≤ 24	≤ 24	0.0
6	cold water extract	850	≤ 24	6.0	75.0
7	hot water extract	1000	≤ 24	5.25	78.1
8	hot water extract	1100	≤ 24	5.4	77.5

The cinnamon extracts table 6 also showed highly activity in degradation of AFB1, where the cold water extract of cinnamon in concentration 1200µg/ml gives the best degradation percentage 96.9%, meaning it caused a reduction in the quantity AFB1 from 24 µg / kg to 0.75, µg / kg, followed by cold water extract of cinnamon 1250µg/ml, alcoholic extract of cinnamon 180µg/ml, hexanic extract of cinnamon 110µg/ml, hexanic extract of cinnamon 100µg/ml and hot water extract of syzygium 1300µg/ml with degradation percentage 78.7, 78.7, 78.1, 62.5 and 53.75% respectively. The alcoholic extract of cinnamon 220µg/ml and hot water extract of cinnamon 1250µg/ml didn't appear any effect in degradation of AFB1.

**Table 6. Effect of Cinnamon extracts in degradation AFB1.**

No.	Cinnamon extracts	Conc. (µg/ml)	AFB1 (µg/Kg)		AFB1 degradation %
1	hexanic extract	100	≤ 24	9.0	62.5
2	hexanic extract	110	≤ 24	5.25	78.1
3	alcoholic extract	180	≤ 24	5.1	78.7
4	alcoholic extract	220	≤ 24	≤ 24	0.0
5	cold water extract	1200	≤ 24	0.75	96.9
6	hot water extract	1250	≤ 24	≤ 24	0.0
7	cold water extract	1250	≤ 24	5.1	78.7
8	hot water extract	1300	≤ 24	11.1	53.75

And the efficiency of these plant extracts were attributed to it containing of flavonoids and volatile oils, as limited studies have shown effectiveness of these compounds in the inhibition or degradation AFB1 in two different ways: First reduce the correlation of AFB1 with DNA as it is characterized by AFB1 high correlation with DNA molecular C≡G especially, when it removes the nitrogen base G and replace, As flavonoids with volatile oils lead to weaken the ability of correlation AFB1 DNA, and the second, the volatile oils correlation with the free radicals molecular of AFB1, which lead to reduced effectiveness of the AFB1 toxicity and protect the living cell from the toxic effects of the AFB1, and this can be seen as flavonoids and volatile oils antioxidants and thus inhibitors of cancer within a living cell (Harbon, 1984 and Alpsoy, 2010), and these results excels on the findings of the Al-wershan (1999) reductase AFB1 in concentration 127.93 µg/kg in the percentage 87.83% by using adsorption materials such as clay, kaolin, Gypsum, crushed stone, peat moss and ash, And the results excels as well as with that of Mughallis (2001), which stated that the reduction AFB1 in concentration 2.413 µg/g with inhibition percentage 92.07% increased when using adsorption bentonite material, and these results excels as well as on the findings of Majeed (1997); Al-Saidy (2004); Al-Nazzal (2004) reductase AFB1 concentrating 20.176 µg/kg and 4.7 µg/g and 6.5 µg/g and 100% respectively by using urea, but there were large significant differences between these treatments and treatment comparison, and these differences attributed to urea that making the feedstuff unpalatable by broilers and affect the health of the animal because of the excess residues on the specified concentration of ammonia in the feedstuff (Hashim et al, 1994). There was an increase in water consumption and a significant reduction in the weights of the broilers in this treatment compared to the treatment of comparison as the surplus of inorganic compounds with a negative effect on the chicks and reduces the consumption of food in spite of the efficiency of urea in degradation of mycotoxins (Al-Saidy, 2004).

## REFERENCES

- Adeday, O., W. Aderson, M. Young, V. Snickus, P. Patil, and Kolawole, D. 2001. Photochemistry and antibacterial activity of sennalota flower pharmuct. Biol.39:1-5.
- Al-Jenabi, S.J. 1998, The effect of some food preservatives in the growth of fungus *Aspergillus flavus* Link ex Fries, production of aflatoxins in the flour. M. Sc. thesis. College of Education, Ibn Al Haytham. University of Baghdad.
- Al-Nazzal, A. I. A. 2004. Methods of degradation ochratoxin -A in maize and its effect On broiler chickens. PhD thesis. College of Agriculture. University of Baghdad.
- Alpsoy, L. A., 2010. Inhibitory of essential oil on Aflatoxin Activities. Dep. Biology, Colloge of Art and Sci. Fatih Universty, *African J. of Bio. Technol.* 9(17): 2474-2481.

- AL-Khazragi , S.M. 1991. Biopharmacological study of *Artemisia herbalba* MSc. Thesis. Univ. Baghdad.
- Al-Saeed, M. S., M. A. A.Yahya, M. E. H. Agaand A.N. Omarin. 2003. Pharmacology for Teresa and Evans. Damascus.
- AL- Sadik, S.M.2006.Effect of Some Active Components Extracted from Lemon grass (*Cymbopogon citratus*) and Thyme (*Thymus vulgaris*) in Candida species and Bacteria Isolated from Children mouths with Oral Thrush. M.Sc. thesis.College of Science. University.of Baghdad.
- Al-Saidy,A.M., N.G. Al-Zebedee and I.K.M. Danbuss. 2012. Inhibition Activity of aplant Extract *Thymus vulgaris* and *Mentha piperita* against *Candida albicans* .J.of Diyala Agri. Sci. 4 (1).
- Al-Rawi, k.S.1984. Vital Statistics, Univ. of Mosul, the Press, the Ministry of Higher Education Scientific Research.
- Al-Werchan, S. H. S. 1999. Using some absorption chemicals to reduce aflatoxin contamination from poultry feedstuff. M. Sc. College of Agriculture University of Baghdad.
- Barkia-Golan ,R. and N. Paster. 2008. Mouldy fruits and Vegetables as a source of mycotoxins the Volcanicenter. Agricultural research organization.Bet Dagan 50250.Israel.
- Davis,N.D.,U.L.Dienerd and D.W.Eldridage.1966. production of aflatoxin B1 and G1 by *Aspergillus flafus* in semi synthetic medium. *Appl.Microbiol.*14:378
- El-Fallal, A. A. and M. H. El-Kattan. 1997. Effect of plant Extracts on the mycelial growth of some cultivated Mushrooms.*Egypt. J.Microbiol.* 32(1):41-48.
- Harborn , J. B. 1984. Phytochemical Methods, Champon and Hall London. (2<sup>nd</sup>).New York.
- Hashim , S., VS. Aboobker , Madhubala R1 RK. Bhattacharya and AR. Rao . 1994. Modulatory Effects of essential oil from spices on the Formation of DNA adducts by aflatoxin B1 *in vitro*.*Nutr.Cancer*,21:169-175
- Huing ,A.; S. Freimund, O. Kappeli and H. Dulter. 2001.Mycotoxin detoxification of animal feed by differentAdsorbents.*Toxicol.Lett.*122:179-188.
- Jaber, H. A. K. 2006. Medicinal plants and plant chemistry. Mars Publishing House. Riyadh. Saudi Arabia.
- Majeed, M. A.1997. Study the effect of urea on the *Aspergillus flavus* fungus and aflatoxin B1 In fooder blocks, M.Sc. thesis. College of Agriculture. University of Baghdad.
- Mughallis, M. A. A. 2001 Modeling of rates of clay minerals added for adsorption of Aflatoxin B1 from poultry feedstuff and nutritional values .M.Sc. thesis. College of Agriculture University of Baghdad.
- Parekh , J. and S. Chanda , 2007. In vitro antimicrobial activity Phytochemical analysis of some india medical plant.*Turk.J. Biol.* 31:53-58.
- Rassoli, I., M. H. Fakoor, A. A. Allameh,M. B. Rezace and P. owlia. 2009. Phytoprevention of aflatoxin production. *J. Med. Plants*, 8(5): 97 – 104.
- Shtayeh , M.S.A. and S.I. Abu-Ghdeib , 1999. Antifungal Activity of plant extract againt dermatophytes.*J.Mycoses.*, 42:665-672 Shihata , I.M.1951.A pharmacological study of *Anagallis arvensis* M.D.Vet.Thesis. Cairo Univ.

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