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The Prevalence of fungi in spices and study the effectiveness of some antimycotics antioxidants in elimination of these fungi and its toxins in vitro and in vivo

Nahed, M. El-Mokhtar[*] ,Nashwa A.H.Ahmedand S. EL-Araby Kammal[**]**

Departments of Mycology[*] and Biochemistry** Dep., Animal Health Research Institute, Dokki.

Abstract

The purpose of the present study is to assess fungi and Aflatoxin content in different spices present in local markets in Egypt and test herbal and chemical materials that have antimycotic antioxidant properties to eliminate or ameliorate these fungi and its toxins in vitro and in vivo. This study compromises three parts. In part I :samples of spices ; black pepper, dry ginger, cumin ,coriander (corundum), red chilly (pepper) and curcumin were collected randomly (20 of each) from local markets and super markets at Cairo governorate for investigation of fungal contamination and detection of aflatoxin . The results indicated that the most prevalent fungi were belonging to genus Aspergillus which was recovered from all samples of cumin and corundum (coriander) (100 %), black pepper and curcumin samples (90%), dry ginger samples (75%) and red chilly (pepper) samples (70%).Other genera of fungi were also recovered in lower rates of frequency namely Penicillium, Scopulariopsis , Mucor ,Cladosporium ,Candida and Rhizopus species. The fungus of *Aspergillus flavus* was recovered from samples of cumin and coriander and produced aflatoxins. The maximum levels of toxin were obtained from *A. flavus* isolated from Cumin (90%) of isolates produced mean level of (4.85±2.35ppb) followed by those isolated from Coriander (corundum) (70%) with the mean level of (2.89±2.21 ppb).The laboratory findings for inhibition of aflatoxinogenic *A.flavus* (part II) showed that zone of inhibition caused by sorbic and benzoic acids, rosemary and thyme were (12.9 - 28.4, 6.9 -30.6, 16.7 - 21.2 and 3.9 - 27.4 mm , at concentrations of 0.25% and 1.0% ,respectively for *A. flavus*).On the other hand ,the in vivo application of laboratory findings in rats using thyme,rosemary,sorbic and benzoic acids to ameliorate aflatoxicosis were undertaken(part III). One hundred rats (150-170 g) were divided into 10 equal groups. Rats of the first group were given healthy commercial pelleted basal diet and kept as a negative control. Rats of groups 2, 4, 6,8and 10 were injected intraperitoneal with a single dose of AFB1 1.5 mg/kg body weight **.**Then on the second day the diet of rats were supplemented with 5% commercial thyme powder for groups 3 & 4, 2.5 % commercial rosemary powder for groups 5&6, (2 % sorbic acid for groups 7&8) and (2% benzoic acid for groups 9&10).The period of feeding was continued for 4 weeks. The biochemical investigation of sera of the aflatoxicated group 2 showed a significant increase in ALT and no changes of AST, urea,

creatinine, TAC,T3 and T_4.Rosemary powder,sorbic and benzoic acids exhibited a hepatoprotective effect .Herbal materials improved urea and T_3(active form of thyroid hormones) levels.Sorbic and benzoic acids affect negatively TAC and may it correlated with elevation in T_4 . Aforementiond results showed that most prevalent fungi were belonging to genus Aspergillus in spices marketed in Egypt. *Aspergillus flavus* was the most predominant member.Though hepatoprotective effect of antimycotics antioxidants studied, more studies recommended on pure extracts and different doses of thyme and rosemary to exert more benefits.

Introduction

Traditionally, spices and herbs are valued for their distinctive flavors, colors and aromas and are the most versatile substances widely used all over the world **(Hashem and Alamri, 2010).**

Fungi are the predominant contaminants of spices but most such microbial populations are probably regarded as commensally residents on the plant. Soil and air are the main inoculums sources for causing contamination in crude spices in field. Other practices like harvesting, handling and packing cause additional contamination. Moreover, spices are collected in tropical areas by simple methods and are commonly exposed to many contaminants before, being enough to prevent microbial growth. They are also stored in conditions favoring contamination by insects, rodents, and other vermin **(Arshad et al., 2012)**. The most frequent fungal contaminants of spices are species from the genera Aspergillus and Penicillium **(Kocic´ -Tanackov et al.,2007).**

Aflatoxins are the most important mycotoxins, recognized as ubiquitous contaminants of food throughout the developing world **(Kamkar et al., 2013)**.The major aflatoxins are AFB1, AFB2, AFG1, AFG2 and two more additional metabolic products, M1 and M2 **(Samuel et al.,2013)**.Among them, aflatoxin B1 (AFB1) is the most potent cause of human carcinogen; hence, the International Agency for Research on Cancer (IARC) classified AFB1 into a primary group of carcinogenic compounds **(Reddy et al., 2009b and Tavakoli et al.,2013)**. At least 100 countries have regulations to control major mycotoxins, especially aflatoxins, in commodities and food, so that the maximum tolerable mycotoxins levels vary greatly among the countries **(Reddy et al., 2009a).** European Union has established the maximum tolerable limits for AFs in spices as 10 μg/kg for total aflatoxins (B1 + B2 + G1 + G2) and 5 μg/kg for AFB1 **(Commission Regulation, 2002).**

A variety of data suggests a role for oxidative stress, including lipid peroxidation, in the pathogenesis of aflatoxicosis **(Abdel- Wahhab et al., 2006 & Umarani et al., 2008).** Chemoprevention of toxicoses and/or cancer using nutrients is the subject of intense study. Among the many compounds examined, antioxidants are being

investigated because of their ability to reduce disease formation by either induction or inhibition of key enzyme systems **(Guarisco et al., 2008)**.

In oxidation,electrons are transferred from substance to oxidizing agent which leads to production of free radicals and initiates chain reaction. Antioxidants are the agents capable of slowing or preventing non-enzymatic oxidation. Phenolic acids and their derivatives like 3,4,5-trihydroxy benzoic acid, Propyl 3,4,5-trihydroxybenzoate, Octyl 3,4,5-trihydroxybenzoate, 2,4-dihydroxy benzoic acid are known to possess antioxidant capacity and are used in food products to scavenge reactive oxygen species ROS **(Katti and Ranjekar,2014)**. Sorbic acid may act as chemical antioxidants detoxifying ROS by suppressing effects mediated by interferon- γ(IFN-γ) and on the other hand, they may also reduce the formation of ROS **(Winkler et al .,2006).**

Rosemarinus officinalis L. an evergreen perennial aromatic shrub belonging to the family *Labiatae*, commonly called Rosemary, native to the north and south coasts of the Mediterranean Sea and is a common house hold plant **(Al-Sereiti et al., 1999)** . Rosemary is commonly used as a spice and flavoring agent in food processing **(Saito et al., 2004).** It is composed of dried leaves and flowers contain some antioxidant phenolics that have been shown to provide a defense against oxidative stress from oxidizing agents and free radicals **(Matkowski, 2006)**. The antioxidant potential of rosemary and its constituents has predominantly been derived from *in vitro* and *in vivo* studies **(Saber and Hawazen, 2012).**

Thymus vulgaris L. is a perennial herb indigenous in central and southern Europe, Africa and Asia that are rich in essential oils and antioxidative phenolic substances **(WHO, 1999)**. It is widely used in folk medicine for the treatment of a variety of diseases including gastroenteric, bronchopulmonary disorders, anthelmintic, antispasmodic , carminative, sedative and diaphoretic **(Rustaiyan et al., 2000).** It has been reported that thyme possesses numerous biological activities including, antimicrobial **(Marino et al., 1999)**, antioxidant **(Miura et al., 2002)** and antifungal **(Pina-Vaz et al., 2004).**

The spices and herbs are basic supplements in daily food of most people, even infants, some infant feeding specialists recommended to give them daily boiled herbs (cumin, anise….etc.). The purpose of the present study is to assess fungi and Aflatoxin content in different spices. The information will be helpful for higher authorities to establish regulations and safe limits for this toxin present in spices. This study is aimed at :(1) estimating the prevalence of fungi in spices of Egyptian markets and (2) studying the effect of some antimycotic antioxidant compounds, natural (thyme and rosemary) and chemical(sorbic and benzoic acids) to eliminate or ameliorate fungi and its toxins in vitro and in vivo.

Materials and Methods

This study compromises three parts:

1-Part I :Estimation of the prevalence of fungi in spices of some Egyptian markets.

-Collection of samples

Samples of spices ; black pepper, dry ginger, cumin coriander (corundum), red chilly (pepper) and curcumin were collected randomly (20 of each) from local markets and super markets at Cairo governorate for investigation of fungal contamination and detection of aflatoxin . The collected samples were put into sealed plastic bags and brought to the laboratory and stored in refrigerator until analysis.

-Isolation and identification of fungi:

Total fungal count was carried out according to the techniques recommended by **ISO (217-1-2:2008).** Isolated fungi were further identified according to macro and microscopic characteristics as described by **Pitt and Hocking (2009)**.

-Cultivation , extraction and estimation of aflatoxins:

Production and estimation of aflatoxins from isolated strains of *A.flavus* were carried out**.** Isolated strains of *Aspergillus flavus* from the collected samples were inoculated into flasks containing 50 ml of sterile yeast extract solution 2% containing 20% Sucrose (YES). The inoculated flasks were incubated at 25ºC for 7-10 days. At the end of the incubation period, extraction and purification of produced aflatoxins using immunoaffinity column and quantitatively estimated by fluorometric method according to **(AOAC, 1990) and (Hansen, 1993).**

2-Part II: Determination of the efficacy of different antifungus antioxidants as mould inhibitors

-Preparation of spore suspension of A. flavus according to Gupta and kohli (2003). The effect of chemical and natural herbs antimycotics antioxidants as mould inhibitors against fungal isolates was determined by disc diffusion technique **(Nakashima et al., 2002)**: A filter paper discs of 0.6 cm diameter were impregnated for 10 minutes with different concentrations of the tested mould inhibitor (sorbic acid,benzoic acid, thyme and rosemary **(**2.0, 1.0, 0.50 and 0.25µg).The prepared discs were dried by heating at 40-50 ºC for one hour. One ml of spore suspension (10^5/ml) was added to sterile plates and over layered with SDA. The plates were rotated to mix the content and allowed to solidify at room temperature. On the surface of plates the prepared paper discs of the tested chemicals were pressed firmly to be in complete contact with the agar. The discs were distributed evenly in a manner that not to be closer to each other, 15mm from edges of dishes, 20 mm between each 2 discs and 24mm from center of plates.Then incubated at 25ºC for 2-5 days. At the end of incubation period, the sensitivity of

fungi to the tested drug was determined by measuring the area of the growth inhibition zone (mm).

3-Part III: studying the role of different antimycotics antioxidants to ameliorate aflatoxicosis in vivo.

Experimental animals:

One hundred apparently healthy albino rats weighted (150-170 g) were housed under hygienic conventional conditions in suspended stainless steel cages. Prior to experiment; rats fed on healthy commercial pelleted basal diet free from any cause of disease. Drinking water was supplied in glass bottles, *ad libitum*.

Experimental Design

One hundred rats were divided into 10 equal groups. Rats of the first group were given healthy commercial pelleted basal diet and kept as a negative control. Rats of groups 2, 4, 6,8and 10 were injected intraperitoneal with AFB1, 1.5 mg/kg body weight freshly prepared in dimethyl sulphoxide **(Bao, 2002)**. Then on the second day the diet of rats were supplemented with 5% commercial thyme leaves powder **(Al Badr, 2011)** for groups 3 & 4, 2.5 % commercial rosemary leaves powder (**Abd El-Ghany et al.,2012)** for groups 5&6 , (2 % sorbic acid for groups 7&8) and (2% benzoic acid for groups 9&10). The second group was left without any treatment and kept as positive control. The period of feeding was continued for 4 weeks**.**

Blood samples:

At the end of the experiment the animals were fasted for 12 hr. Blood samples were collected from the retro-orbital venous plexus from each animal under ether anesthesia. Blood samples were left to clot and the sera were separated using cooling centrifugation at 3000 rpm for 15 min and stored at -20ºC until analysis .The sera were used for the determination of alanine transaminase (ALT), aspartate transaminase (AST),urea and creatinine by using kits purchased from Randex Laboratories (San Francisco, CA, USA). . Thyroid hormones; triiodothyronine (T3), thyroxine (T4)&thyroid stimulating hormone (TSH) were determined by using kits purchased from Immunospec Corporation and total antioxidant capacity (TAC) kit was purchased from Biodiagnostic Co. (Cairo, Egypt) and determined according to the kits instructions. After collection of blood samples, animals of groups 1,2,4&6 were killed by cervical dislocation and samples of liver and kidneys preserved for aflatoxin residue estimation.

Statistical analysis:

Data obtained were statistically analyzed using analysis of variance (ANOVA) using F- test according to **SPSS-18 (2009).**

Results and discussion

Moulds are considered as one of the indicators for hygienic status of food and food premises. The good quality of food depends greatly on the quality of fresh food and the environment. The current results in Table (1) indicated that the most prevalent fungi were belonging to the members of genus Aspergillus which were recovered in the same percent from cumin, corundum (coriander) samples (100 %), black pepper and curcumin samples (90%), dry ginger samples (75%) and red chilly (pepper) samples (70%). The genus of Penicillium was recovered from (50%) of black pepper and corundum (coriander) samples .But (25%) from dry ginger samples and red chilly (pepper) samples. Followed by Scopulariopsis and Mucor species, red chilly (pepper) samples (50%) and the same percent of Cladosporium species were recovered in cumin. Candida species were isolated from dry ginger samples (40%) followed by curcumin samples (25%). Rhizopus species were isolated at relatively lower frequency from black pepper samples (25%) and then (20%) from cumin samples. The previous studies revealed that *Aspergillus* species, *Penicillium* species and yeasts were the most common fungi present in spices **(Taniwaki and Dender, 1992 and Arshad et al., 2012)**.

The isolation of such fungi in the present samples may be due to their exposure to environmental condition as high temperatures and humidity during harvesting, transportation, handling, processing and/or storage which lead to fungal pollution by different genera of fungi such reports were previously published before by many authors as **Hassan and Omran (1996) and Hassan et al. (2009).**

The obtained data in (Table, 2) showed that members of Aspergillus were isolated in various frequencies. *Aspergillus flavus* was the most predominant member of *Aspergillus species* that recovered from samples of cumin and coriander (corundum) (100 %).Dry ginger samples (75%) followed by black pepper and red chilly (pepper) (50%) and then curcumin samples (25%). Followed by *A. niger* which was recovered at the rates of (100%, 90%, 40% and 15%), respectively. Other members of *Aspergillus* were isolated at relatively lower frequency **(Sampayo *et al.*, 1995; Aly, 1999 and Hassan et al., 2010b).**

The current results in (Table, 3) showed that total count of members *Aspergillus flavus* was that recovered from samples of Cumin with the mean count of (**$5.0\times10^3\pm2.0\times10^3$**). Other total count of members of *Aspergillus flavus* were in significant various frequencies, total count of members *Aspergillus flavus* was that recovered from samples of Dry ginger and Curcumin samples **($2.10\times10^4\pm1.0\times10^4$)** .Followed by Black pepper (**$2.0x10\pm1.0x10^2$**). Then (**$2.0x10^3\pm1.0x10^2$)** total count of Red

chilly (pepper) and Coriander (corundum) with the mean count of(**$1.0x10^2$±0.5x10**) .The similar results were previously reported by **Wafia and Hassan(2000).**

Significant levels of aflatoxin were produced by *A. flavus* isolated from collected samples (Table, 4). Where, the maximum levels of toxin were obtained from *A. flavus* isolated from Cumin (90%) of isolates produced mean level of (4.85±2.35ppb) followed by those isolated from Coriander (corundum) (70%) with the mean level of (2.89±2.21 ppb). However samples of black pepper are recorded (50%) of isolates produced mean level of AF (1.0±0.1 ppb) ,but dry ginger and red chilly(pepper) are recorded the same percent (40%) with a different levels of (0.55±0.2 ppb) and (0.17±0.09 ppb) ,respectively .Strain of A. flavus isolated from curcumin samples were found to be non-toxigenic in agreement with **Hassan and Hamad (2001).** These mycotoxins were proved to be etiological agents in some outbreaks of food-born diseases of human and animals and induced haemorrhage, hepatotoxic, nephrotoxic, neurotoxic, dermatotoxic, genotoxic, teratotoxic, mutagenic, carcinogenic or have hormonal effects and immunosuppression **(Andrew and Christopher, 1994; Smith et al., 1994 ; Hassan, 2003 and Hassan et al.,2009).**

The chemical and natural antimycotics antioxidans showed that the areas of inhibition zones were increased when the concentration of antimycotics was elevated. The obtained fresh cultures of the common fungal isolates used for the evaluation of some antifungals by determination of disc diffusion technique (DDT). The minimal inhibition concentration can be used to estimate the most economical and efficient application doses of antifungals or disinfectant to disinfect in animate objects. As shown in table(5), zone of inhibition caused by sorbic acid ,benzoic acid, rosmary and thyme were (3.2-13.0 ,3.9-11.2 , 11.9-18.9 and 3.9-13.6 mm ,respectively) at concentration of 0.25 µg/ml to (19.7-50.4, 19.9-30.6 , 16.4-40.5 and 24.7-33.6, respectively) at concentration of 1.0µg/ml. The present findings came in accordance with the findings (**Nahed et al.,2007 and Hassan et al., 2009**).

It is interesting to report here that the aflatoxicated rats that treated with rosemary and Thyme (Table,6) showed a significant diminution the levels of aflatoxins residues in kidney and liver organs. Whereas, nearly degradation of aflatoxin residues was detected in rosmary than thyme. Similar results were obtained by **(Awad et al., 2011)** who detected that the treatment of aflatoxicated rats with herbal extracts resulted in a significant degradation of aflatoxins from vital organs particularly from liver and kidney in these aflatoxicated rats that treated with rosemary.

Table(7) shows the effect of different antimycotics antioxidants (natural and chemical) on the activities of some serum biochemical parameters (liver &kidney

functions),total antioxidant capacity (TAC) and thyroid hormones (triiodothyronine "T3"thyroxine "T_4" & thyroid stimulating hormone "TSH") of rats treated with AFB1 in a dose of 1.5 mg/kg body weight freshly prepared in dimethyl sulphoxide (single intraperitoneal dose).

The liver functions were examined through the determination of alanine aminotransferase (ALT) and aspartate amino transferase (AST) activities which known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage, therefore both enzymes are used as indicator for hepatic damage **(Andallu and Vardacharyulu,2001)**. Whereas increased levels of urea and creatinine may indicate protein catabolism and/or renal dysfunction **(Abdel-Fattah *et al.*, 2010).**

Data shows a significant increase in ALT due to aflatoxin injection and insignificant change in AST compared to control. Urea and creatinine levels were not affected by AFB1. Elevation of ALT was in accordance with that reported by (**Hamzawy et al.,2012** and **Abdel- Fattah et al.,2014)**.Several studies on the mechanisms of aflatoxins induced liver injury have demonstrated that in animals fed diets contaminated with toxicants, the serum levels of these enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum **(Sherif et al.,2009).**Unexpected , in present study there were no effect of aflatoxin on AST,urea and creatinine in disagreement with **Hamzawy et al.(2012)** and **Abdel- Fattah et al.(2014)**.On the other hand, these results are in concomitant with **Barton et al.(2000)** as they reported that a dose of 1 mg AFB1 / Kg resulted in no elevation in serum ALT or AST and little or no histologic change. These findings may be proved by observations of **U I healthcare (2015)** who recorded that the liver is able to replace damaged tissue with new cells. If up to 50 - 60 percent of the liver cells may be killed within 3 - 4 days in an extreme case like a Tylenol overdose, the liver will repair completely after 30 days if no complications arise. This may explain a slight increase in ALT and no effect on AST comparing to control group.

In recent years, the possible correlation between impaired thyroid gland function and reactive oxygen species has been increasingly taken into consideration **(Vitale et al., 2000).** Obtained data revealed no significant effect of aflatoxin injection on thyroid hormones. Insignificant effect of AFB1 on T_3& T_4 is disagreed with statistically significant decrease were observed in the levels of blood T3 and T4 reported by **Salem and Selim(1994)** , **Eraslan et al.(2005)** & **Hassan et al. (2010a)** .There is a significant decrease in TSH which may be due to pituitary disorder.

Total antioxidant capacity may provide more relevant biological information compared with that obtained by measurement of its individual components, because it considers the cumulative effect of all antioxidants present in plasma and body fluids **(Ghiselli et al., 2000)**. No significant changes due to AFB1 injection.These results were different at all from those previously recorded by **El-Kady et al. (2010)** in serum and **El-Nekeety et al. (2011)** &**Hassan et al. (2013)** in liver tissues) which proved a significant decrease in total antioxidant capacity due to aflatoxicosis.This finding though it is against the proposal but it is agreed with liver and kidney markers and supports aflatoxicosis dose dependence **(Barton et al., 2000)**.

In this study data revealed that commercial thyme leaves powder supplemented to the basal diet of rat has no significant effect on liver function ,urea and total antioxidant capacity but showed a bad significant increase in creatinine level and favour thyroid activity comparing to the control group. These findings approaches that reported by **(AL Badr ,2011**) concerning thyme powder while the same author reported a significant effect of thyme extract on total antioxidant status in concomitant with **Vitaglione et al.,2004 and Kruma et al.,2008 .**Intoxicated rats fed on thyme powder supplemented diet exhibited significant improvement in kidney function and T_3 level that may be agreed with ethanolic extract of thyme which exhibited hepatorenoprotective properties against aflatoxin in a dose dependant manner due to its antioxidant ,free radical scavenging activity and antiinflamatory properties**(EL Nekeety et al.,2011,Abdel Kader and Mohamed,2012 and Hamzawy et al.,2012).**

Rosemary leaves gave a significant favour effect on ALT and no effect on AST, urea,thyroid hormones and TAC comparing control group, while, negatively affected creatinine level. Aflatoxicated rats fed on diet supplemented with 2.5% rosemary powder had a favour decrease in ALT and no effect on AST, urea, T3, T4 .A bad effect on creatinine and TAC and an increase in TSH level. Although decrease in TAC, the favour effects are in concomitant with the ameliorating effect of rosemary on injured liver that concluded by **Abd El-Ghany et al.(2012).**The ameliorative effect of rosemary extract may be due to its antioxidant properties in combating free radical-induced oxidative stress and tissue injury **(Sakr et al.,2015).**

Concerning the effect of chemical antimycotics antioxidants (sorbic and benzoic acids) had the same pattern either in aflatoxicated rats or non-toxicated rats compared with aflatoxin group or control group respectively. Both improved ALT, AST and urea .No effect on active form of thyroid hormones (T_3).Bad effect on total antioxidant capacity and T_4.These results may be in parallel with studies that showed no histological abnormalities in internal organs due to small doses (1.5 %) sorbic acid and (16 -1090 mg/kg /day over a 30-day period)sodium benzoate (**FAO, 1974**) but

disagreed with **(Daoud and Griffin,1980)** who reported that sorbic acid exerted no protective effect against hepatocarcinogenesis .Benzoic and Sorbic acids may act as chemical antioxidants detoxifying reactive oxygen species ROS by suppressing effects mediated by IFN-γ, and on the other hand, they may also reduce the formation of ROS. **(Murr et al., 2005).** Elevation in thyroxine may be contributed to decrease in TAC because the synthesis of thyroid hormones crucially depends on H_2O_2, which works as a donor of oxidative equivalents for thyroperoxidase **(Corvilain et al., 1991).** Regarding the way in which thyroid gland hyperfunction influences antioxidant defence capacity, the organism can defend itself against the effects of oxidative stress by increasing superoxide dismutase SOD activity as a protection mechanism, but **Petrulea et al., 2012** observed a decreased SOD activityfollowing L-thyroxine treatment**.**

Aforementiond results showed that the most prevalent fungus was genus Aspergillus in spices marketed in Egypt. *Aspergillus flavus* was the most predominant member.Though hepatoprotective effect of antimycotics antioxidants studied, more studies recommended on pure extracts and different doses of thyme and rosemary to exert more benefits.

Table(1) Frequency of isolated fungi in spices examined samples:

Genera of isolated fungi	**Types of examined samples**											
	Black pepper (20)		**Dry ginger** (20)		**Cumin** (20)		**(corundum coriander)** (20)		**Red chilly (pepper)** (20)		**curcumin** (20)	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Aspergillus species	**18**	**90**	**15**	**75**	**20**	**100**	**20**	**100**	**14**	**70**	**18**	**90**
Penicillum species	**10**	**50**	**5**	**25**	**-**	**-**	**10**	**50**	**5**	**25**	**-**	**-**
Rhizopus species	**5**	**25**	**-**	**-**	**4**	**20**	**-**	**-**	**-**	**-**	**-**	**-**
Scopulariopsis species	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**10**	**50**	**-**	**-**
Mucor species	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**10**	**50**	**-**	**-**
Cladosporium species	**-**	**-**	**-**	**-**	**10**	**50**	**-**	**-**	**-**	**-**	**-**	**-**
Candida species	**-**	**-**	**8**	**40**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**25**

Table (2): Incidence of Aspergillus species isolated from examined samples

Genera of isolated fungi	Prevalence of *Aspergllus* spices											
	Black pepper (20)		Dry ginger (20)		Cumin (20)		Coriander (corundum) (20)		Red chilly (pepper) (20)		Curcumin (20)	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
A.flavus	10	50	15	75	20	100	20	100	10	50	5	25
A.niger	20	100	-	-	18	90	8	40	-	-	3	15
A.fumigatus	-	-	-	-	5	25	-	-	-	-	-	-
A.terreus	10	50	-	-	-	-	-	-	-	-	-	-
A.candidus	5	25	-	-	14	70	-	-	-	-	-	-

Table (3): *A.flavus* count in examined samples

Types of samples	No. of Examined Samples	+ve samples		Total mould count (c.f.u. /g)		
		No	%	Max	Min	Mean± SE
Black pepper	20	10	50	$1.0x10^3$	1.0x10	$2.0x10\pm 1.0x10^2$
Dry ginger	20	15	75	$2.5x10^5$	$2.0x10^2$	$2.1x10^4\pm1.0x10^4$
Cumin	20	20	100	$4.8x10^4$	5.0x10	$5.0x10^3\pm2.0x10^3$
Coriander (corundum)	20	20	100	4.0x10	$2.0x10^2$	$1.0x10^2\pm0.5x10$
Red chilly (pepper)	20	10	50	$1.4x10^5$	$1.0x10^2$	$2.0x10^3\pm1.0x10^2$
Curcumin	20	5	25	$2.5x10^5$	$2.0x10^2$	$2.1x10^4\pm1.0x10^4$

Table (4): Levels of aflatoxins production by *A. flavus* isolated from samples

Type of samples	No. of tested isolates	+ ve samples		Levels of AF ppb		
		No.	%	Max	Min	Mean ± SE
Black pepper	10	5	50	2.0	1.00	1.00±0.1
Dry ginger	15	6	40	2.50	1.80	0.55±0. 2
Cumin	20	18	90	7.20	2.50	4.85±2.35
Coriander(corundun	20	14	70	5.17	0.98	2.89±2.21
Red chilly(pepper)	10	4	40	2.50	1.00	0.17±0.09
curcumin	20	0	0	0.00	0.00	0.00±0.00

Table (5): Influence of fungal growth by different doses of sorbic acid, benzoic acid,rosemary and thyme :

Fungal Isolates	Zone of inhibition to different concentrations of chemicals (µg/ml) (mm)															
	Sorbic Acid				Benzoic Acid				Rosemary				Thyme			
	0.25 %	0.5 %	0.75 %	1.0 %	0.25 %	0.5 %	0.75 %	1.0 %	0.25 %	0.5 %	0.75 %	1.0 %	0.25 %	0.5 %	0.75 %	1.0 %
A. flavus	12.9	20.1	19.7	28.4	6.9	17.3	29.5	30.6	16.7	15.0	16.7	21.2	3.9	13.6	20.4	27.4
A. niger	7.2	13.4	39.6	38.6	11.2	19.3	27.5	25.9	14.5	14.4	14.5	35.6	13.6	29.0	28.2	28.6
A.fumigatus	13.0	29.1	28.4	38.6	7.40	18.7	20.3	28.1	18.9	18.2	18.2	29.8	7.2	12.7	12.7	28.8
A.ochraceus	12.7	20.1	19.7	50.4	3.9	13.6	20.2	27.4	14.7	12.9	14.7	16.4	6.9	21.3	29.5	33.6
A.terreus	3.2	3.67	13.0	19.7	9.00	9.9	15.5	19.9	11.9	10.9	16.9	40.5	7.4	18.7	20..3	28.1
A candidus	7.2	12.7	12.7	28.8	10.9	16.1	19.8	26.7	18.4	17.0	18.4	21.3	10.9	16.0	19.8	24.7

Table (6): Detection of aflatoxins residues in the internal organs of rats after administration of aflatoxin alone or in combination with rosemary and thyme.

Organs	Levels of Af. Residues in organs of treated groups of rats(mg/Kg)			
	Control -ve	Aflatoxicated group	Aflatoxicated treated with rosemary group	Aflatoxicated treated with thyme group
Liver	0	1.0	0.3	0.6
Kidney	0	1.0	0.2	0.5

Table,7 :Effect of different antimycotics antioxidants on serum biochemical, thyroid hormones and total antioxidant capacitiy in aflatoxicated rats.

	ALT (U\L)	AST (U\L)	UREA (mg\dl)	CREA (mg\dl)	T3 (ng\ml)	T4 (µg\dl)	TSH (µIU\ml)	TAC (µmol\ml)
Control	32.3±0.65 c	95.6±2.49 a	54.0±1.4 abc	0.70±0.04 cd	0.98±0.01 cde	3.0±0.2 c	0.50±0.03 ab	1.67±0.003 a
AF+	35.4±0.93 ab	95.3±0.65 a	55.0±0.52 ab	0.70±0.02 d	1.01±0.02 cde	1.92±0.03 c	0.31±0.04 de	1.72±0.01 a
THYM	34.6±0.54 bc	90.9±5.93 a	56.3±1.32 a	0.82±0.02 ab	1.26±0.07 a	8.92±0.9 b	0.40±0.03 cd	1.68±0.03 a
AF+ THYM	37.4±0.65 a	94.2±1.58 a	51.1±0.67 d	0.78±0.02 bcd	1.21±0.04 ab	2.7±0.2 c	0.24±0.02 d	1.67±0.01 a
ROSE	25.6±0.64 de	90.9±1.8 a	52.8±1.8 bc	0.87±0.02 a	1.10±0.01 bc	3.24±0.09 c	0.36±0.03 cd	1.71±0.05 a
AF+ ROSE	27.7±0.37 d	90.9±2.5 a	52.4±1.11 bc	0.78±0.03 bc	1.04±0.04 cde	3.7±0.08 c	0.50±0.03 ab	1.59±0.01 b
SORB	24.3±1.07 ef	73.0±1.32 c	47.6±0.5 d	0.84±0.01 ab	0.98±0.08 cd	12.22±0.87 a	0.42±0.06 bc	1.4±0.03 d
AF+ SORB	21.9±1.40 g	82.6±1.8 b	44.6±1.15 de	0.72±0.02 cd	0.93±0.04 e	11.53±1.58 a	0.53±0.01 a	1.44±0.03 cd
BENZ	22.0±0.26 fg	72.4±0.88 c	43.0±0.47 e	0.72±0.03 cd	0.95±0.01 de	11.11±0.22 a	0.53±0.02 a	1.57±0.01 b
AF+ BENZ	21.6±1.08 g	72.6±2.68 c	46.3±0.79 d	0.77±0.01 bcd	1.06±0.01 cde	11.65±0.45 a	0.37±0.02 cd	1.5±0.01 c

- Values are expressed as mean ± SE (n=10) within the same column with different superscripts are significantly different ($p < 0.05$). **AF+:**A group that injected I.P.with 1.5mg\kg bodyweight aflatoxin B1;**control:**A control group fed on basal diet; **THYM:** A group fed on basal diet supplemented with 5%thyme **;AF+THYM:** A group that injected I.P. with 2mg\kg bodyweight aflatoxin B1and fed with basal diet supplemented with 5%thyme; **ROSE:** A group fed on basal diet supplemented with 2.5%rosemary;**AF+ROSE:** A group that injected I.P. with 2mg\kg bodyweight aflatoxin B1and fed with basal diet supplemented with 2.5% rosemary; **SORB:** A group fed on basal diet supplemented with 2%sorbic acid; **AF+SORB:** A group that injected I.P. with 2mg\kg bodyweight aflatoxin B1and fed with basal diet supplemented with 2%sorbic acid; **BENZ:** A group fed on basal diet supplemented with 2% benzoic acid; **AF+BENZ:** A group that injected I.P. with 2mg\kg bodyweight aflatoxin B1and fed with basal diet supplemented with 2%benzoic acid.**ALT:**alanine transaminase;**AST**:aspartate transaminase; **CREA:**creatinine; **T_3:**triiodothyronine;**T_4**:thyroxine**; TSH**:thyroid stimulating hormone; **TAC:**total antioxidant capacity.

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