ANTIFUNGAL ACTIVITIES OF SOME PLANT EXTRACTS AGAINST SOME MOULD FUNGI AND THEIR TOXICOLOGICAL EFFECT ON JAPANESE QUAIL (*COTURNIX COTURNIX*).

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ABSTRACT

Antifungal activity of seven plant extracts i.e. Brazilian peppertree, Pick-tooth, Fleabane, Black pepper, Fenchel, Cumin and Black cumin were tested on growth of three mould fungi isolated from poultry and stored foodstuffs. The isolated fungi were *Pencillium sp*, *Mucor sp* and *Rizopus stolonifer*. Furthermore, the toxicological effects of the most effective plant extracts on the Japanese quail were also investigated. Black pepper extract was the most effective one on *Pencillium sp* with IC_{50} of 167.7 ppm, while Black cumin and Cumin were the most effective extract against *Mucor sp* with IC_{50} of 26.05 and 41.36 ppm, respectively. Fleabane followed by Pick-tooth was the highly effective extracts against *Rizopus stolonifer* with IC_{50} value of 6.06 and 73.17 ppm, respectively.

Some biochemical parameters in Japanese quail were determined after treating with the most effective plant extracts Fleabane, Black pepper, Black cumin, Cumin and Pick-tooth inhibited the growth of mould fungi even at the lowest IC_{50} values. From the obtained data, it concluded that, in general enzymes activities were increased in all samples after 24 hours of treatment except cholinesterase. However, after 21 days the activities of some enzymes were reached to the normal level. All tested plant extracts reduced significantly the activity of Acetylcholinesterase (AChE) after 24 hours of treatment but the AChE activity reached to the normal level after 21 days of treatment. Transaminases activities (AST and ALT) were significantly increased after 24 hours and continued up to 21 days of treatment compared with control except that of pick-tooth extract. This study suggests that, the tested plant extracts especially Black pepper; Cumin, Black cumin and Fleabane are promising agent for controlling the tested fungi and thus becoming more safety compared to chemical control particularly if the treatment is done on edible crops.

INTRODUCTION

In the last few decades, scientists all over the world tried to minimize usage of synthetic chemicals for management of plant pathogens, insects and weeds. Synthetic pesticides are the chemicals which accumulated in the environment causing several deleterious effects. To avoid hazards of the environmental pollution, several investigations studied long persistence period (Beye, 1978), pollutive effects (Dubey and Mall, 1972), phytotoxicity (Fawcett and Spencer, 1970), teratogenicity (Javoraska, 1978) and carcinogenicity of these chemicals (Epstein *et al.*, 1967) were focused. Therefore, the new methods to control diseases became very important task (Wilson and Otto, 1987).

In addition to the target pathogen, pesticides may also kill various beneficial organisms. Moreover, developing of resistance pathogenic strains was another problem caused by synthetic pesticides. Due to these problems, great much attention was offered to find alternatives, such as natural plant products that are biodegradable and much safe to our environment. Various attempts have been carried out to search extensively for new biologically active terpenoids as a potential source for agrochemicals.

In recent years, plant extracts showed potential source of biological activities against several pests such as micro-organisms (Gayoso *et al.*, 2005; Maksimovic *et al.*, 2005 and Rasooli *et al.*, 2005), insects (Traboulsi *et al.*, 2002 and El-Zemity *et al.*, 2002), mollusca (El-Zemity, 2001 and El-Zemity *et al.*, 2001a,b) and weeds (Duke *et al.*, 2000). However, the role of higher plants as a source of fungitoxic compounds and their importance in controlling different plant pathogens (Al-Abed *et al.*, 1993 and Baharat et al., 1997 and in inhibition growth of the mould fungi by Zommara and Rashed, 2005) was stated.

As source of carbon and energy, a wide range of substrates can be used by many fungal species. Some are strictly growing on fruits as pathogenic fungi causing serious transited diseases leading to reduction in the yield. Others are so-called saprophytes that have found in soils, in dung and in organic matter. There seems to be a negative influence of the saprophytic fungi with their spores on foodstuff conversion, making it unsuitable for feed. *Penicillium sp.*, *Rizopus stolonifer* and *Mucor sp.* are of these fungi growing on the organic matter and isolated from poultry foodstuffs. *Penicillium sp* can also be growing on citrus and on other fruits causing green and blue moulds (Alexopoulos and mims, 1979). *Rizopus stolonifer* is a weak widespread fungs belonging to Mucoracae. A serious transite diseases of strawberries, designated as leak, and a soft rot of sweet potatoes were caused by this fungus. It is also found in decaying fruits and caused mainly black mold of our bread (Ajello, 1976).

Another more common species of Mucoraceae is *Mucor sp.*. The activity of this fungus is of direct importance in human affairs. *Mucor sp.* is the main causal agent of Mucor rot of pome and stone fruits during cold storage (Michailides and Spotts, 1990) soil mainly as sporangiospores (Michailides and Ogawa, 1987 and 1989).

Enzyme activities vary greatly among tissues and species of birds. It is important to realize that the activity of a particular enzyme may be high in one organ or tissue, or even specific for that tissue. Alterations in serum enzyme activity due to malfuctioning of the liver occur as a result of three processes: (1) An elevation of enzyme due to disruption of hepatic cells as a result of necrosis or as a consequence of altered membrane permeability. This enzymes are GPT, GOT, arginase, glutamic dehydrogenase (GD), sorbitol dehydrogenase (SD), and lactic dehydrogenase (LDH). (2) A decrease in concentration in the serum resulting from impaired synthesis by the liver (cholin esterase). (3) An elevation in enzyme levels due to cholestasis (alkaline phosphatase,and γ glutamyl transferase), Ebeid *et al* 2005.

Not only the efficiency of plant extracts against pests is the limiting factor but also evaluating its toxicological effect on mammals is source of major concern. The aim of this study was to decrease the applied pesticides concentration in order to reduce the cost of chemical control strategy and minimize hazarded of human, animals and natural enemies of pests as well as to reduce the environmental pollution. Therefore, the present study was attempted to evaluate the antifungal activities of some plant extracts against some decayed foodstuff fungi and its toxicological effects on Japanese quail.

MATERIALS AND METHODS

1- Plant materials:

Seven plant species belonging to five families were used throughout these experiments. Samples were purchased from local market. Fleabane and Brazilian peppertree were collected from the farm of Faculty of Agriculture Kafr El-Sheikh University. They were identified according to the taxonomic characters detailed by Tackholm, (1974) and Chiej, (1988). The English name, scientific name, Family name as well as the used part are shown in Table (1).

2- Extraction procedure:

The tested plant materials was dried, ground and batches of 100 gm from powdered leaves of Brazilian peppertree and Fleabane were macerated in 500 ml of ethanol and acetone at ratio of 1: 1 (v/v) for five days. During the maceration periods the samples were shaken for 5 hours using an electric shaker. Extracts were filtered, dried over anhydrous sodium sulphate and evaporated to dryness. The residues were weighed and dissolved in acetone to make the desired concentrations. Volatile oils of dry fruits of cumin, black pepper, Pick-tooth, Fenchel and Black cumin were extracted from the powder using the method described by El-Hamady, 1989.

Table (1): English name, scientific name, Family name and used part of the tested Plant materials

No.	English name	Scientific name	Family name	used part
1	Brazilian peppertree	*Schinus terebinthifoius* Raddi	Anacardiaceae	Leaves
2	Pick-tooth	*Ammi visnaga* L.	Apiaceae	Dry fruits
3	Fleabane	*Conyza dioscoroides* L.	Compositae	Leaves
4	Black pepper	*Piper nigrum* Linn	Piperaceae	Dry fruits
5	Fenchel	*Foeniculum vugare* Miller	Apiaceae	Dry fruits
6	Cumin	*Cuminum cyminum* L.	Apiaceae	Dry fruits
7	Black cumin	*Nigella sativa* L.	Ranunculaceae	Seeds

3- Isolation, purification and identification of the tested fungi:

From the practical point of view foodstuffs storage occurred under bad conditions particularly in the developing countries. Each sample was washed thoroughly under running tap water to remove any adhering solid particles and washed several times with sterilized water. The samples were then blotted to dry between sterilized filter papers. In sterile water, different diluted suspensions of the dried samples were prepared and plated on a potato dextrose agar (PDA) medium. The plates were incubated at 28°C ± 2°C for 3-7 days. The isolates were purified by hyphal tip technique. The developing colonies were identified to genus level and sometimes to species level according to the method developed by Barnett and Hunter (1979) and singh (1982). Three isolated fungi were selected for this study namely *Pencillium sp, Mucor sp* and *Rizopus stolonifer.*

Isolates of each genus were grown on PDA in Petri-dishes, then transformed to PDA slants and kept in a refrigerator at 4°C as stock cultures.

4-Biological tests:

A laboratory study of the antifungal activity was carried out by using four concentrations of each plant extract, i.e. 250, 500, 1000 and 2000 ppm calculate as crude substance. The required concentrations were obtained by adding the appropriate amount of stock plant extract (3 ml) to 42 ml portions of autoclaved PDA medium cooled to about 45°C. A non amended check were also prepared as control for each fungus. Approximately 20 ml of non amended (control) or amended PDA for each treatment were poured into each of five Petri dishes, 9 cm diameter as replicates for each concentration. After solidification of the medium each dish was inoculated centrally with a mycelia disk (0.5 Cm in diameter) taken from the cultures of each fungus. Dishes were incubated at 28°C ± 2°C and colony diameters were measured till the untreated controls had just covered plate. After the untreated controls had just covered the plate percentage of inhibition (I%) was calculated according to the formula suggested by Topps and Wain (1957).

$$I\% = [(A - B)/A] \times 100$$

Where:-

I% = Percent of inhibition.

A = Mean diameter growth in the control.

B = Mean diameter growth in a given treatment.

Linear regression lines were fitted to logarithmic probability data of plant extract concentration and percentage of growth inhibition for each plant extracts fungus treatment so that slope values and IC_{50} values could be interplotted (Finney, 1971).

5-Biochemical tests of plant extracts:

The most effective plant extracts against the tested fungus (Cumin, Pick-tooth, Black cumin, Black pepper and Fleabane) were selected to evaluate its toxicological effects on Japanese quail at concentration levels of IC_{50} (41.36, 73.17, 26.05, 100.2 and 6.06 ppm) of each plant extract respectively. The biochemical parameters used to investigate the toxicological effects of plant extracts were Acetylcholinesterase (AChE) activity, Aspartate Amino Transferase (AST) (Formerly GOT), Alanine Amino Transferase (ALT) (Formerly GPT), alkaline phosphatase (ALP), Creatinine, Albumin and total protein.

In order to evaluate the toxicological effects of the plant extracts, six experimental groups of male Japanese quail (n=36) were used. The five groups were given single oral dose of plant extracts mentioned previously and the last group was used as control. After 24 hours three birds from each group were slaughtered and rest of birds were slaughtered after 21 day.

Blood samples were centrifuged for 15 minutes at 4000 rpm at 4°C. Blood serum was used to determine the different enzymes described above. The activity of Acetylcholinesterase was determined according to the method of Ellman *et al.,* (1961). This method is based on the hydrolysis of acetyle thiocholine Iodide (ASChI) as substrate by the (ChE) enzyme to produce thiocholine and acetic acid. Thiocholine reacts with 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) to produce the yellow color anion of 5-thio-2-nitro benzoic acid. The rate of color formation (as a function of enzyme activity) is measured at 412 nm. Transaminases activity (AST and ALT), alkaline phosphatase, Creatinine, Albumin and total protein were determined by Bio-diagnostics kits. The colorimetric method were used according to Reitman and Frankel (1957), Belfield and Goldberg (1971), Barham and Trinder (1972), Doumas and Watson (1971) and Gornall *et al.*, (1949) respectively.

6- Statistical Analysis and Equations:

Differences among groups were tested by one-way ANOVA using SPSS statistical software package for windows version 11.0. Duncan's multiple-range test was used to find out the group effects. $P \leq 0.05$ was set as limit of significance.

1. $\% \text{ of control} = \dfrac{\text{Activity of enzyme in treatment}}{\text{Activity of enzyme in control}} \times 100$
2. % of inhibition = 100 - % of control

RESULTS AND DISCUSSION

1-In vitro studies:

Fungi are significant destroyers of foodstuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Synthetic chemicals commonly control storage fungi; however, most of the fungicides of this group create several side effects in the forms of carcinogenicity, teratogenicity, and residual toxicity (Chandra, *et al.*, 1982; Dikshit *et al.*, 1983 and Dube, *et al.*, 1989). Therefore, some alternative biodegradable chemical control measures should be discovered to replace synthetic pesticides for pest management without creating any pesticidal pollution.

With regards to the importance of natural products as promising substitution for traditional pesticides, the of seven plant species of Brazilian peppertree, Pick-tooth, Fleabane, Black pepper, Fenchel, Cumin and Black cumin were selected to study their antifungal activity against growth of some mould fungi. The effect of different concentrations of plant extracts on *Pencillium sp.*, *Mucor sp.* and *Rizopus sp* (radial growth) were estimated under laboratory conditions. To evaluate the in vitro antifungal activities, IC_{50} values, slope of data fitting and confidence concentration limits of each extract against the isolated mould were summarized in Table (2).

The data showed that the extract of Black pepper was the most effective one on *Pencillium sp.* with IC_{50} of 167.6 ppm, followed by the extracts of Pick-tooth, Brazilian peppertree, Cumin and Fleabane with IC_{50} values of 377.5, 499.3, 513.8 and 515.3 ppm, respectively. While the extracts of both Fenchel and Black cumin were the lowest effective extracts against *Pencillium sp.* Extracts of Black cumin, Cumin and Black pepper showed high efficiency on *Mucor sp.* with IC_{50} values of 26.05, 41.36 and 100.2 ppm, respectively. While, extracts of Brazilian peppertree and Pick-tooth had a moderate effect against *Mucor sp.* Extracts of Fleabane and Fenchel were the lowest effective against *Mucor sp.* with IC_{50} of 409.5 and 487.6 ppm, respectively.

Fleabane and Pick-tooth showed the most effective extracts against *Rizopus sp.* with IC_{50} values of 6.06 and 73.17 ppm respectively. Extracts of Black pepper, Fenchel and Black cumin were effective against the same fungus with IC_{50} values of 167.4, 227.5 and 250 ppm, respectively.

Based on the results recorded, the antifungal activities of the tested biocide agents' versus growth of the mould fungi can be divided into gradient groups. Black pepper fruits were the most effective extracts group inhibited growth of all isolated fungi. It characterized by small IC_{50} equal 145.07 ppm against growth of the three moulds. This result was in full agreement with the findings of Alice and Rao (1987) who stated that *piper nigrum* extract was the most effective among of 31 extracts against Drechslera oryzae. Growth of *Rhizoctani solani* was also inhibited with black pepper fruits extracts (Ismail and Ahmed 2000). Mould fungi found on Ras cheese surface belonging to genus *Penicillium* and *Aspergillus* were also inhibited by using black pepper extract (Zommara and Rashed 2005).

It is worth noting, that extracts of Pick-tooth fruits and fleabane leaves came in the second category with mean of IC_{50} about 236.96 and 310.25 ppm, respectively. El-Shoraky, 1998 reported that the extract of pick-tooth strongly inhibited the radial growth of some pathogenic fungi. Due to the antimicrobial activity caused by high percent of sesquiterpens and alcohols, fleabane oil extract can be used for pharmaceutical purposes (Said, *et. al.*, 1993).

Concerning the data revealed brazilian peppertree and black cumin extracts showed moderately toxic effect against all tested fungi with mean of IC_{50} equal 371.52 and 382.15 ppm, respectively. The obtaind effect of black cumin extract was in full agreement with the findings of Rathee, *et. al.*, (1982). The authors indicated that, the essential oil extracts from *N. Sativa* exhibited strong antimicrobial activity against *Pythium Vexaus, Rizoctonia solani* and *Colletotrichum Capsici*. These data are in contrast to the results observed by Ismail, *et al.*, (2003). The authors stated that, brazilian peppertree extract inhibited the radial growth of some pathogenic fungi. Allthough, cumin fruits extract inhibited growth of *Mucor sp* strongly, the general effect against the other tested fungi was relatively mild. Essential oils were reported to have fungitoxic effects. Garg and Siddiqui (1992) found that, Cumaldehyde isolated from essential oil of *Cuminum cyminum* and other constituents of certain volatile oils were of high fungicidal against the fungi, *Absidiaglauca, Alternaria alternate, Aspergillus fumigatus, Aspergillus niger, Fusarium moiliforae, Phytophora* *parasiticae, Helminthosporium oryzae, Penicillium expansum* and others. In addition to Cumin and Fenchel extract were the lowest potential substances against growth of the mould fungi.

Table (2): Efficiency of different plant extracts against tested fungi.

Tested compounds	*Penicillium Sp.*			
	IC_{50}	Slop	Confidence Limits	
			Lower	Upper
Brazilian Pep.	499.3	0.2	345.3	657.20
Pick-tooth	377.5	0.2	295.7	455.18
Fleabane	515.2	0.2	301.8	676.98
Black pepper	167.6	0.3	96.65	231.51
Fenchel	672.5	0.5	325.4	832.73
Cumin	513.8	1.0	88.90	2025.9
Black cumin	870.4	0.2	680.2	1162.9
	Mucor sp.			
Brazilian pep.	250	0.35	89.5	451.3
Pick-tooth	260.2	0.33	176	326.8
Fleabane	409.5	0.55	258.2	689.2
Black pepper	100.2	0.42	39.2	154.7
Fenchel	487.6	0.71	369.5	725.2
Cumin	41.36	0.61	10.6	102.8
Black cumin	26.05	0.27	12.4	83.71
	Rizopus stolonifer			
Brazilian pep.	356.25	0.37	158.2	422.8
Pick-tooth	73.17	0.3	15.53	141.1
Fleabane	6.06	0.2	0.25	82.35
Black pepper	167.4	0.3	105.0	221.5
Fenchel	227.5	0.2	94.42	346.3
Cumin	1520	0.4	747.3	>2000
Black cumin	250	0.5	74.25	425.2

Brazilian Pep. = Brazilian peppertree

The ethanolic extract was more effective and possessed broad spectrum fungitoxicity than aqueous extract (Mishra and Tewari, 1992). Volatile oils isolated from Pick-tooth, Fenchel and Cumin were found also to have a fungistatic effect on fungal mycelial growth of *Fusarium oxysoprum*, *Macrophomina phaseolina* and *Botrytis cinerea* (Shimon, *et. al.*, 1993).

In general, treatment of stored and poultry foodstuffs with black pepper fruits extract will be able to decrease the growth of all fungi decayed foodstuffs. The extract of Pick-tooth fruits and Fleaban leaves had less effective inhibition, while Brazilian peppertree and Black cumin extracts inhibited growth of the mould fungi moderately. The other treatment with Cumin, Fenchel was the lowest antifungal activities. These results were confirmed with the exhibit the findings of Hassan and El-Deeb (1988), Carcia and Lawas (1990), Ismail and Ahmed (2000) and Zommara and Rashed (2005). They reported that, plant extracts were able to inhibit growth and sporulation of the mould fungi.

Plant extracts used in this study showed higher efficiency for controlling the tested fungi than some recommended fungicides in this way. Since, the IC50 of these fungicides such as agrosan (1000 ppm) ogrozim (2000 ppm) dithane (2000 ppm) and cersan (2500 ppm) as reported by Mishra and Dubey (1994) were clearly much higher comparing with the same values of our tested plant extracts.

2- Biochemical Effect of plant extracts on some Japanese quail enzymes:

The enzymatic reactions are of two types: phase I: reactions, involving oxidation, reduction and hydrolysis, phase II: reactions, consisting of conjugation or synthesis. Phase I reactions generally convert foreign compounds to derivatives that can undergo phase II reactions (Neal, 1980).

Some toxicological effects of the above mentioned plant extracts were determined using Japanese quail as in vivo experimental model. The activity of Acetylcholinesterase, Transaminases activity (AST and ALT), alkaline phosphatase, Creatinine, Albumin and total protein were determined in the serum.

The results of the effect of plant extracts on some enzymes after 24 hours and 21 days of treatment are summarized in Table (3 and 4) respectively. From the obtained data, it can be concluded that, in general the enzymes activity were increased in all samples after 24 hours of treatment except cholinesterase. However, after 21 day the activities of some enzymes were return back to their normal level.

It could be notesed that, the total protein for each bird varied considerably from 2.59-4.67 g/dI. This variation might cause significant variations within treatment. Accordingly, it is more preferable to minimize this error by recalculating the data in the form of specific activity. Base on the specific activity of tested enzyme the currant results reveled that, a part of Black pepper no significant different were found after 24 hr and 21 days.

Precise investigation of Table (3 and 4) could be notes that, the total protein extend from 2.70 to 4.67 and 2.59 to 4.64 respectively, with wide conceder relatively rang. Accordingly, in is not fair to compare our results the base of enzyme activity. In the other words, estimation on the base of specific activity will be more accurate and more preferable. Base on the specific activity of tested enzyme the currant results reveled that, a part of Black pepper no significant different were found after 24 hr and 21 days.

Results presented in Table (3) indicated that total serum protein concentration was significantly increased in Japanese quail treated with Cumin, Black cumin and Fleabane after 24 hrs of treatment compared with control. This might be due to loss of body water (dehydration). The percent of control for these extracts were 135.02, 143.53 and 147.32, respectively, but treatment with Pick-tooth after 24 hrs was not significant. Black pepper was significantly decreased with 85.17% of control. After 21 days (Table 4) the activity as percent of control for Black cumin and Fleabane were 106.86 and 122.43 respectively. On the other hand the total protein was decreased in treated with Pick-tooth and Black pepper, the % of inhibition was 26.39 and 31.66 respectively. The treatment with cumin after 21 days reached the normal level compared with control.

Aspartate aminotransferase (AST) catalyzes the transfer of the amino group of aspartic acid to α-ketoglutaric acid, forming glutamic and oxaloacetic acids, Alanine aminotransferase glutamic and pyruvic acids. Transaminases are important and critical enzymes in the biological processes. They play a role in amino acids metabolism and biosynthesis. Transaminases activities (AST and ALT) were significantly increased in all treatments compared with control except cumin and pick-tooth respectively (Table 3). The activity of AST with Cumin, Pick-tooth, Black cumin, Black pepper and Fleabane were 100.33, 111.82, 136.73, 145.04 and 144.44% of control respectively. The same results can be found with ALT activity, with respect to control were 119.81, 98.02, 117.50, 125.51 and 154.85 compared with control.

Table (3): Effect of tested plant extracts on some biochemical parameters of Japanese quail after 42hr. of the treatment.

Treat-ment	Total protein g/dl	GPT (AST)		GOT (ALT)		ChE		ALP		Albumin		Creatinine	
		U/ml	S.P.	U/ml	S.P.	Activity*	S.P.	U/100ml	S.P.	(g/dl)	S.P.	mg/dl	S.P.
Control	3.17^{c}	33.08^{d}	10.43	30.85^{d}	9.73	3.52×10^{-04a}	1.11	6.87^{b}	2.17	0.31^{b}	0.098	0.13^{d}	0.041
Cumin	4.28^{b}	33.19^{d}	7.75	36.96^{c}	8.63	3.33×10^{-04a}	0.78	8.88^{a}	2.07	0.55^{a}	0.13	0.28^{c}	0.065
P.T.	3.19^{c}	36.99^{c}	11.59	30.24^{d}	9.47	3.28×10^{-04a}	1.02	5.85^{c}	1.83	0.15^{c}	0.047	0.23^{c}	0.072
B. C.	4.55^{b}	45.23^{b}	9.94	36.25^{c}	7.96	2.17×10^{-04b}	0.47	5.99^{c}	1.32	0.35^{b}	0.76	0.10^{d}	0.022
B. P.	2.70^{d}	47.98^{a}	17.77**	38.72^{b}	14.34**	1.85×10^{-04c}	0.69	7.95^{a}	2.94**	0.32^{b}	0.12	0.34^{b}	0.13
Fleabane	4.67^{a}	47.78^{a}	10.23	47.77^{a}	10.22	1.79×10^{-04c}	0.38	8.54^{a}	1.83	0.21^{c}	0.045	0.45^{a}	0.096

Table (4): Effect of tested plant extracts on some biochemical parameters of Japanese quail after 21days of the treatment.

Treat-ment	Total protein g/dl	GPT (AST)		GOT (ALT)		ChE		ALP		Albumin		Creatinine	
		U/ml	S.P.	U/ml	S.P.	Activity*	S.P.	U/100ml	S.P.	(g/dl)	S.P.	mg/dl	S.P.
Control	3.79^{b}	32.15^{d}	8.48	29.70^{c}	7.84	1.45×10^{-04a}	0.38	5.94^{c}	1.56	0.11^{b}	0.059	0.19^{b}	0.050
Cumin	3.46^{b}	33.40^{d}	9.65	35.86^{b}	10.36	1.35×10^{-04a}	0.39	8.16^{a}	2.35	0.31^{a}	0.089	0.20^{b}	0.057
P.T.	2.79^{c}	36.57^{c}	13.11	29.70^{c}	10.65	1.66×10^{-04a}	0.59	5.09^{c}	1.82	0.12^{b}	0.043	0.17^{b}	0.061
B. C.	4.05^{a}	44.82^{b}	11.07	35.22^{b}	8.69	1.47×10^{-04a}	0.36	5.21^{c}	1.28	0.28^{a}	0.069	0.18^{b}	0.044
B. P.	2.59^{c}	47.51^{a}	18.34**	38.43^{a}	14.83**	1.38×10^{-04a}	0.53	6.88^{b}	2.66	0.14^{b}	0.054	0.45^{a}	0.17**
Fleabane	4.64^{a}	46.26^{a}	9.97	47.08^{a}	10.15	1.66×10^{-04a}	0.36	7.21^{b}	1.55	0.11^{b}	0.024	0.53^{a}	0.11

P.T.= Pick-tooth, B.C.= Black cumin, B.P.= Black pepper, ChE = Cholinesterase, *Activity = μ moles AChE/min/mg protein, ALP=Alkaline phosphates and S.P.=Specific activity. ** Significant ($p<0.05$). S.P. for ChE x 10^{-4}

Acetylcholinesterase (AChE) is one of the hydrolytic enzymes for acetylcholine. The inhibition of the enzyme disturbs the normal nervous function (Eto and Olkaw, 1970). Significant inhibitory effect was noticed with Black cumin, Black pepper and Fleabane after 24 hours with % of inhibition 38.35, 47.44 and 49.15 respectively. In other words, AchE in all treatments after 21 days reached the normal level and the activity was not significant in all treatments compared with control.

Alkaline Phosphatase catalyzes the hydrolysis of various phosphate esters, transferring the phosphate group to an acceptor molecule. ALP has a pH optimum between 9 and 10. This enzyme is widely distributed in the body, and is found in high concentrations in bone (in the osteoblasts), intestinal mucosa, renal tubule cells and liver (Kaplan and Pesce 1989; Calbreath 1992).

Cumin, Black pepper and Fleabane significantly increased the activity of Alkaline phosphates (Table 3) as percent of control as follow 129.26, 115.72 and 124.31 respectively however Pick-tooth and Black cumin decreased the activity after 24 hr of treatment as % of control as follow 85.15 and 87.19 respectively. Furthermore after 21 days treatment with Pick-tooth and Black cumin did not show significant difference compared with control, on the other hand the effect of Cumin, Black pepper and Fleabane continuously increased (Table 4) until 21 day from treatment. The activities as percent of control for these extracts were 137.37, 115.82 and 121.38 respectively.

Albumin is the largest protein fraction in normal avian serum. Avian albumin is similar in structure to mammalian albumin. Albumin binds and transports anions, cations, fatty acids, and thyroid hormones (Ivins et al. 1978). Seventy-five percent of the plasma T4 is attached to albumin and 50 percent of plasma T3 is associated with albumin in chickens (Butler, 1983).

Results after 24 hrs recorded in Table (3) showed that, serum albumin was decreased with Pick-tooth and Fleabane, while it was increased with Cumin, Black cumin and Black pepper compared with untreated Japanese quail, but the increased with Black cumin and Black pepper was not significant compared with control. The data after 21 days (Table 4) indicated that all treatments not significant compared with control except cumin and Black cumin with % of control 281.82 and 254.55 respectively.

Creatinine is not a major nonprotein nitrogen component of avian blood .Avian urine contains very little creatinine but much more creatine. Therefore serum creatinine has questionable value in the evaluation of renal function in birds. Many investigators think that serum creatinine may become elevated in birds with renal failure, but less reliably than uric acid.

The normal serum creatinine for most birds 0.5- 1.5 mg/dl. High serum creatinine values may be seen in psittacine birds fed with high levels of animal protein (Campbell and Coles, 1986).

Creatinine activity after 24 hours of treatment with Cumin, Pick-tooth, Black pepper and Fleabane was significantly increased. The activity of creatinine as percent of control were as follow 215.38, 176.92, 261.54 and 346.15, respectively. Also creatinine activity after 24 hours was decreased with Black cumin, % of control was (76.92). On the other hand creatinine activity after 21 days of treatment started to return to the normal level except Black pepper and Fleabane extracts. Percent of control for these plant extracts were 236.84 and 278.95, respectively.

The results of Black pepper agreed fully with the previous findings of El-Naggar (2000) who found that, Transaminases activities (AST and ALT), alkaline phosphatase, albumin and creatinine were significantly increased in rats treated with Black pepper extracts.

In conclusion, the tested promising plant extracts might be a good source of materials that have fungicidal properties against the tested fungi.

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الملخص العربى

النشاط الابادى الفطرى لبعض المستخلصات النباتية على بعض الفطريات المترممة والتاثيرات السامة لها على طائر السمان.

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تم اختبار قدرة سبع مستخلصات نباتية (الفلفل العريض ، الخلة ، البرنوف ، الفلفل الاسود ، الشمر ، الكمون ، حبة البركة) على تثبيط نمو ثلاثة انواع من فطريات العفن التى تنمو على علائق الدواجن والاغذية المخزنة وهى *Pencillium sp* و *Rizopus stolonifer* و *Mucor sp* . كما تم ايضا دراسة التاثيرات السامة لافضل المستخلصات تاثيرا على الفطريات على طائر السمان.

واوضحت النتائج ان الفلفل الاسود كان افضل المستخلصات كفاءة على فطر *Pencillium sp* حيث كان التركيز القاتل لـ ٥٠% هو ١٧٦,٥ جزء فى المليون بينما حبة البركة والكمون كانت افضل المستخلصات كفاءة على فطر *Mucor sp* حيث كان التركيز القاتل لـ ٥٠% هو ٢٦,٠٥ و ٤١,٣٦ عل الترتيب. مستخلص البرنوف والخلة كانت لهم تاثيرات قوية عل نمو فطر *Rizopus stolonifer* حيث كان التركيز القاتل لـ٥٠% هو ٦,٠٦ و ٧٣,١٧ جزء فى المليون عل الترتيب.

تم تقدير نشاط بعض الانزيمات فى طائر السمان بعد المعاملة بافضل المستخلصات تاثيرا على الفطريات وهى (البرنوف ، الفلفل الاسود ، حبة البركة، الكمون ، الخلة). اوضحت النتائج ان نشاط الانزيمات عموما زاد بعد ٢٤ ساعة من المعاملة فيما عدا انزيم اسيتايل كولين استريز. ولكن بعد ٢١ يوم من المعاملة عادت الانزيمات الى المستوى الطبيعي كما فى الكنترول. وبالنسبة لانزيم الاسيتايل كولين استريز انخفض نشاطه انخفاضاً معنويا بعد ٢٤ ساعة من المعاملة وبعد ٢١ يوم عاد الى نشاطة الطبيعى. انزيمات الترانس امينيز حدث لها زياد معنوية بعد ٢٤ ساعة و ٢١ يوم من المعاملة مقارنة بالكنترول فيما عدا مستخلص الخلة.

وتوضح هذا الدراسة دور المستخلصات النباتية خصوصا مستخلصات الفلفل الاسودو الكمون وحبة البركة و البرنوف كمستخلصات واعدة فى مكافحة الفطريات مع درجه عالية من الامان بالمقارنة بالمكافحة الكيماوية وذلك فى حالة استخدامها على المحاصيل التى تؤكل طازجة.