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THE EFFICACY OF LICORICE EXTRACT ON BOTH MOULD GROWTH AND AFLATOXIN(S) PRODUCTION.

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ABSTRACT

The efficacy of licorice extract (*Glycyrriza glabra*) on growth and aflatoxin(s) production of both *Aspergillus parasiticus* and / or *Aspergillus flavus* were studied in Yeast Extract Sucrose medium (YES).Seventy two YES containing flasks were divided into 2 groups, the 1 st group of flasks were inoculated with a suspension of *A.flavus* containing 10^6 spores, each, while the 2 nd group of flasks received similer count, each from *A. parasiticus* spores. Simultanously, the licorice extracts were prepared at concentrations of 0.0, 0.1, 0.2, 0.4, 0.6, and 0.8 mg/ ml medium. Each group of flasks were divided into 6 subgroups to correspond the 6 studied concentrations of licorice extract. Consequently, each treated subgroup was divided into 2 equal number of flasks to be incubated at either 14 or 28^0C. The parameters of mycelial dry weight, pH and aflatoxin(s) production were carried-out at the intervals of 3, 7, 10 and 30, 45 , 90 days in different treatments incubated at 14 or 28^0C, respectively. Data showed an obvious inhibitory effect on both mould growth and aflatoxin(s) production at concentrations of 0.2 and / or 0.4 mg/ ml medium. Moreover, a lethal effect was obtained at concentrations 0.8 mg / ml medium in both *A. flavus* and *A.parasiticus* media,while the concentration of 0.6 mg/ ml medium showed lethal effect in media flasks previously inoculated with *A. flavus* only.

Keywords*: licorice, fungi, aflatoxin(s), Glycyrriza glabra, mycotoxins, detoxification.*

INTRODUCTION

Herbs and spices have traditionally been used to extend the shelf life of foods a activities of plant extracts has been rekindled as a subject of intense scientific investigation. Several studies indicated that essential oils drived from many plants possess an important role as antimicrobial and / or antiaflatoxigenic agents (Kivanc and Akgul, 1986; Paster *et al.*,1990 and Yadava and Saini,1991). Oils of sweet marjoram, spearmint and thyme herbs were reported to possess antifungal activities against *A. ochraceus, A. niger,* and *A. parasiticus* (Omer *et al.,* 1997).

Licorice is an economically important plant that has been used for centuries as a medicine because of its wide ranging therapeutic properties including relife of reheumatic and other pain and healing effect on ulcers. Licorice contains glycyrrhizin, which is a biologically active triterpene glycoside present at 5-24 %, and also contains flavonoids,isoflavonoids and coumarins (Fenwick *et al.,*1990) Flavonoids are considered as natural plant components with antioxidant activity (Nieto *et al.,* 1993). Extracts of licorice are known to possess antioxidant activity (Takagaki, 1989).

Aim of wark: Reviewing the chemical composition of licorice, it appears that the 4 biological active compounds reported by Fenwick *et al.,(*1990), could act – synergestically- as antimycotic agent(s). This leads to suggest the recent proposal of study, even licorice as antimicrobial agent did not get the attention of the scientists as our best of knowledge.

MATERIALS AND METHODS

Plant material and solvent extracts:

Licorice root (*Glycyrriza glabra*), was purchased from local market as a commercial sample used for the preparation of herbal extracts .Solvent extraction was performed according to the method of Michael and Jing (1995). Dried licorice powder was extracted with hexane in a soxhelt extractor. Then, the powder extracted with methanol to yield an extract corresponding to 27.3% by mass (w/w) of the original powder . The methanol extract was then extracted twice with chloroform at room temperature using an ultrasonic bath, to yield an extract corresponding to 3.9% by mass (w / w). Extraction was done as previous because of chloroform extract is more effective as antioxidant than fractions extracted with hexane or methanol (Michael and Jing , 1995) . Chloroform extract was then evaporated using a rotary evaporator and temprature ranging between 50 and 55 ^{0}C untel chloroform phase was completely evaporated.The resulting residue was then weighed, emulsified using known volumes of a sterile 0.01% solution of Tween 20 and kept untel used.

Organisms :

A. Parasiticus and *A. flavus* were obtained from the mycotoxin lab., National Research Center ,Dokk i ,Cairo ,Egypt . These strains were cheked for purity and identity according to Raper and Fennel(1955) .

Inoculum :

Cultures of the toxic moulds were grown on potato - dextrose – agar (PDA) slants for 10 days at 25 ^{0}C until well sporulated . The spores were washed from the slants with a sterile 0.01% solution of Tween 80 . The spores from two slants of each organism were combined to form a single heavy spore suspension . The harvested spores were suspended in 100 ml of sterile tween 80 and aseptically filtered through sterile cheese cloth to remove mycelial debres . The number of conidia was estimated by plate count and the suspension was adjusted to contain approximately 10^6 spores / ml .

Antifungal Assay (substrate)

Yeast- extract– sucrose medium (YES) was used as a basal medium for mould growth and aflatoxin(s) production in stationary cultures (Davis *et al.,* 1966). Seventy two YES containing flasks were adjusted the initial pH to 5.5 using a pH meter and 0.1 N KOH . Then, flasks autoclaved at 121 ^{0}C for 15 min ., cooled and divided into 2 groups; the 1 st group of flasks were inoculated with a suspension of *A.flavus* containing 10^6 spores, each, while the 2 nd group of flasks received similar count, each from *A.parasiticus* spores. Simultanously, the licorice extracts were prepared at concentrations of 0.0, 0.1, 0.2, 0.4, 0.6 and 0. 8 mg/ ml medium. Each group of flasks, were divided into 6 subgroups to correspond the 6 studied concentrations of licorice extract. Consequently, each treated subgroup was divided into 2 equal number of flasks to be incubated at either 14 or 28^{0}C. The parameters of mycelial dry weight, pH and aflatoxin(s) production were carried –out at the intervals of 3, 7, 10, and 30, 45, 90 days in different treatments incubated at 14 or 28^{0}C, respectively.

Analysis:

At the end of the incubation period, contents of flasks (with and without licorice solvent extract) were analyzed in triplicate for dry weight of mycelium, and aflatoxin production as follow : dry weight of mycelium was carried out according to the method of Yousef and Marth (1981). Extraction of aflatoxin(s) were performed as described by Bauer *et al*. (1983). Determination of aflatoxins was carried out according to the method of Nabney and Nesbitt (1965).The final residue of each vial was dissolved in 1 ml benzene: acetonitrile mixture (98 : 2, v/v). Aflatoxin B_1, B_2, G_1 and G_2 were qualitatively identified on 20x20 thin layer chromatographic plates (0.5 mm thickness) by comparing the R_f values with those of standards (10 ug/ml of each) dissolved in benzene : acetonitrile (98 : 2, v/v). Crude aflatoxin(s) (0.2 ml) were applied in a line approximately 6 cm wide across a chromatoplate , 3 cm from the bottom , and developed in trichloroethylene – chloroform – methanol (8+1+1) mixture . This solvent system cleanly separates aflatoxin(s) B_1 and B_2 as one band (R_f= 0.5) and aflatoxin(s) G_1 and G_2 as another band (R_f = 0.4) . Aflatoxin(s) were located by viewing under U V light (365 nm) and each band was scraped separately from the plate. . Aflatoxin(s) were stimated as aflatoxin B_1 (the blue band) while those of green band as aflatoxin G_1 using the appropriate molar extinction coefficients . The absorbance values for each group were recorded using a Perkin – Elmer UV / VIS spectrophotometer, model Lambda 3 . The results were recorded as mean values of triplicated.

Calculations

Average rate of net aflatoxin (μg/day) or mycelial dry weight (g/day) produced in one day by the mould during incubation period were calculated as follow:

Average rate = net (aflatoxin or mycelial dry weight produced after incubation period)/ incubation period

Average rate of increases or decrease in mycelial dry weight (g/day)= (net mycelial dry weight at a period B- net mycelial dry weight at a period A)/ period(B-A)

Average rate of accumulation or degradation in aflatoxin (μg/day) preduced during incubation period =(net aflatoxin produced at a period B- net aflatoxin at a period A)/ period (B-A)

Average rate of net aflatoxin (μg/day) preduced by 1gram of mycelial dry weight in one day was calculated by equation: A/B

where:

A= average rate of net aflatoxin produced in 1 day during incubation period

B= average rate of net mycelial dry weight produced in 1 day during incubation period

The percentage of inhibitory or stimulatory effect of licorice extract was calculated as follow:

$$\% \text{ Inhibition or stimulation of aflatoxin production} = \frac{D - C}{C} \times 100$$

where:

C= average rate of net aflatoxin produced by 1 gram of dry mycelium in 1 day by the mould in a medium containing no licorice extract (control)

D= average of rate of net aflatoxin produced by 1 gram of dry mycelium in 1 day by the mould in a medium containing licorice extract

RESULTS AND DISCUSSION

Effect of solvent extracts of licorice in a medium incubated at 28˚C.

Mycelial dry weight : As can be seen from Table (1), all of concentrations of licorice extract exhibited a variable antifungal activity against the two tested fungal strains. Data presented in Table (1) show that there were a gradual decrease in growth rate as affected by increasing licorice extract, specially at concentrations of 0.2, 0.4, 0.6 or 0.8 mg/ml medium during incubation period (10 days). These results agree with those of Baratta *et al*., (1998) , who studied the antioxidant properties of some commercial essential oils against *A. niger* and other organisms. Results obtained by Mehmoud *et al.,* (1997) on *Aspergillus* and *Fusarium* using the essential oils from lemongrarss, cinnamons and mint , also agree with our results.

From the results in Tables (1and 2) , the mould growth was affected by the extract added during the period of incubation from 0 to 3 days. The average amount of mycelial dry weight produced in cultures containing licorice extract was less than in cultures free of these extracts (i.e. 0.6 mg/ml concentration completely inhibited mould growth in *A. flavus* cultures during the 0- to 3 days interval). Whereas, the extract concentration(0.8 mg/ml medium) completely inhibited the mould growth in all cultures at the same period. Maximum mycelial growth occurred after 7 days in the media containing licorice extract 0.1 mg/ml medium . Whereas extracts concentration > 0.1 mg /ml medium, decreased the mould growth in all tested cultures during the 3 - 7 - day interval of incubation (Tables 1and 2). These results in agreement with those observed by EL-Gazzar and Marth (1986) who studied the role of H_2O_2 the prevention of growth and aflatoxin production by *A. parasiticus*. The observed increase in dry mycelial weight in the media supplemented with licorice extract of 0.1 mg/ml medium , can be attributed to an extension of the lag phase of the mould caused by licorice extract in the medium. Results in Tables (1 and 2) further indicate that there was a decrease in mycelial weight during the interval from 7- to 10 days in the presence or absence of 0.1, 0.2 or 0.4 mg/ml medium of added extract, whereas in the presence of 0.6 mg/ml of the extract in the medium, the *A flavus* failed to grow . At the concentration of 0.8 mg/ml, both mould strains failed to grow during the period from 7- to 10 day interval. Our results, in terms of change in mycelial growth, are similar to those observed by Horberg (1998), who studied the antifungal effect of the vapours of the essential oils of the caraway, spermint, thyme and garlic against 3 important post-harvest pathogens of carrots. The decrease in dry weight of mycelium (Table 2) during the final stages of the growth cycle can be explained bioautolysis, which may have occurred at that time and resulted in loss of soluble intracellular solutes which were released through hydrolysis of the mycelium during the filtration step of the analysis (Doyle and Marth, 1978).

Table (1):Mycelial dry weight (g/ 25 ml medium), pH and aflatoxin content (μg / 25 ml medium) in cultures containing licorice extract and incubated at 28 ºC for up to 10 days.

Extract added (mg /ml medium)	Incubation time (days)	*A.parasiticus*					*A.Flavus*				
		Mycelial dry weight	pH	Aflatoxin content			Mycelial dry weight	pH	Aflatoxin content		
				B_1	G_1	Total			B_1	G_1	Total
0.0		1.57	3.21	66	686	752	1.38	3.46	59	556	615
0.1		1.31	3.11	89	600	689	1.06	3.28	61	397	458
0.2		0.94	5.12	15	20	35	0.91	4.73	ND	25	25
0.4	3	0.69	5.38	NT	NT	NT	0.49	5.40	NT	NT	NT
0.6		0.44	5.49	NT	NT	NT	NG	5.43	T	NT	NT
0.8		NG	5.50	NT	NT	NT	NG	5.45	NT	NT	NT
0.0		1.41	7.05	127	1065	1192	1.20	7.05	1.18	859	977
0.1		1.49	6.98	156	1114	1270	1.20	7.00	114	750	869
0.2	7	0.87	6.88	99	342	441	0.83	5.95	NT	508	583
0.4		0.65	5.47	NT	NT	NT	0.31	5.53	NT	NT	NT
0.6		0.32	5.48	NT	NT	NT	NG	5.49	75	NT	NT
0.8		NG	5.45	NT	NT	NT	NG	5.47	NT	NT	NT
0.0		1.27	7.18	106	617	723	1.14	7.26	104	492	596
0.1		1.38	7.15	120	855	975	0.98	7.11	96	583	679
0.2	10	0.83	7.06	58	250	308	0.73	6.98	33	309	342
0.4		0.64	5.53	NT	NT	NT	0.27	5.51	NT	NT	NT
0.6		0.31	5.45	NT	NT	NT	NG	5.47	NT	NT	NT
0.8		NG	5.43	NT	NT	NT	NG	5.33	NT	NT	NT

ND: Not determine
NG: No growth for up to 90 days of incubation at 28 ºC
NT: No aflatoxin.

pH of medium:

Data in Table(1) indicated that the initial pH was decreased after 3 days and This decrease was influenced by the licorice extract added , mould strain tested and amount of mould growth that occurred. For *A .parasiticus*. The rate of decrease in the pH value was slower in the medium with extract, 0.2 mg /ml medium than in the media with added licorice extract , 0.0 or 0.1 mg /ml medium. But for *A . flavus* strain , the rate of decrease in pH was slower in the medium supplemented with extract 0.4 mg / ml medium than in the media contained extract concentrations , 0.0 , 0.1 or 0.2 mg /ml medium. After 7 days of incubation, the pH increased in the media supplemented with extract, 0 0, 0.1 and 0.2 mg /ml medium for both all mould tested. The rate of increase in pH was slower in the medium contained extract, 0.2 mg /ml medium than in the media contained extracts, 0.0 or 0.1 mg/ml medium. After 10 days of incubation, another increase in the rate of pH occurred in the media had concentrations, 00, 0.1 or 0.2 mg/ ml medium, the rate of increase probably was influenced by the amount of mycelium that autolysed during the 7-to 10 day incubation interval . The rate of increase in pH in both tested cultures during this period was greater in the medium had extract 0.2 mg/ml medium than was observed when the media had extract concentrations ,0.0 or 0.1mg/ml medium .These results, in terms of change in pH, are similar to those observed by Yousef and Marth (1981) on *A. parasiticus* in the presence of sorbic acid, those obtained by EL-Gendy and Marth (1981) on *A .parasiticus* in the presence of *Lactobacillus casei,* Buchanan (1976) on *A .parasiticus* NRRL 2999 and Utpal *et al.,* (1996) on *Lactococcus lactis subsp.*

Table (2): Change in mycelial dry weight (g/day) produced by *A. parasiticus* and *A. flavus* in 25 ml YES broth medium containing solvent extract of licorice incubated 28°C for up to 10 days.

Incubation time (days)	*A. Parasiticus* [a]					*A. flavus* [a]			
	Extract added to medium (mg/ml)					Extract added to medium(mg/ml)			
	0.0	0.1	0.2	0.4	0.6	0.0	0.1	0.2	0.4
0-3d	0.520	0.44	0.31	0.23	0.15	0.46	0.35	0.03	0.16
3-7d	0.04[c]	0.05[b]	0.018[c]	0.01[c]	0.03[c]	0.045[c]	0.035[b]	0.02[c]	0.045[c]
7-10d	0.05[c]	0.037[c]	0.013[c]	0.003[c]	0.003[c]	0.02[c]	0.037[c]	0.03[c]	0.01[c]
Average rate of net mycelial dry weight (g/day) during cubation period (10 days)	0.13	0.14	0.08	0.06	0.03	0.11	0.1	0.07	0.03

a : No growth for up to 90 day at 28°C when cultures contained licorice extract concentrations, 0.6 or 0.8 mg/ml medium (for A.flavus) or 0.8 mg/ml medium (for all cultures) .

b :Average rate of increase in mycelial dry weight .

c : Average rate of decrease in mycelial dry weight due to bioautolysis .

Aflatoxin production:

The data presented in Table (1) indicate that aflatoxin(s) production by *A flavus* strain was relatively high at the lower levels of added extract (0.1 mg/ml medium). For *A.parasiticus*. 0.1 mg of extract produced approximately 50.6% , 64% , 54.5% reduction in aflatoxin(s) production compared to that of control after 3 , 7 , and 10 days of incubation , respectively . Increasing the concentration of extract above 0.1 mg/ml medium significantly altered aflatoxin (s) production for the two strains and a complete inhibition was occurred at concentrations of licorice extract above 0.2 mg/ml medium These results agree with those obtained by Shimoyamada *et al.* (1996), who studied the inhibitory effect of steroid saponin isolated from the bottom part of white asparagus (*Asparagus officinalis* L.) and those reported by Bratta *et al.,* (1998), Omer *et al.,* (1997) and EL-Gazzer and Marth (1986). It has been reported that a compound is considered as a positive inhibitor if it reduces aflatoxin formation to 50% of that of control (Masimango *et al.,* 1978).

The amount of aflatoxin B_1 produced by both mould strains (Table 1) after 3, 7 or 10 days at 28°C was less than that of aflatoxin G_1. These results agree with those obtained by Bullerman *et al.* (1969); when they studied aflatoxin (s) production in aged dry salami and aged country cured hams.

The data in Table (1) further indicate that the extend of inhibition by licorice extract of aflatoxins B1 plus G_1 during the entire period of incubation (10 days) increased as the concentration of licorice extract increased from 0.1- to 0.8 mg/ml medium. These results, in terms of change of aflatoxin(s) production, are similar to those obtained by Omer *et al.* (1997).

It is important to consider the average rate of net aflatoxin(s) produced during the period of incubation (10 days) (Table 3). The data in Table (3) indicate that for the 0- to 3 day incubation interval , the average rate of aflatoxin (s) production was greater in control media than in the treatment with 0.1 to 0.2 mg of added extract. No aflatoxin (s), B_1 or G_1, were produced

when the media contained 0.4, 0.6 or 0.8 mg/ml medium of added extract. A decrease in the average amount of aflatoxin (s) produced by both mould strains, occurred after 10 days in the presence or absence of 0.1 to 0.2 mg/ml medium of added extract.

Aflatoxin production per unit of growth (Table 4) is a more precise measure of the mold's ability to produce aflatoxin than are the absolute values for aflatoxin accumulation. The extent of stimulation by licorice on production of aflatoxin(s) B_1 plus G_1 during the entire period of incubation (10 days) decreased from 0.0 to 0.8 mg/ ml medium. This is also true for aflatoxin(s), B_1 or G_1 (Table 4). From the foregoing it seams that this calculation was useful to make relative comparisons to demonstrate the effect of additive on aflatoxin product .

Results in this part of the study (Tables 1-4) indicate that the biosynthesis and accumulation of aflatoxin(s) were influenced by concentration of licorice extract in the media, mycelial growth and length of incubation period. These results also indicate that extract at concentration 0.6 mg/ml medium completely inhibited growth by *A. flavus* up to 90 days of icubation at 28°C and the extract concentration 0.8 mg/ml medium completely inhibited mould growth and aflatoxin(s) production for all mould strains tested, up to 90 days at 28°C . However, small concentrations (0.1 mg/ml meduim) stimulated mould growth during the 10- day of incubation at 28°.

Effect of solvent extract of licorice in the medium incubated at 14°C.
Myceliel dry weight:

As indicated from Tables (5 and 6), the amounts of dry mycelium produced by the mould during 90 day incubation was affected by the extract added in the media. A gradual decrease in mycelial dry weight was observed by increasing concentration of added extract during the incubation period (90 day). Mould growth was completely inhibited for up to 90 days in cultures with 0.6 mg/ml medium (for *A. flavus)* and with 0.8 mg/ml medium (for all mold cultures tested).

Our results were similar, in terms of change in mycelial dry weight with those obtained by Farag *et al.* (1989),who studied the influence of some spice essential oil on *A. Parasiticus* growth and production of aflatoxin in a synthetic medium.

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pH of medium:

In the media incubated at 14 °C for 90 days, the initial pH of the medium was adjusted to 5.5. Data in Table (5) indicate that the pH increased after 30 days in the media with extract: 0.0, 0.1 or 0.2 mg/ ml medium. The change of pH was influenced by concentration of extract, amount of mold growth that occurred and the mold strain tested.

After 45 days, the pH of the media with 0.0 , 0.1 and 0.2 mg/ml medium increased. The rate of increase in pH was slower in the media with extract: 0.0, or 0.1 than with 0.2 mg/ml medium, whereas the pH was still decreasing in the media supplemented with extracts > 0.2 mg/ml medium. After 90 days of incubation, the pH increased in the media contained extracts: 0.0 , 0.1 or 0.2-mg/ml medium added extract; the rate of increase was greater in the control than in the media with added extract. The pH of the media with extracts > 0.2 mg/ml medium was still decreasing at the end of the incubation period (90 days). These results, in terms of change in pH value, were similar with those obtained by Utpal *et al.* (1996), who studied the antifungal activity of substances produced by *Lactococcus lactis subsp.,* aginst *A.flavus, A. parasiticus* and *Fusarium SSP.,* when grown at 30°C for 48 hours

Production of aflatoxin :

Aflatoxin(s) production at 14°C was less than that observed at 28°C (Tables 3 and 7). This indicate that temperature is one factor, which affects mould growth and aflatoxin(s) production. This observation was agree with that observed by EL- Gendry and Marth (1980) when they sudied the growth of toxigenic and nontoxigenic *Aspergilli* and *Penicillus* at diffrent temperatures and in the presens of *lactic acid bacteria*.

To demonstrate the activity of the extract in controlling the mould growth and aflatoxin(s) production, relative comparisons were made between the control and treatments and the percentage of inhibition or stimulation of the mould by extract were calculated (Tables 6, 7and 8). Increasing amount of extract from 0.1- to 0.4 mg/ml medium inhibited aflatoxin(s) production by 44.62%, 52.19% and 43%, 49.25% for *A.parasiticus* and *A.flavus,* respectively (Table 8).

Examination of aflatoxin(s) production per unit growth indicated that the decrease in aflatoxin(s) production during incubation period (90 days) was primarily due to inhibition of growth (Table 8).

The inhibitory action of licorice extract might be due to the presence of a phenolic –OH group. It is well known that the – OH group is much more reactive and can easily from hydrogen bonds with the activities of enzymes Michael and Jing (1995) .

Results of our study indicate that 0.6 or 0.8 mg/ml medium of food additive completely prevented growth and aflatoxin(s) production by *A.parasiticus and A.flavus* for up to 90 days of incubation at 14 or 28 °C. It is also evident from our results that , if possible , a sufficient amount of a food additive to prevent mould growth needs to be used if one wishes to prevent aflatoxin(s) production.

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أثر مستخلص نبات العرق سوس على النمو الفطرى وإنتاج الأفلاتوكسينات

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أجريت الدراسة بغرض معرفة تأثير مستخلص نبات العرق سوس على تثبيط نمو وإنتاج الأفلاتوكسينات . تمت الدراسة على نوعى فطر /مختلفين من جنس الأسبرجيللس *A. Flavus, A. Parasiticus* وبأستخدام بيئة مستخلص الخميرة والسكروز كبيئة أساسية وبأستخدام نوعين من المعاملات : المعاملة الأولى : تعرضت فيها السلالات الفطرية المختبرة لتركيزات من المستخلص النباتى عبارة عن ٠,١ ، ٠,٢ ، ٠,٤ ، ٠,٦ ، ٠,٨ ماجم /مل بيئة أساسية و تم التحضين على درجة ٢٨° م لمدة ١٠ أيام تم خلالها إجراء التحاليل لتقدير درجة ال pH ، محتوى الافلاتوكسينات و كمية المادة الجافة الفطرية و ذلك على فترات من التحضين هى ٣ ، ٧ ، ١٠ أيام أما المعاملة الثانية : فقد تعرضت لنفس التركيزات السابقة من المستخلص النباتى و لكن على درجة ١٤° م و لمدة ٩٠ يوم تحضين أجريت خلالها نفس القياسات السابقة فى المعاملة الأولى و لكن على فترات تحضين بلغت، ٣٠ ، ٤٥ ، ٩٠ يوم . أسفرت النتائج أن مستخلص نبات العرق سوس له تأثير مثبط على نمو هذين النوعين من الفطر و كذلك منع أنتاج التوكسيين حيث تبين أن تركيز ٠,٦ ملجم من المستخلص / مل بيئه أساسية كان كافياً لمنع النمو الفطرى و تثبيط إنتاج التوكسين بالنسبة للفطر A.flavus أما التركيز ٠,٨ ملجم / مل بيئه أساسيه كان كافياً لمنع نمو و إنتاج التوكسين بالنسبة لكلا السلالاتين المختبرتين . و هذه النتائج تشجع على أستمرار البحث لتحديد مدى إمكانية تطبيق استخدام هذا المستخلص كمادة حافظه غذائيه حيث أن هذا النبات هو مادة آمنه صحياً و رخيصة الثمن .

Table (3): Average rate of accumulation or degradation of aflatoxin(s) B_1 and G_1 (μg/day) produced *by A. parasiticus* and *A. flavus* in 25 ml YES broth medium containing chloroform extract of licorice incubated at 28 ºC for up to 10 days .

Period of incubation (days)	*A. Parasiticus*[a]						*A. Flavus*[a]					
	Amount of extract added (mg /ml)						Amount of extract added (mg /ml)					
	0.0		0.1		0.2		0.0		0.1		0.2	
	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1
0 – 3d	22	288.7	29.7	200	5	6.7	19.7	185.3	20.3	132.3	ND	8.31
3 –7d	15.3[b]	94.8[b]	16.8[b]	128.5[b]	21[b]	80.5[b]	14.8[b]	75.8[b]	13.25[b]	883[b]	10.7[b]	208[b]
7 – 10d	3[c]	149.3[c]	12[c]	86.3[c]	13.67[c]	30.7[c]	4.7[c]	122.3[c]	6[c]	55.7[c]	14[c]	66.3[c]
Average rate of net aflatoxin produced during period of incubation (10d)	10.6	61.7	12	85.5	5.8	25	10.4	49.2	9.6	58.3	3.3	30.9

a: No aflatoxin produced for up to 90 days at 28ºC when cultures contained licorice extract concentrations, 0.6 or 0.8 mg/ml medium (for *A.flavus*) or 0.8 mg/ml medium (for all cultures) .

b: Average rate of aflatoxin accumulation **c: Average rate of aflatoxin degradation .**

Table (4) : Effect of licorice extract on the average rate of net aflatoxins B_1 and G_1 produced by one gram of mycelial dry weight of *A. parasiticus* and *A. flavus* in one day at 28ºC for up to 10 days .

Item	*A.Parasiticus*[a]			*A.Flavus*[a]		
	Extract added medium (mg /25 m l)			Extract added medium (mg /25 ml)		
	0.0	0.1	0.2	0.0	0.1	0.2
Average rate net aflatoxin produced by 1 gm of mycelial dry wt. in 1 day						
Aflatoxin B_1	81.54	85.71	72.50	94.55	96	62.86
Aflatoxin G_1	474.62	610.71	312.50	447.27	583	441.43
Aflatoxins B_1+ G_1	556.16	696.42	385	541.82	679	504.29
Net amount of inhibition or stimulation (%)						
Aflatoxin B_1		5.11[c]	11.09[b]		1.53[c]	33.52[b]
Aflatoxin G_1		28.8	34.16[b]		30.35[c]	1.31[b]
Aflatoxins B_1 + G_1		25.22[c]	30.77[b]		25.32[c]	6.93[b]

a: No aflatoxin produced for up to 90 days at 28ºC when cultures contained licorice extract concentrations, 0.6 or 0.8 mg/ml medium (for *A.flavus*) or 0.8 mg/ml medium (for all cultures) .

b: % inhibition

c: % stimulation

Table(5): Mycelial dry weight (g/ 25 ml medium), pH and aflatoxin content (μg / 25 ml medium) in cultures containing licorice extract and incubated at 14°C for up to 90 days.

Extract added (mg/ml medium)	Incubation time (days)	*A.parasiticus*					*A.Flavus*				
		Mycelial dry weight	pH	Aflatoxin (μg / 25 ml medium)			Mycelial dry weight	pH	Aflatoxin (μg / 25 ml medium)		
				B_1	G_1	Total			B_1	G_1	Total
0.0		1.66	6.40	289	2382	2671	1.42	6.60	279	1951	2230
0.1		1.63	6.35	127	952	1079	1.33	6.51	89	650	739
0.2		1.41	6.68	85	318	403	1.27	6.38	60	423	483
0.4	30	0.88	5.40	NT	NT	---	0.97	5.33	NT	NT	---
0.6		0.51	5.36	NT	NT	---	0.53	5.37	NT	NT	---
0.8		NG	5.28	NT	NT	---	NG	5.34	NT	NT	---
0.0		1.18	6.85	123	968	1091	0.98	6.85	115	788	903
0.1	45	1.31	0.97	83	442	525	1.04	7.10	55	300	355
0.2		0.75	7.10	61	255	316	0.71	7.25	46	343	389
0.4		0.36	5.19	NT	NT	---	0.53	5.30	NT	NT	---
0.6		0.38	5.17	NT	NT	---	NG	5.30	NT	NT	---
0.8		NG	5.13	NT	NT	---	NG	5.27	NT	NT	---
0.0	90	1.02	7.19	56	635	691	0.65	7.20	55	512	567
0.1		1.13	7.11	47	405	452	0.69	7.15	46	310	356
0.2		0.32	7.00	12	96	108	0.33	7.05	15	136	151
0.4		0.13	5.11	NT	NT	---	0.17	5.27	NT	NT	---
0.6		NG	5.15.1	NT	NT	---	NG	5.22	NT	NT	---
0.8		NG	5	NT	NT	---	NG.	5.20	NT	NT	---

NG: No growth
NT: No aflatoxin

Table (6): Average rate of increase or decrease in dry mycelial weight (ml/day) produced by *A. parasiticus* and *A. flavus* in 25 ml YES broth medium containing solvent extract of licorice incubated for up to 90 day at 14 ºC.

Incubation time (days)	*A. Parasiticus* [a]					*A. flavus* [a]			
	Extract added to medium (mg/ml)					Extract added to medium(mg/ml)			
	0.0	0.1	0.2	0.4	0.6	0.0	0.1	0.2	0.4
0-3d	0.06	0.054	0.047	0.029	0.017	0.047	0.044	0.042	0.032
3-7d	0.032[b]	0.02[b]	0.044[b]	0.035[b]	0.009[b]	0.03[b]	0.02[b]	0.037[b]	0.029[b]
7-10d	0.004[b]	0.004[b]	0.01[b]	0.006[b]	0.008[b]	0.007[b]	0.008[b]	0.008[b]	0.008[b]
Average rate of net mycelial dry weight (g/day) during incubation period (10days)	0.011	0.013	0.0036	0.03	0.00	0.007	0.0077	0.0037	0.0019

a : No growth for up to 90 day at 14ºC in cultures contained licorice extract concentrations, 0.6 or 0.8 mg/ml media (for *A. flavus*) and 0.8 mg/ml media (for all cultures).

b : Average rate of decrease in mycelial dry weight due to bioautolysis .

Table (7) : Average rate of accumulation or degradation of aflatoxin(s) B_1 and G_1 (µg /day) produced by *A.parasiticus* and *A. flavus* in 25 ml YES broth medium containing solvent extracts of licorice incubated at 14 ºC for 90 days

Period of incubation (days)	*A. Parasiticus*[a]						*A. Flavus*[a]					
	Amount of extract added (mg /ml)						Amount of extract added (mg /ml)					
	0.0		0.1		0.2		0.0		0.1		0.2	
	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1	B_1	G_1
0 – 30d	9.6	79.4	4.2	31.7	2.8	10.6	9.3	53	3	21.7	2	14.1
30 –45d	11.1[b]	94.3[b]	2.9[b]	34[b]	1.6[b]	4.2[b]	10.9[b]	53.5[b]	2.3[b]	23.3[b]	0.9[b]	5.34[b]
45 – 90d	1.5[b]	7.4[b]	0.8[b]	0.82[b]	1.09[b]	3.53[b]	1.3[b]	6.13[b]	0.2[b]	0.2[b]	0.67[b]	4.6[b]
Average rate of net aflatoxin produced during period of incubation (90d)	0.62	7.05	0.52	4.5	0.13	1.07	0.61	5.69	0.51	3.44	0.18	1.51

a: No aflatoxin produced for up to 90 days at 14ºC when cultures contained licorice extract concentrations, 0.6 or 0.8 mg/ml medium (for *A.flavus*) or 0.8 mg/ml medium (for all cultures) .

b: Average rate of aflatoxin degradation .

Table (8} : Effect of licorice extract on the average rate of net aflatoxin(s) $B_1$and G_1 produced by one gram of mycelial dry weight of *A.parasiticus* and *A. flavus* in one day at 14°C for up to 90 days .

Item	*A.Parasiticus*			*A.Flavus*		
	Extract added medium) (mg /25 ml)			Extract added medium) (mg /25 ml		
	0.0	0.1	0.2	0.0	0.1	0.2
Average rate net aflatoxin(s) produced by 1 gm of mycelial dry wt. in 1 day						
Aflatoxin B_1	56.3	40	36.11	87.14	66.23	48.65
Aflatoxin G_1	640.91	346.15	297.22	812.86	446.75	408.11
Aflatoxin(s) B_1+G_1	697.27	386.15	333.38	900	512.98	456.76
Net amount of inhibition[b] or stimulation[c] (%)						
Aflatoxin B_1		29.03[b]	35.93[b]		23.99[b]	44.17[b]
Aflatoxin G_1		45.99[b]	53.62[b]		45.03[b]	49.79[b]
Aflatoxin(s) B_1+G_1		44.62[b]	52.19[b]		43.00[b]	49.25[b]

a: No aflatoxin produced for up to 90 days at 14°C when cultures contained licorice extract concentrations, 0.6 or 0.8 mg/ml medium (for *A. flavus*) or 0.8 mg/ml medium (for all cultures) .