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INHIBITION OF *ASPERGILLUS PARASITICUS* GROWTH AND AFLATOXIN PRODUCTION BY SOME ESSENTIAL OILS

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ABSTRACT

The essential oils of lemon grass, geranium, sweet margorum, Brazilian pepper tree, santinica and hazanbil leaves were obtained by steam distillation. The chemical composition of these oils was elucidated by gas-liquid chromatography. The essential oils under study were distinguished by having basic components belonging to different organic classes, i.e., aliphatic aldehydes, aliphatic alcohols, cyclic monoterpene alcohol, aromatic hydrocarbons and aliphatic hydrocarbons for lemon grass, geranium, sweet margorum, Brazilian pepper tree, gantonica and hazanbal, respectively. The influence of these essential oils at different levels on *Aspergillus parasiticus* growth and aflatoxin production was evaluated. The fungus was allowed to grow on a yeast extract-sucrose media in a stationary culture incubated in the dark at 26 ± 1 °C for 7 days. Administration of the essential oils caused a concomitant decrease in both mycelial dry weight and aflatoxin production. This was entirely dose dependent. In general, the inhibition effect of the oils on *A. parasiticus* growth and aflatoxin production followed the sequence: lemon grass > geranium > sweet margorum > hazanbal > santonica > Brazilian pepper tree. It appears that there is a relationship between the chemical structure of the most abundant substances in the essential oils under study and the antifungal potency.

Key Words: Antifungal potency, chemical structure, organic compounds

RÉSUMÉ

Les huiles essentielles des feuilles de citronelle, geranium, margorum sucré, arbre à poivre brésilien, santinica et d'hazanbal étaient obtenues par distillation à la vapeur. La composition chimique de ces huiles étaient déterminée par la chromatographie à gaz liquide. Les huiles essentielles étudiées étaient distinguées grâce à leurs composantes de base appartenant à différentes classes organiques telsque les aldéhides aliphatiques, les alcohols aliphatiques, l'alcohol cyclique monoterpène, les hydrocarbones aromatiques et les hydrocarbones aliphatiques respectivement pour la citronelle, le geranium, le margorum sucré, l'arbre à poivre brésilien, le santonica et l'hazanbal. L'influence des huiles essentielles à différents niveaux de production d'*Aspergillus parasiticus* et d'aflatoxine était évaluée. Le champignon poussait sur une lévure d'un milieu d'extract de sucrose en culture stationaire incubée à 26 ± 1 °C à l'ombre pendant 7 jours. L'administration d'huiles essentielles a causé une réduction concomitante du poids sec mycelial et de la production d'aflatoxine. Cette découverte dépendait de la dose utilisée. En général, l'effet d'inhibition des huiles sur la croissance d'*A. parasiticus* et la production d'aflatoxine suivait la séquence citronelle > geranium> margorum sucré >hazanbal> santonica> arbre à poivre brésilien. Il semblerait qu' il y a une relation entre la structure chimique de la plus part de substances abondantes dans les huiles essentielles étudiées et la potence antichampignon.

Mots Clés: Potence antichampigon, structure chimique, composants organiques

INTRODUCTION

Aflatoxins are toxic secondary metabolites that can be produced on different seeds by the widely distributed fungi *Aspergillus flavus* and *A. parasiticus*. Several investigators have demonstrated that aflatozins are acutely toxic, teratogenic, carcinogenic and mutagenic (Ciegler, 1915; Goldblatte, 1969; Hayes, 1978). Although the aflatozins are closely related chemically, they differ greatly in their biological effects. There are four common types of aflatoxins, i.e., B_2, B_2, G_1 and G_2; aflatoxin B_1 is the most carcinogenic. In this respect, sensitive rat strains develop tumors at aflatoxin B_1 dietary levels of 1 ppb (Wogan *et al.*, 1971). It appears that there is a positive correlation between the consumption of aflatoxin contaminated foods and the increase of liver cancer in several countries (Shank *et al.*, 1972; Peers and Linsell, 1973; Shank, 1976; Groopman *et al.*, 1988).

Preventing the contamination of foods by the toxigenic fungi *A. flavus* and *A. parsiticus* and detoxification of aflatoxin containing foods are the most rational way to avoid the hazards. In this respect, a number of reagents have been examined for their ability to reduce aflatoxin levels in contaminated agricultural commodities. These reagents are generally acids, bases and oxidising agents (Dollear, 1969; Mann *et al.*, 1970, 1971; Goldblatt, 1971; Brekke, 1978; Marth and Doyle, 1979).

It has been shown that NaOH can destroy aflatoxins in peanut meal at elevated temperatures (Dollar *et al.*, 1968). Gaseous ammonia, with and without heat and pressure, has also been used to eliminate aflatoxins from cottonseed and peanut meals (Dollear and Gadener, 1966; Dollear *et al*, 1968; Nofsinger and Anderson, 1979).

Recently, there has been increasing interest in using naturally occurring compounds especially essential oils to limit fungal growth and toxin production. In this respect, Hitokoto *et al.* (1980) demonstrated that mustard, green garlic, cinnamon bark and hops inhibited fungal growth, while pepper, clove, thyme and green tea only inhibited toxin production. Farag *et al.* (1989) found that some essential oils caused complete inhibition of both mycelial growth and aflatoxin production. The effectiveness of the oils followed the sequence: thyme > cumin > clove > caraway > rosemary > sage. Daw (1989) studied the effect of flavoured additives on growth and aflatozin production in chick pea. Some compounds at high concentrations controlled aflatoxin formation. The inhibitory effect of these compounds on aflatoxin formation was arranged in the sequence : lemon juice < hot pepper < cumin < NaCl < mixture of all. The main objective of the present study was to evaluate some essential oils obtained from certain plants for prevention of *A. parasiticus* growth and aflatoxin formation.

MATERIALS AND METHODS

Mould. *Aspergillus parasiticus* (ATCC 120920) was obtained from Tropical Products Institute, London, England. This strain was checked for purity and identity (Raper and Fennell, 1965). The mould was grown on a potato - dextrose - agar (Difco) slant for 10 days at 28°C. Spores were harvested by adding sterilised Tween 80 solution (0.01%, v/v); filtered through several layers of sterilised cheese cloth; centrifuged (300 x g); washed three times with sterilised distilled water; and re-suspended in a sterilised Tween 80 solution (0.01%, v/v). The number of conidia was estimated by plate count and the suspension was adjusted to contain approximately 10^6 spores ml^{-1}.

Essential oils. Leaves of lemon grass (*Andropogon citratus*), geranium (*Pelargonium graveolens*), sweet margorum (*Majorana hortenois*), (Achillea *specific name needed*), Brazilian pepper tree (*Schinus terebinthifolius* and santonica (*Artemisia cince*), were collected from the Agricultural Farm, Cairo University. The plant materials, cut into small pieces (ca 100 g), were placed in a flask (2 L) with double distilled water (1.5 L). A steam distillation continuous extraction head was attached to the flask. After steam distillation (ca 3 hr), the oil was isolated and dried over anhydrous sodium sulfate.

Authentic volatile compounds. A set of 25 standard materials with a stated purity of 99% by GLC was obtained from Dragoco Company (Hozminden, Germany). The standard materials were - pinene, - myrcene, -phellandrene, P - cymene, limonene, elemene, - terpinene, -

phellandrene, citronellal, terpinolene, linalool, camphor, linalyl acetate, cadinene, 4 - terpineol, methyl chavicol, citronellol, batchoulene, neral, geranial, geraniol, geranyl acetate, elemol and nerolidol.

Identification and determination of essential oil composition. The constituents of the essential oils under study were determined qualitatively and quantitatively by gas-liquid chromatography. A Pye Unicam PU 4550 chromatograph equipped with dual flame ionisation detectors and glass column (1.4 m x 4 mm) packed with 10% PEGA, was employed. The oven temperature was programmed at 4 °C for 15 min. Detector and injector temperatures were 220 °C min^{-1} from 60 to 190 °C, and was held at 190 °C for 15 min. Detector and injector temperatures were 220 and 300 °C, respectively. Gas flow rates for N_2, H_2 and air were 30, 33 and 330 ml min^{-1}, respectively. Peak identification was performed by comparing the relative retention times of each peak with those of known compounds. Also, the essential oils were mixed with their major compounds and injected into GLC to verify peak identity. The peak and relative area percentages were calculated using PU 4810 computing integrator (Philips). All samples were analysed in triplicates and used if results agreed within 2%. Mean values are presented in the text.

Culture conditions. Yeast-extract-sucrose medium (YES) was used as a basal medium for aflatoxin production in stationary cultures (Davis *et al.*, 1966). One ml of spore suspension was added to 40 ml YES medium in a 250 ml Erlenmeyer flask. Appropriate amounts of various essential oils were added to the culture medium to give concentrations of 0.2, 0.4, 0.8, 1.0 and 2 mg ml^{-1} medium. After the addition of each essential oil, the culture was incubated in the dark at 26 ± 1 °C for 7 days.

Determination of mycelial dry weight. At the end of the incubation period, the cultures were filtered under vacuum through Whatman No. 1 filter paper. The paper containing the mycelium was washed several times with distilled water and discarded. The mycelium was dried at 100 ° C for 14 hr and weighed.

Determination of aflatoxins. An aliquot from the culture filtrate (10 ml) was extracted twice with chloroform (20 ml each time). The combined chloroform extract was filtered through anhydrous sodium sulfate and the solvent evaporated to dryness. Aflatoxins were separated by thin layer chromatographic technique using precoated plates with silica gel 60 (0.2 mm thickness) and developed using a mixture of chloroform / acetone (9:1, v/v). The chromatoplates were air-dired and viewed under UV light (365 mm). Aflatoxins were measured by comparing the fluorescence intensity of the samples with the fluorescence of known concentrations of standard aflatoxins. Each determination was conducted in triplicate (and the data accepted within 2%).

RESULTS AND DISCUSSION

Table (1) presents the constituents of the steam - distilled essential oils of geranium, sweet margorum, Brazilian pepper tree, limon grass, santonica and hazonbal. The identification of each essential oil component was made by comparing the retention times of these components with those of the available authentic materials and with a collection of literature chromatogrammes which had nearly similar fractionation conditions. Eleven compounds, amounting to about 80% of the essential oil components of the geranium oil, were characterised. The genenium oil was very rich in the alcoholic compounds, i.e., geraniol (33.50%), citronellol (23.68%), Linalool (10.14%) and terpineol (5.81%).

The main constituents of sweet margorum oil were alcohols, esters and hydrocarbons. The conspicuous components were terpineol (26.85%), linalyl acetate (22.38%) and limonene (9.32%). The basic components of the Brazilian pepper tree oil were monoterpenes, sesquiterpens and hydrocarbons (> 70% of total) such as myrecene (22.68%), elemene (22.34%), limonene (16.72%) and -phellandrene (6.05%).

The lemon grass oil and santonica were very rich in aldehydic substances, the most abundant aldehydes being geranial (43.56%) and neral (31.96%), whilst citroellal (46.20%) was the most abundant compound in santonica oil. Hazonbal essential oil was distinguished by having the highest level of -phellandrene (59.68%) compared

TABLE 1. The chemical constituents of some essential oils

Component	Geranium	Sweet morgorum	Brazilian pepper tree	Lemon grass	Santonica	Hazanbal
- Pinene	-	-	-	0.73	-	-
- Pinene	0.06	-	-	0.10	0.06	2.69
Myrcene	0.20	1.45	22.68	1.46	-	-
- phellandrene	-	4.84	-	0.05	0.90	0.90
- Cymene	-	-	-	-	1.42	-
Limonene	1.17	9.32	16.72	7.20	5.78	9.73
Elemene	-	-	22.34	-	-	-
Terpinene	1.37	7.81	-	0.30	11.77	2.51
Phellandrene	-	6.83	6.05	0.70	5.79	59.68
Citronellal	-	-	-	0.79	46.20	0.06
Terpinolene	0.23	4.59	-	-	-	2.90
Linalool	10.14	-	0.53	0.16	-	2.30
Camphor	-	-	-	-	-	2.40
Linalyl acetate	-	22.38	0.33	0.82	12.87	6.30
Cadinene	-	-	4.06	0.59	-	3.29
Terpineol	5.87	26.85	1.28	1.38	1.65	-
Methly chavical	-	2.56	0.63	-	-	-
Citronellol	23.68	2.70	6.10	-	-	-
Batchoulene	-	2.07	2.44	-	-	-
Neral	-	-	-	31.96	-	1.50
Geranial	5.37	-	-	43.56	-	0.16
Geraniol	33.50	-	1.80	-	-	-
Gerznyl acetate	-	-	-	3.20	-	0.40
Elemol	1.61	0.20	4.60	-	-	-
Nerolidol	-	0.15	0.10	-	-	-
Unidentified Compounds	-	8.25	9.84	7.00	13.56	5.78

to the other oils under study. In general, all the essential oils showed remarkable qualitative and quantitative differences. Similar results were obtained by other authors (Masada, 1976; Yashphe *et al.*, 1979).

The influence of some essential oils on *A. parasiticus* growth and aflatoxin production in yeast extract sucrose media is shown in Table 2. The results demonstrated that fungal activity was largely dependent upon the type of essential oil and its concentration. For instance, lemon grass oil at low concentrations of 0.2 and 0.4 mg ml^{-1} caused a reductions in mycelial growth of 41 and 84% and aflatoxin production of about 52 and 96%, respectively. The high levels of lemon grass oil (0.8-2 mg ml^{-1}) inhibited both the fungal growth and aflatoxin production.

Similar activity data were recorded for geranium oil. The application of geranium oil at 0.2 and 0.4 mg ml^{-1} reduced the mycelial growth by 8% and 45% and aflatoxin production by 11% and 79%, respectively. At high levels (0.8 - 2 mg ml^{-1}), it completely inhibited fungal growth and aflatoxin formation. Sweet margorum oil at 0.2 mg ml^{-1} induced an increase in both fungal growth and aflatoxin production. The administration of other oil concentrations (0.4, 0.8 and 1.0 mg ml^{-1}) caused a gradual decrease in both fungal growth and aflatoxin synthesis. The highest level of sweet margoram oil (2.0 mg ml^{-1}) prevented fungal growth and aflatoxin production.

Hazanbal, santonica and Brazilian pepper tree oils caused a gradual decrease in both the fungal mycelium dry weight and aflatoxin production with increasing levels of applied oil.

There was a concomitant decrease in mycelial dry weight and aflatoxin production in all cases. In general, the inhibition effect of the oils on *A. parasiticus* growth and aflatoxin production followed the sequence: lemon grass > geranium > sweet margorum > hazanbil > santonica > Brazilian pepper tree. The essential oils under study are distinguished by certain basic compounds, i.e., aliphatic aldehydes (neral, geranial, 74.5%),

TABLE 2. The chemical constituents of some essential oils

Conc. (mg ml^{-1})	Mycelial wt (g/40 ml)	Inhibition %	aflatoxin conc. (µg ml^{-1}) B^1	B^2	G	G^2	Total	Inhibition %
Control	1.55	-	78.4	2.8	62.1	2.1	145.4	
0.2	0.92	41	47.0	0.3	11.6	T*	58.9	52
0.4	0.25	84	4.3	0.0	1.4	0.0	5.7	96
0.8	0.0	100	0.0	0.0	0.0	0.0	0.0	100
1.0	0.0	100	0.0	0.0	0.0	0.0	0.0	100
2.0	0.0	100	0.0	0.0	0.0	0.0	0.0	100
0.2	1.43	8	62.7	2.8	62.1	2.1	129.7	11
0.4	0.86	45	15.7	T	15.5	T	31.2	79
0.8	0.0	100	0.0	0.0	0.0	0.0	0.0	100
1.0	0.0	100	0.0	0.0	0.0	0.0	0.0	100
2.0	0.0	100	0.0	0.0	0.0	0.0	0.0	100
0.2	1.46	6	47.0	2.2	23.3	1.6	74.1	79
0.4	1.38	11	31.4	1.7	15.5	0.6	49.2	66
0.8	1.24	20	15.7	0.6	11.6	0.3	28.2	81
1.0	1.20	23	15.7	0.6	11.6	0.3	28.2	81
2.0	0.51	67	4.3	0.0	1.4	0.0	5.7	96
0.2	1.88	+21	125.4	3.3	108.8	2.6	240.1	+65
0.4	1.39	10	94.1	2.8	93.1	2.1	192.1	+32
0.8	1.19	23	47.1	1.1	23.3	0.6	72.0	50
1.0	0.70	55	2.9	0.0	1.4	0.0	4.3	97
2.0	0.0	100	0.0	0.0	0.0	0.0	0.0	100
0.2	1.51	3	31.4	1.1	23.3	0.6	56.4	61
0.4	1.5	3	39.2	1.1	23.3	0.6	64.2	56
0.8	1.48	5	31.4	1.1	23.3	0.6	56.4	61
1.0	1.41	9	31.4	1.1	15.5	0.6	48.6	67
2.0	1.42	8	31.4	1.1	15.5	0.3	48.3	67
0.2	1.53	1.0	47.0	2.2	23.3	1.6	74.1	49
0.4	1.57	+1.0	39.2	2.2	23.3	1.6	66.3	54
0.8	1.28	17	31.4	1.1	15.5	0.8	48.8	66
1.0	1.26	19	31.4	0.6	11.6	0.0	43.6	70
2.0	1.04	33	8.5	0.0	2.8	0.0	11.3	92

T* = Trace
\+ = In excess of the control

aliphatic alcohols (geraniol, citronellol, 56.68%), cyclic monoterpene alcohol (terpineol, 26%), aromatic hydrocarbons (-phellandren, 59.64%) and aliphatic hydrocarbons (myrecene, 22.68%). There seems to be a relationship between the chemical structure of the most abundant substances in the essential oils under investigation and the antifungal potency. It is well known that aldehydes (e.g. formaldehyde) and alcohol (e.g. ethanol) possess remarkable disinfectant property. In addition, it is well established that - CHO and - OH groups are much more reactive and can easily form hydrogen bonds with the active sites of enzymes (Farag *et al.*, 1989). Accordingly, lemon grass and geranium oils which contain aldehydic and hydroxylic groups, respectively, possessed higher inhibitory effect. On the other hand, hazanbil and Brazilian pepper tree oil which contain aromatic and aliphatic hydrocarbons caused the least inhibitory action. Similar results

were obtained by Bullerman *et al.* (1977), Yashphe *et al.* (1979), Hitkoto *et al.* (1980), Tharib *et al.* (1983), and Hussein Ayoub (1990).

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REFERENCES

Brekke, O.L., String Fellow, A.C. and Peplinski, A.J. 1978 Aflatozin inactivation in corn by ammonia: Laboratory trials. *Journal of Agricultural Food Chemistry* 26:1383-1389.

Bullerman, B., Lieu, F. Y. and Seier, S.A. 1977. Inhibition of growth and aflatoxin production cirnamcn and clove oils. Cinnamic aldehyde and eugenol. *Journal of Food Science* 42:1107 - 1109.

Ciegler, A. 1975. Mycotoxins: Occurrence, chemistry, biological activity. *Lloydia* 38: 21-35.

Davis, N. D., Diener, U.L. and Eldridge, D.W. 1966. Production of aflatoxin B, and G, by *Aspergillus flavus* in a semi-synthetic medium. *Applied Microbiology* 14:378-380.

Daw, Z. Y. 1989. Some factors affecting aflatoxin production on Lupine, chick pea and nut sedge by *Aspergillus parasiticus. Chemical Microbiology Technology Lebensm* 12:24-30.

Dollear, F. G. 1969. Detoxification of aflatoxin in food and feeds. In: *Aflatoxin - Scientific background, Control and Implications.* Goldblatt, L. A. (Ed.), pp. 329-391. Academic Press, New York and London.

Dollear, F. G. and Gardener, H.K. 1966. Activation and removal of aflatoxin. Pages 72 - 81. *Proceeding 4th National Peanut Research Conference*, Tifton, Georgia, July 14-15.

Dollear, F.G., Mann, G.E., Codifer, L.P., Gardener, H. K., Koltun, Jr. S. P. and Vix, H.L.F. 1968. Elimination of aflatoxins from peanut meal. *Journal of American Oil Chemical Society* 54:862-865.

Farag, R.S., Daw, Z.Y. and Abo-Raya, S.H. 1989. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *Journal of Food Science* 54:74-76.

Goldblatt, L. A. (Ed.). 1969. *Aflatozin-scientific background, Control and Implications.* Academic Press. Inc., New York and London.

Goldblatt, L.A. 1971. Control and removal of aflatoxin. *Journal of American Oil Chemical Society* 48:605-608.

Groopman, J. D.,Cain , L. G. and Kensler, T. W. 1988. Aflatozin exposure in human population: measurement and relationship to cancer. *CRC Critical Reviews in Toxicology* 19:113-146.

Hayes, A. W. 1978. Mycotozin teratogenicity. In: *Toxins: Animal, Plant, and Microbial.* Proceedings of the 5th International symposium. Rosenberg, P. (Ed.), pp. 739-758. Pergamon Press, New York.

Hitokoto, H., Morozumi, S., Wauke, T., Sakai, S. and Kurata, H. 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Applied Environmental Microbiology* 39:818-822.

Hussein Ayoub, S. M. 1990. Antibacterial and antifungal activities of some Libyan aromatic plants. *Planta Med.* 56:644-645.

Mann, G.G., Gardener, H. K., Booth, A. N. and Grumbmann, M.R. 1971. Aflatoxin inactivation. Chemical and biological properties of ammonia and methylamine-treated cottonseed meal. *Journal of Agricultural Food Chemistry* 19:1155-1158.

Mann, G.E., Godifer, L.P, Gardener, H. K., Kotteen, S.P. and Dollear, F.G. 1970. Chemical inactivation of aflatoxins in peanut and cottonseed meals. *Journal of American Oil Chemical Society* 47:173-176.

Math, E.H. and Doyle, M.P. 1979. Update on mold: Degradation of aflatoxin. *Food Technology* 33:81-87.

Masada, Y. (Ed.). 1976. *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry.* John Wiley and Sons. Inc., New York, London.

Nofsinger, G.W. and Anderson, R.A. 1979. Note on inactivation of aflatoxin in ammonia-treated shelled corn at low temperatures. Cures. *Cereal Chemistry* 56:120-122.

Peers, F.G. and Linsell, C.A. 1973. Dietary

aflatoxins and liver cancer population based study in Kenya. *British Journal of Cancer* 27:473- 484.

Raper, K. B. and Fennel, D. I. 1965. *The Genus Aspergillus*. Williams and Wilkins Company, Baltimore, MD.

Shank, R. C. 1976. The role of aflatoxin in human disease. In: *Mycotoxins and Other Fungal Related Food Problems*. Rodricks, J. V. (Ed.), pp. 51-57. Am. Chem. Soc. Washington. DC.

Shank, R.C., Wogan, G.N., Gibson, J.B. and Nondasuta, 1972. Dietary aflatoxins and human liver cancer II. Aflatoxins in market food and food stuffs of Thailand and Hongkong. *Food Cosmet. Toxicology* 10:61-69.

Tharib, S.M., Gana, S.O. and Veitch, G.B.A. 1983. Antimicrobial activity of compounds from *Artemisia campestris*. *Journal of Food Protection* 64:185-187.

Wogan, G.N., Edwards, G.S. and Newberne, P.M. 1971. Structure-activity relation in toxicity and carcinogenicity of aflatoxin analogs. *Cancer Research* 31:1936-1942.

Yashphe, J., Segal, R., Breuer, A. and Naflali, G.E. 1979 Antibacterial activity of Artemisa herba ala. *Journal of Pharmaceutical Science* 68:942-944.