Comparative studies of antifugal potentialities for some natural plant oils against different fungi isolated from poultry

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RESUMEN

Estudios comparativos de posibles actividades antifúngicas de aceites de plantas naturales frente a diferentes aislados de hongo de aves de corral

El efecto inhibidor de ocho aceites naturales sobre diez aislados de hongos patógenos de los tractos digestivo y respiratorio de pollos muertos en "Kena Governorate" mostró que el aceite de menta crudo tiene un mayor efecto frente a algunos aislados y una repuesta menor frente a otros. Aunque sus concentraciones en aceite al 10% y 2% consiguieron dar algún efecto frente a todos los hongos ensayados. Aceites de geranio y manzanilla crudo mostraron efecto moderado frente a todos los aislados de hongos. El efecto de disoluciones diferentes de aceites de manzanilla, comino y apio dieron como resultado que la concentración al 10% era más efectiva que el aceite crudo. Aceites de lemongras y albahaca tienen casí el mismo comportamiento con respecto a los aislados de hongo ya que los aceites crudos y las concentraciones al 10% les afectaron grandemente. Por otro lado aceite de albahaca al 2% no dió ningun efecto. Se calcularon las concentraciones críticas de los aceites con eficacia frente a aislados de hongo. Los aceites más eficaces fueron lemongras frente *Aspergillus flavipes,* Manzanilla frente a *A. fumigatus* y comino frente a *A. nidulans,* mientras que comino frente a *A. glacus,* clavo frente a *A.flavus,* manzanilla frente a *A. flavus* y clavo frente a *A. flavipes* fueron los aceites con menor eficiencia.

PALABRAS–CLAVE: Aceite de planta — Actividad antifúngica — Ave de corral — Hongo.

SUMMARY

Comparative studies of antifungal potentialities for some natural plant oils against different fungi isolated from poultry

The inhibitory effect of eight natural oils on ten pathogenic fungi isolated from the digestive and respiratory tracts of dead chickens in Kena Governorate showed that crude peppermint oil only has a highest effect against some isolated fungi and a low response against others. While its 10% and 2% oil concentrations failed to give any effect against all the tested fungi. Crude chamomile and pelargonium oils showed moderate effect against all isolated fungi. The effect of different dilutions of chamomile, cumin and celery oils appeared that the 10% concentration showed more effective than the crude oil. Lemongrass and basil oils have almost the same behaviour towards the isolated fungi as the crude oils and the 10% concentration affected them greatly. On the other hand 2% basil oil gave no effect at all. Critical concentrations of the efficient oils against isolated fungi were calculated. The most efficient oils were lemongrass against *Aspergillus flavipes*, chamomile against *A fumigatus* and cumin against *A. nidulans,* while cumin against *A. glaucus,* clove against *A. flavus* were chamomile against *A. flavus* and clove against *A. flavipes* were the lowest efficient oils.

KEY–WORDS: Antifungal potentiality — Fungi — Plant oil — Poultry.

1. INTRODUCTION

One of the main problems in the poultry farms is the increase in mortality rate due to fungal infections. The fungal infections of poultry causes economic losses particularly when associated with other infections. Mycotic diseases in birds have higher incidence in open and dusty yards because spores are ubiquitous in nature, so birds get frequently infected by inhalation and/or ingestion (El-Badri and Shatta, 1988). Pathogenic fungi have been isolated by many researchers from chickens and the surrounding environment Refai *et al.* ,1971; Safi, 1976; Abou-Gabal *et al.*, 1977, El-Batrawi, 1980 and El-Badri and Sokkar (1988).

Sprecher (1984) had reported that about 150 of natural occurring and semi synthetically derived antibiotics saled in markets. Their outstanding clinical value, their limitations in view of changing patterns of resistance and their side effects together with a demand on improving their pharmacokinetic properties provide a powerful incentive to continue research. However, the increasing problems with the side or adverse effects of antibiotics initiated from unicellular prokaryotes was attributed to their pharmacodynamics with organized multicellular communities *in vivo.*

Essential oils, like many other plant extracts have been found to be equipped with a number of pharmacodynamic and antiseptic properties. Some of the essential oils have been found to be effective; non phytotoxic and easily biodegradable, as well as being antifungal agents (Garg and Dengre, 1988).

The present investigation aimed to illustrate the in-vitro activity of some natural plant oils on controlling the growth of some pathogenic fungi isolated from the digestive and respiratory organs of dead chickens in Kena Governorate.

2. MATERIAL AND METHODS

2.1. Isolation and identification of fungi

A total of 1038 samples were taken from the digestive and respiratory organs of 352 dead chickens collected from different farms in Kena. Samples were taken from

the esophagus, proventriculus, trachea and lungs, as well as dead in shell chicken embryos. Swabs were streaked on slope Sabouraud's agar containing 250 mg streptomycin and 250 mg chloramphenicol/liter. All the cultured media were incubated at 37ºC for 7 days before recording the result.

Ten mould cultures were isolated and identified morphologically and physiologically on the Sabouraud's agar tubes and Czapek's agar plates (El-Badri & Sokker, 1988) as: *Aspergillus niger, A. fumigatus, A. flavus, A. flavipes, A. ochraceus, A. nidulans, A. glaucus, A. terrus, Paecilomyces* spp. and *Penicillium* spp.

2.2. Microbiological techniques

Pure growth from each fungal strain was obtained on Sabouraud's agar medium after 7 days incubation at 28ºC (Jacobs and Gerstein, 1960). A part from the fungal growth was transferred by a sterile solid platinium loop to a 100ml. Capacity conical flask containing 20ml Ringer's solution and 5 grams of sterile coarse sand. The mixture was agitated at 200 RPM for half an hour after which it was left for sedimentation at room temperature for 15 mins. The supernatent was aspirated asceptically to a sterile test tube for Sabouraud's agar inoculation (1 ml/100 ml medium).

The antifungal activities of tested oils were carried out by disc diffusion technique, as described in British Pharmacopoeia (1968). The sterile 5 mm diameter Whatman No.3 filter paper discs completely moistened in the raw tested oils or dilutions 10% and 2% acetone was used as a solvent for all the tested oils (vol/vol). The treated discs were aseptically transferred and left for evaporation of the solvent and then placed upon the surface of the inoculated plates, which were kept in a refrigerator for one hour to permit diffusion of the antifungal substances, then incubated at 28ºC for 3 days. The zone of inhibition was measured in mm. The mean value of inhibition zones were calculated from triple reading in each test.

2.3. Extraction of natural oils

Oils investigated were extracted from seeds of cumin, basil and celery. Also oils derived from the buds of clove, and from the whole plant of peppermint, lemongrass and pelargonium; and from natural flowers of chamomile (table I). The oils were freshly prepared to the water distillation procedure according to Guenther (1950).

The inhibitory effect of the previous eight natural oils were tested at different concentrations (crude oil, 10% and 2%) against the ten isolated fungi. Diameter zone of inhibition and critical concentration were recorded for each natural oil (Maruzzella, 1958 and Ab-El Raoof, 1986).

2.4. Estimation of critical concentration

Critical concentration is that concentration of oil at the margin of inhibition zone or the border of microbial growth.

Table I. **Extraction of some natural oils from different plant organs**

Family and Plant spp.	Extracted organ	Oil name	Crude (active) ingredient	Solvent used
(1) Compositae: *Matricaria chamomilla L.*	Flowers	Chamomile	Chamazulene	Steam distill.
(2) Geraniaceae: *Pelargonium graveolens*	Leaves and stem	Geranium	Citronellol and geraniol	Watery distill.
(3) Graminae: *Cymbopogon citratus* (D.C.) stapf.	Leaves	Lemongrass	Citral and citronellol	Watery distill.
(4) Labiatae: *Mentha piperita* L.	Whole plant	Peppermint	Menthol and menthone	Watery distill.
Ocimum basilicum L.	Whole plant	Basil	Cineole	Watery distill.
(5) Myrtaceae: *Eugenia caryophyllata* Thunb = *Caryophyllus aromaticus* L.	Leaves and buds	Clove	Eugenol	Watery distill.
(6) Umbelliferae: *Apium graveolens* L.	Whole plant	Celery	Linalerol	Watery distill.
Cuminum cyminum L.	Seeds	Cumin	Perillaldehyde	Boiling water and water distill.

It was calculated for each oil according to the equation of Cooper and Woodman (1946). It is important to realize that critical concentration is usually 2-4 times the minimal inhibitory concentration of the same organism in the liquid media due to the direct contact between the causative agent of the organisms (Cooper, 1963).

3. RESULTS

Data (Table II) indicated that crude oil of peppermint showed the highest inhibitory effect (50mm) against *A.fumigatus* followed by *A. nidulans* (27mm), *Penicillium spp.* (25mm) and *A. flavus* (24mm), while the other isolated fungi gave a low response. On the other hand, 10% and 2% oil concentrations failed to give any inhibitory effect against all tested fungi.

Crude clove oil and 10% concentration showed marked inhibitory effect against all isolated fungi. The effect of different dilutions gave a general trend of reduction in the activity as the dilution was increased. *A.flavus, A. nidulans, A. glaucus, A. terreus, A. fumigatus, A. ochraceus and Penicillium spp.* were the most sensitive organisms against crude clove oil followed by *A. niger* and *Paecilomyces spp.* Clove oil at 2% concentration was non effective against *A. fumigatus, A. glaucus, Penicillium spp., Paecilomyces spp., A. niger* and *A. nidulans*, while the same concentration of the oil gave a faint effect against other isolated fungi.

Generally crude chamomile oil showed moderate, inhibitory effect against all tested fungi. The effect of different dilutions of chamomile oil against the tested fungi showed that the activity was reduced as the dilution was increased except with *A. flavus; A. flavipes; A. ochraceus* and *A. glaucus,* where the activity of 10% chamomile oil

Table II. **Effect of different oils concentrations on the growth of isolated fungi**

Oil	Clove				Camomile				Pelargonium				Peppermint				Lemongrass				Basil				Cumin				Celery			
Concentrations	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%	Crude	10%	2%	C.C.%
Organisms																																
A. fumigatus	37	26	0	2.0	17	15	10	0.55	17	10	0	2	50	0	0	0	45	0	0	0	37	10	0	2	30	40	10	0.8	10	30	12	1.5
A. flavus	45	28	8	1.7	15	23	8	1.6	10	8	0	2	24	0	0	0	24	0	8	10	18	13	0	2	29	14	8	0.9	12	16	0	2.0
A. flavipes	25	22	8	1.6	10	18	10	1	8	8	0	2	8	0	0	0	50	12	10	0.05	20	10	0	2	40	10	7	0.4	10	13	7	1
A. ochraceus	35	20	10	1.2	12	15	8	1.1	20	10	0	2	14	11	0	2	27	8	0	2	22	0	0	0	20	8	8	0	10	11	0	2
A. glaucus	42	40	0	2	14	16	0	2	12	8	0	2	18	0	0	0	28	14	7	1.2	24	27	0	2	24	40	12	1.7	10	40	0	2
A. terreus	37	37	8	1.9	13	11	0	2	14	8	0	2	15	0	0	0	35	20	0	2	32	22	0	2	24	30	10	1.5	11	20	10	1.2
Penicillium spp.	33	22	0	2	16	11	0	2	11	0	0	0	25	0	0	0	22	10	0	2	18	8	0	2	22	18	0	2	16	18	0	2
Paecilomyces spp.	28	20	0	2	10	9	0	2	10	0	0	0	16	0	0	0	18	11	0	2	21	9	0	2	20	20	0	2	10	21	0	2
A. niger	30	20	0	2	20	12	0	2	10	0	0	0	12	0	0	0	17	12	0	2	14	0	0	0	15	18	0	2	12	16	0	2
A. nidulans	45	18	0	2	15	12	0	2	12	0	0	0	27	0	0	0	33	0	7	10	26	0	0	0	26	15	12	0.1	9	12	0	2

C.C. = Critical concentration
Inhibition zone (mm)
Diameter of disc = 5 mm

concentration was higher than crude chamomile oil. On the other hand, 2% chamomile oil had no/or slight effect against the tested fungi.

The inhibitory effect of pelargonium oil was moderate against the tested fungi while 10% concentration gave slight effect and 2% concentration did not give any effect at all against any of the tested fungi.

Crude lemongrass oil appeared to have marked inhibitory effect especially against *A. flavipes* and *A. fumigatus* followed by *A. terreus* and *A. nidulans.* However, the effect of 10% and 2% concentrations ranged between slight to completely uneffectiveness against the tested fungi.

Crude basil oil gave high inhibitory effect against *A. fumigatus* and *A terreus,* while gave moderate effect against other tested fungi. The effect of 10% dilution showed a general trend of reduction in the activity, while 2% concentration did not give any antagonistic effect against any of the tested fungi.

Unlike the other natural oils tested, cumin oil, antagonistic effect against *A. fumigatus, A. glaucus, A. terreus* and *A. niger* was increased with the use of 10% concentration of the crude oil. However, like the others, crude cumin oil inhibited the remaining fungi higher than at 10% concentration, while 2% concentration gave very slight effect against all isolated fungi (zone of inhibition 0-12mm).

Crude celery oil appeared to have a faint inhibitory effect against all examined fungi. But 10% concentration of the oil showed higher effect against *A. fumigatus* and *A. glaucus* and at the same time moderate effect against *Paecilomyces spp.* and *A terreus.* The remaining isolated fungi were inhibited by 10% celery oil concentration, but higher than the effect of crude celery oil. However, 2% celery oil concentration is very faint (zone of inhibitory 1 1/2-2mm).

Critical concentrations of the efficient oils against examined fungi were calculated. The lower the critical concentration of the oil the higher its efficiency (Cooper and Woodman, 1946 and Cooper, 1963).

Efficient oils could be arranged descendingly according to their efficiencies against tested fungi in table II as follows: Lemongrass against *A. flavipes* (0.05%), chamomile against *A. fumigatus* (0.55%), cumin against *A. nidulans* (0.1%), cumin against *A. flavipes* (0.4%), cumin against *A. fumigatus* (0.8%), cumin against *A. flavus* (0.9%), chamomile against *A. flavipes* (1.0%), celery against *A. flavipes* (1.0%), chamomile against *A. ochraceus* (1.1%), clove against *A. ochraceus* (1.2%), lemongrass against *A. glaucus* (1.2%), celery against *A.terreus* (1.2%), celery against *A. fumigatus* (1.5%), cumin against *A. terreus* (1.5%), clove against *A. flavipes* (1.6%), chamomile against *A. flavus* (1.6%), clove against *A. flavus* (1.7%) and cumin against *A. glaucus* (1.7%). The critical concentration of other oils showed no promising values against the tested fungi.

Data revealed also that celery oil was efficient against *A. flavipes* (cr. concn. 1.0%), *A. terreus* (cr. concn. 1.2%) and *A. fumigatus* (cr. concn 1.5%) while pelargonium, peppermint and basil oils have no effect against any of the tested fungi.

4. DISCUSSION

Antimicrobial agents from higher plants are few (14%) compared to the total obtained from the different forms of life (Kurylowicz, 1972 and Alicia *et al.*, 1981). They may be less toxic to the host, cheaper, more available especially during war time since they are from local sources (Ikram and Inamul-HaQ, 1980).

Concerning the antifungal agents obtained from plant oils, scanty are known or available in the market as compared to the antibacterial agents. They are mostly unsatisfactory in controlling the fungal infections, (Dikshit and Husain, 1984). Stock (1981) and Lorenti *et al.* (1981) emphasized the need for discovering powerful and specific antimycotic agents on an increasing scale to control fungal infections, and provide a useful tool for the medical and pathological applications.

Thus, antifungal activity of eight natural oils at different concentrations were studied against ten fungi which were isolated from the digestive and respiratory tracts of dead chickens in Kena Governorate.

Most crude oils showed good inhibitory activity against all tested fungi, this may be due to the oil vapours which accumulate in the Petri dishes and prevent fungi growing.

Generally the effect of different dilutions of most tested oils against most tested fungi showed a general trend of reduction in the activity as the dilution was increased. This was true for all investigated oils except cumin and celery oils where the diameter of inhibition zones were greater at 10% concentration than that of their crude oils. This effect may be due to the highest diffusion rate of the oil component in agar at 10% dilution or due to the greater up-take of celery and cumin oils by some isolated fungi at 10% concentration (Abd-El-Raoof, 1986).

Although the zone of inhibition indicates the sensitivity of the organism to the oils, the area or width of the inhibition zone does not necessary correlate with the effectiveness of the oils, since there is no direct relationship between the diffusion of the oils into agar and in its *in vivo* effectiveness (Prescott and Dunn, 1959).So, the zones of inhibition were estimated in three successive dilutions and critical concentration was calculated for each oil according to the equation of Cooper and Woodman (1946). The oils which showed clear zones of inhibition at the lowest concentration (2%) were considered efficient against the test organism. Efficient oils were compared in their antimicrobial activity according to their critical concentrations.

Results given in Table II showed that clove oil was the most effective against *A. ochraceus* (cr concn.1.2%), *A. flavipes* (cr. concn. 1.6%), *A. flavus* (cr. concn. 1.7%) and *A. terreus* (cr. concn.1.9%). Martindale (1910) reported that clove had a phenol coefficient of 8.88 against *E. coli*. The germicidal effect of clove and its active main component Eugenol (84.4%) against bacteria and fungi was reported by many investigators (Marsh and Maus, 1930; Dold and Knapp, 1948 and Galloway, 1952).

Also data showed that chamomile oil has the highest effect against *A. fumigatus* (cr. concn. 0.55%), followed by *A. flavipes* (cr. concn. 1.0%), *A. ochraceus* (cr. concn. 1.1%) and *A. flavus* (cr. concn.1.6%). Gottshall *et al.* (1949) found that the water extract of chamomile showed an inhibitory effect against *M. tuberculosis* and *E. coli*. Frisbey et al., (1954) found that ethanolic extract of chamomile showed an inhibitory effect against *S. typhimurium* in dilution below 1:20. Aggag and Yousef (1972) reported that Gram positive bacteria were more sensitive to the action of chamomile oil than the Gram negative bacteria. This oil showed a marked fungicidal activity against *C. albicans* (Szalontal *et al.* 1976).

Cumin oil was the most effective oil against *A. nidulans* (cr.concn. 0.1%), *A. flavipes* (cr. concn. 0.4%), *A. fumigatus* (cr. concn. 0.8%), *A. flavus* (cr. concn. 0.9%), *A. terreus* (cr. concn. 1.5%) and *A. glaucus* (cr. concn. 1.7%). El-Hamidi and Richter (1965) reported the occurrence of cumin alcohol, cumin aldehyde, perillaldehyde and unsaturated ketone as well as unidentified terpenes. Gallaway (1952) stated that cumin oil as vapour showed antibiotic activity against certain moulds. Singh and Singh (1970) showed that seeds of cumin inhibited the growth of ten rhizosphere fungi, such effect was attributed to volatile substances which caused stunt mycelial growth, granulation, swelling lysis of the hyphae.

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(Recibido: Agosto 1993)