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IN VITRO EFFECTS OF SOME CITRUS OILS ON SOME PATHOGENIC BACTERIA CAUSING POULTRY DISEASES

BY

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SUMMARY

Volatile oils of peel fruits of Balady orange and Grapefruit were extracted by steam distillation and cold pressed methods, those that of Mandarin and Lemon were extracted by steam distillation only. Bacteriological experiments showed that volatile oils obtained by steam distillation method are strong inhibitors of the in vitro development of *Pasteurella multocida, Salmonella gallinarum, Salmonella pullorum* and *Escherichia coli* than that of pressed method and chloramphenicol.

INTRODUCTION

In Egypt, control of bacterial diseases of poultry relies heavily on antiboitics, while providing effective control, chemical antiboitics produced major well known problems: health hazards to human, destruction of natural biotic control agents and increased resistance of major microbial species. This situation accelerates the movement towards more sound control method of which the bacterial control proved to be most effecient.

The most promising agent to date to satisfy this goal is the essential oils of some citrus fruits.

In 1969, Marth reviewed the literature on the potential use of essential oils in the inhibition of *Salmonella,* Nagy and Tengerdy (1967 and 1968) studied their effect on rumen bacteria of sheep and deer Dabbah et al. (1970) studied the antimicrobial action of some citrus fruit oils on selected food borne bacteria; while Dupaigne (1967) showed that the degree of inhibition of bacterial growth by citrus oils varies considerably with the bacteria tested.

This paper studied the in-vitro effect of some citrus fruit oils on some common poultry pathogens.

MATERIAL AND METHODS

Source of volatile oils:

Steam distilled Grapefruit, Balady orange, Mandarin and lemon oils were obtained individually from mature fruits of adult trees grown at 23 July experimental Horticultural station El-Marg, Ministry of Agriculture, whereas cold-pressed Grapefruit and Balady orange oils were obtained from Al-Mohands National Food Products (Schweppes), Ismailia, Egypt.

Bacterial strains:

Four strains of pathogenic bacteria isolated from different sources were selected for this study. *Pasteurella multocida* was isolated from cases of fowl cholera; *Salmonella gallinarum* from fowl typhoid; *Salmonella pullorum* from *pullorum* disease and *Escherichia coli* from cases of colibacillosis. The isolated bacteria were identified by using the biochemical and serological tests according to Sonnenwirth (1980).

Disc assay was performed on nutrient agar media. Bacteria were spread on the media and the plates were allowed to dry for 5 minutes. Six millimeter sterile filter paper discs were dipped into the appropriated oil concentration (0.1, and 1 mg/ml absolute ethanol) until saturated, the alcohol being evaporated after introducing each portion on the disc.

The saturated discs were sterilized under a quartz

lamp and put on the plates after inoculation.

The control substance used to compare the potency of the effect of the investigated preparations was chloramphenicol disc (30 µg/disc-oxoid), antibiotic of relatively high bacteriostatic activity and widely used in the treatment of the forementioned diseases.

After 24 hours incubation at 37°C, zone of inhibition around discs used were measured to the nearest millimeter.

The results of bacteriological investigations are represented in table (1) in which the values of zones of growth inhibition of the particular examined strains are compiled and a comparison is made of the bacteriostatic of the volatile oil with the activity of chloramphenicol (control antibiotic).

The degree of sensitivity of the bacteria to chloramphenicol was determined according to Bauer (1982) as follows.

Zone of inhibition above 18 mm: is considered sensitive.
Zone of inhibition ranged from 13 to 18 mm: is considered intermediate sensitive.
Zone of inhibition below 13 mm: is considered resistant.

In the estimation of the bacteriostatic action of the citrus oil preparations, the relative bacteriostatic activity (Arel) was taken into account, being determined from the proportion as cited by Musial and Stanice (1965) as follows.

$$\frac{\text{Ø exam.}}{\text{Ø Co.}} = \text{Arel}$$

Where Ø exam. = diameter of inhibition zone of bacteria (in mm) caused by the given preparation of determined concentration.

Ø Co.= diameter of inhibition zone of bacteria (in mm) caused by chloramphenicol.

The numeral values of Arel were the basis for comparing the bacteriostatic activity of determined doses of citrus oils with the action of chloramphenicol (control) toward the particular bacteria.

The following determinations were applied in this comparison.

- For values of Arel >1 :- activity higher than chloramphenicol.
- For values of Arel 0.96 -1:- activity near to chloramphenicol.
- For values of Arel < 0.81. :- weak activity.

RESULTS

The results recorded in Table (1) show that, volatile oils of citrus peel fruits had a considerable positive inhibitory actions on the growth of all

Table (I) : Values of growth inhibition zones of the isolates with citrus oils preparation.

Inhibition zones (mm)													
Method		Steam distillation								Cold pressed			
Substance	Co.	balady orange		Lemon		Grape fruit		Mandarin		Balady orange		Grape fruit	
Dose / Strain	30µg	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml
Past. multocida	22**	19**	25**	16*	25**	8*	20**	10*	12*	7*	21**	7*	15*
S. Gallinarum	10*	19**	23**	14*	20**	11*	16*	8*	11*	10*	22**	9*	12*
S. pullorum	9*	23**	25**	19**	21**	14*	18*	9*	10*	7*	25**	7*	10*
E. coli	10*	25**	27**	21**	25**	13*	17*	7*	9*	7*	23**	7*	12*

Co. = Chloramphanicol (control).
** = Sensitive (< / 8 mm).
* = Intermediate (13-18) or (resistant <13)

four bacteria. Basing the estimation on the values of growth inhibition zones, it was found that citrus oils obtained by steam distillation had a much stronger bacteriostatic activity on the four bacteria than that obtained by cold pressed method.

Pasterurella multocida was the most sensitive organism to chloramphenicol while *Salmonella gallinarum, Salmonella pullorum.* and *Escherichia coli* were the most resistant strains toward the drug.

The results given in Table (2) indicated that, by steam distillation method, the relative bacteriostatic activity of Balady orange oil at a concentration of I mg/ml. was higher than the bacteriostatic activity of chloramphenicol; while at a concentration of 0.1 mg/ml, it was higher in 3 out of 4 and this result is equivalent to the result of the same oil obtained by cold pressed but at a concentration of Img/ml. At a concentration of o.1 mg/ml. (cold pressed method), the oil had bacteriostatic activity equal to the chloramphenicol on *Salmonella gallinarum* and weaker than chloramphenicol on the other three species.

The results recorded in Table (3) show that the relative bacteriostatic activity of Grapefruit oil extracted by steam distillation at a concentration of 0.1 and 1 mg/ml was higher than chloramphenicol in 3 out of 4, but by cold pressed the same result obtained from the concentration of 1 mg/ml only and at a concentration of 0.1 mg/ml the relative bacteriostatic activity of the oil on the four species of bacteria was lower than chloramphenicol.

Consulting the results recorded in Table (4), it is evident that, the relative bacteriostatic activity of Lemon oil on the four bacteria at a concentration of 1mg/ml was higher than chloramphenicol while that of Mandarin oil at the same concentration, it was lower in two out of four.

At concentration of 0.1 mg/ml the relative activity of Lemon oil was lower than chloramphenicol in I out of 4 but that of Mandarin oil it was lower in 3 out of 4.

Table (2) :Comparison of bacteriostatic activity of citrus oils separated by the two methods of extraction from peels of balady orange with the activity of chloramphenicol.

	Steam distillation				Cold pressed			
	Arel I		Bacteriostatic activity of B.O.I compared with Chloramphenicol.		Arel II		Bacteriostatic activity of B.O.II compared with Chloramphenicol.	
Dose / Strain	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml
Past. multocida	0.86	1.14	<Co	>Co	0.32	0.95	weak	<Co
S. Gallinarum	1.9	2.3	>Co	>Co	1	2.2	≠ Co.	>Co
S. pullorum	2.6	2.8	>Co	>Co	0.78	2.8	weak	>Co
E. coli	2.5	2.7	>Co	>Co	0.7	2.3	weak	>Co

Arel I = The relative activity of Balady orange oil separated by steam distillation against isolates
Arel II= The relative activity of Balady orange oil separated by cold pressed against isolates.
> Co. = Higher than chloramphenicol.
< Co. = Lower than chloramphenicol.
≠ Co. = Equal to chloramphenicol.
B.O. = Balady orange.

Table (3) :Comparison of bacteriostatic activity of citrus oils separated by the two methods of extraction from peels of Grape fruit with the activity of chloramphenicol.

			Steam distillation				Cold pressed	
	Arel I		Bacteriostatic activity of G.F. compared with Chloramphenicol.		Arel II		Bacteriostatic activity of G.F. compared with Chloramphenicol.	
Dose / Strain	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml
Past. multocida	0.36	0.91	weak	<Co.	0.32	0.68	weak	weak
S. Gallinarum	1.1	1.6	>Co	>Co.	0.9	1.2	<Co	>Co
S. pullorum	1.6	2.0	>Co	>Co.	0.78	1.1	weak	>Co
E. coli	1.3	1.7	>Co	>Co.	0.7	1.2	weak	>Co

Arel I = The relative activity of Grape fruit oil separated by steam distillation against isolated.
Arel II= The relative activity of Grape fruit oil separated by cold pressed against isolates.
> Co = Higher than chloramphenicol.
< Co = Lower than chloramphenicol.
G.F. = Grape fruit.

Table (4) :Comparison of bacteriostatic activity of citrus oils separated by the two methods of extraction from peels of Mandarin and Lemon with the activity of chloramphenicol.

	Arel I		Bacteriostatic activity of lemon compared with Co.		Arel II		Bacteriostatic activity of Mandarin compared with Co.	
Dose / Strain	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml	0.1 mg/ml	1 mg/ml
Past. multocida	0.73	1.14	weak	>Co	0.45	0.55	weak	weak
S. Gallinarum	1.4	2.0	>Co	>Co	0.8	1.1	weak	>Co
S. pullorum	2.1	2.3	>Co	>Co	1.0	1.1	≠	>Co
E. coli	2.1	2.5	>Co	>Co	0.7	0.9	weak	<Co

Arel I =Rrelative activity of citrurs oil of Lemon.
Arel II= Relative activity of citrus oil of Mandarin
> Co = Higher than chloramphenicol.
< Co = Lower than chloramphenicol.
≠ = Equal to chloramphenicol.

DISCUSSION

Volatile oils of peel fruits of citrus species had been characterized by their activities against some pathogenic bacteria such as *Pasteurella multocida, Salmonella gallinarum; Salmonella pullorum* and *Escherichia coli.* Nearly similar observation was reported by Ross et al. (1980) and Kivanc and Akgul (1986). They concluded that essential oils of Lemon and Orange peel fruits were active against *Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa.* In addition, susceptibility of baceria to volatile oil activity depended upon citrus species, rates of volatile oils and method of extractions. Accordingly, volatile oil from peel fruits of Balady orange was the best effective in inhibiting these bacteria, followed by Lemon and Grapefruit oils, whereas who were resistent to the Qil obtained from peel fruits of Mandarin. These results were similarly agreeable with the findings of Dupaigne (1967), who indicating that, the degree of inhibition of

bacterial growth by citrus oils varied considerably with the bacteria tested, and considerable differences existed in the susceptibility of bacteria to the activity of the rates of volatile oils of peel fruits, because the sensibility of bacteria were gradually increased by increasing the rates of volatile oils.

Comparing the susceptibility of bacteria to the volatile oils obtaiend by both methods of extraction, it is clear that, the four species of bacteria used were highly sensitive to the volatile oils distilled than that of volatile oils pressed, and this may be due to the fact that , the process of distillation of volatile oil affected the composition of oil either by isomerisation, saponification or polymerisation of the more labile constituents as (Koedam et al., 1979).

This observation was confirmed by the largest quantity of oxygenated terpenic constituents of volatile oil resulted from distillaion method, whereas the volatile oils obtained from press method bearing the largest quantity of lipids, yellow carotenoids, greenchlorophylls and flavonoid, besides the terpenic constituents of volatite oils.

Although the mechanism of the antimicrobial effect of these citrus oils was not known, lipid solubility and surface activity of the oils at the surface of the bacteria had been implicated (Nagy and Tengerdy 1967).

Comparing the activity of chloramphenicol with that of volatile oils distilled from peel fruits of Balady orange, Grapefruit and Lemon, it is clear that these oils had a strong bacteriostatic activity than that of the chloramphernicol (control) when tested at the rates ranged from 0.1 mg/ml to 1.0 mg/ml, whereas volatile oil of peel fruit of Mandarin had the smallest activity.

Generally, the bacteriostatic activity of citrus oils separated by the two methods of extraction showed a largest values over the chloramphenicol, and the higher the rate of volatile oils tended to be the larger bacteriostatic activity when compared with that of antiboitic. Based on the presented data one might be concluded that oils obtained from peel fruits of some citrus species had a relatively bacterostatic effect on some pathogenic bacteria as compared with that of the most popular medicament, such as chloramphenicol and these oils should be used in medicine as therapeutic preparations for treatment of diseases resulting from bacterial infection.

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