

EFFECT OF CINNAMON AND CLOVE OILS ON FOOD POISONING AND SPOILAGE BACTERIA IN VITRO AND POULTRY MEAT PRODUCT

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SUMMARY

Various concentrations of either cinnamon or clove oils and mixtures of both oils (1 : 1 v/v) were tested for determination of minimum inhibitory concentrations (MIC_s) and measuring the extinction time of both oils concentrations of food poisoning and spoilage bacteria (*Salmonella typhimurium*, *Staphylococcus aureus*, *Yersinia enterocolitica*, enteropathogenic *E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Saccinia lutea*). The results obtained showed that they have broad spectrum activities against all tested bacteria. Comparison of the antimicrobial activity of cinnamon, clove oils and mixture of both showed that the mixture of oils were the most active against the tested microorganisms. The effect of 0.5% cinnamon oil, 0.4% clove oil and 0.3% mixture of both oils (1 : 1 v/v) on the behaviour of food poisoning bacteria in poultry meat products (sausage and chickenburger) held at 25°C, 10°C, 5°C and -20°C were investigated. The results revealed that the behaviour of all inoculated organisms in both products were similar. It is of interest that 0.3% combination of cinnamon and clove oils (1 : 1) produced a more pronounced effect in inhibiting growth of food poisoning bacteria at all storage temperature.

INTRODUCTION

In all probability the human population will increase drastically world-wide in the next decades and at the same time food stuffs and specially meat will become more scarce and precious, because even by using advanced methods in breeding, feeding and production of livestock not enough meat will be available for the

many people who like to eat it (World Conference on Animal Production, 1983). Therefore, it will become even more necessary than to-day to prevent spoilage of meat and processed meat products (Leistner et al., 1984).

Processed meat products may at times constitute a public health hazard either due to presence of food poisoning or spoilage microorganisms. In this regard it is generally accepted that many herbs and spices are known to exhibit antimicrobial activity and influence the keeping quality of foods to which they have been added. The preservative action of herbs and spices in addition to widely used to import flavour to meat products has only recently received attention in the literature where studies (Sharman et al., 1979; Hitokoto et al., 1980; El-Khateib et al., 1984, 1985 and 1986; El-Khateib and Abd El-Rahman, 1987), have been reported and showed that bacteria and mycotoxin producing mould may be inhibited by some herbs and spices.

Cinnamon and clove were reported to cause inhibition of some microorganisms as aflatoxin producing mould (Bullerman, 1974; Bullerman et al., 1977; Azzouz and Bullerman, 1982). However, it remains unclear as to whether oils of cinnamon and clove have any antibacterial activity specially the food poisoning and spoilage bacteria in meat and meat products. The study reported here deals with the inhibitory effects of cinnamon and clove oils on the growth and survival of food poisoning and spoilage bacteria in vitro and poultry meat products (Sausage and chickenburger).

Table (1) : The minimum inhibitory concentrations (MIC_s) of cinnamon and clove oils on spoilage and food-poisoning bacteria.

Microorganisms	Cinnamon oil	Clove oil	oils of cinnamon & clove
	%	%	(1 : 1 v/v) %.
<i>Salmonella typhimurium</i>	0.4	0.2	0.1
<i>Staph. aureus</i>	0.45	0.45	0.3
<i>Y. enterocolitica</i>	0.35	0.25	0.05
Enteropathogenic <i>E.coli</i>	0.12	0.25	0.06
<i>Pseudomonas aeruginosa</i>	0.30	0.20	0.10
<i>Proteus vulgaris</i>	0.20	0.20	0.10
<i>Sarcina lutea</i>	Very sensitive		

Table (2) : The extinction time of 0.5 % concentration of cinnamon and clove oils on food poisoning and spoilage bacteria.

Microorganisms	Cinnamon oil			Clove oil		
	Time / minutes					
	10	30	60	10	30	60
<i>Salmonella typhimurium</i>	-	-	-	-	-	-
<i>Staph. aureus</i>	-	-	-	-	-	-
<i>Y. enterocolitica</i>	-	-	-	-	-	-
Enteropathogenic <i>E.coli</i>	+	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	-	+	+	-
<i>Proteus vulgaris</i>	-	-	-	+	+	-
<i>Sarcina lutea</i>	-	-	-	+	-	-

- No growth.
 + Growth.

Fig : (1) Scheme for study the effect of cinnamon and clove oils on the behavior of food-poisoning bacteria in poultry meat products.

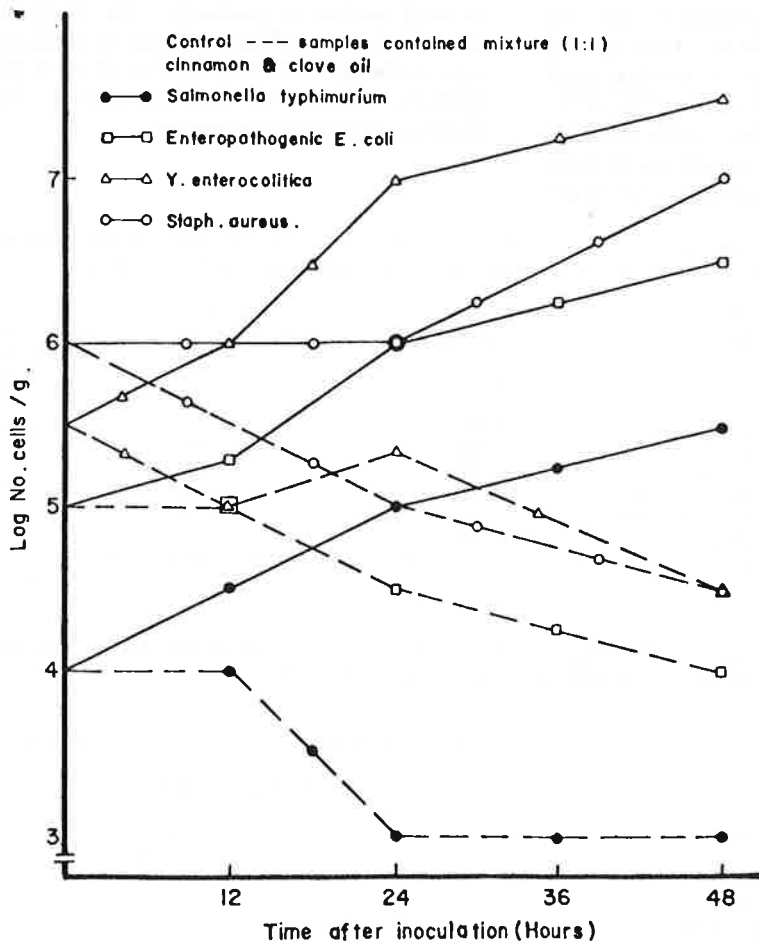
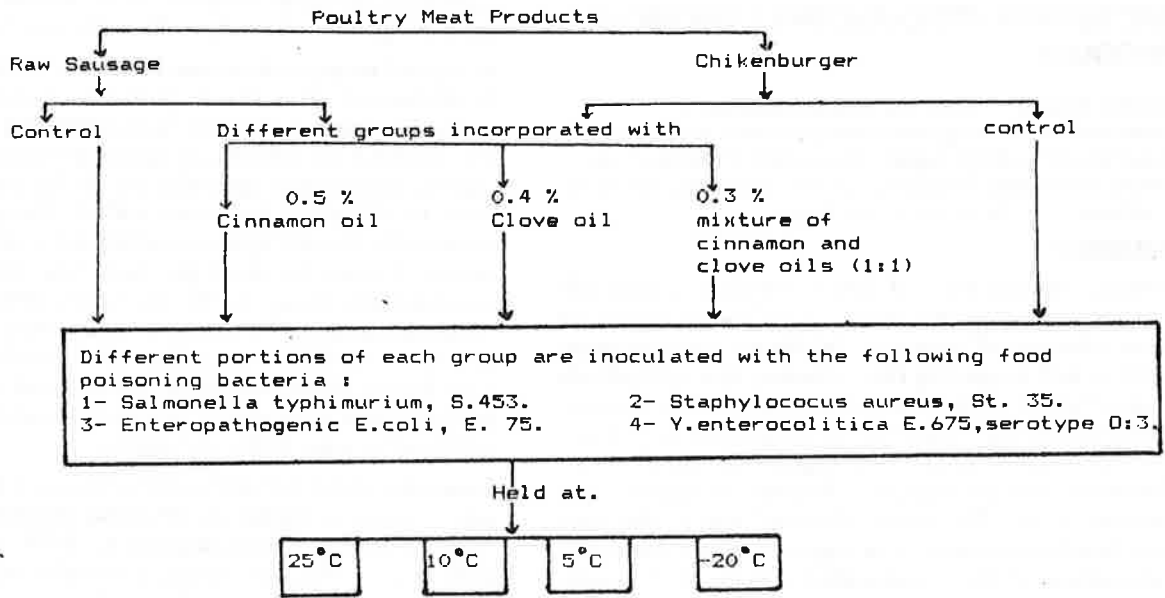


Fig.(2) : Effect of 0.3% cinnamon and clove oils (1:1 V/V) mixture on food poisoning bacteria inoculated into poultry meat products, and stored at 25°C.

MATERIAL AND METHODS

Substance tested

Two spices were chosen for this study on the basis of their reported antimicrobial activity or from the results obtained from preliminary studies. The spices used were as follows: cinnamon (*Cinnamomum cassia*) and clove (*Eugenia caryophyllus*), were prepared as: 125 gm from each spices were extracted with hexane in a continuous extraction apparatus. The volatile oil solution obtained was evaporated under reduced pressure, where the volatile solvent will be evaporated, leaving the volatile oil.

ORGANISMS

The organisms used in this study were (*Salmonella typhimurium* S 453; *Staphylococcus aureus* St 35; enteropathogenic *E. coli* E 75) obtained from Federal Centre for Meat Research, 8650 Kulmbach, Federal Republic of Germany. *Yersinia enterocolitica* E 675 serotype 0:3 was obtained from the Food Research Institute, University of Wisconsin, Madison, US. The spoilage bacteria (*Pseudomonas aeruginosa*; *Proteus vulgaris* and *Sarcinia lutea*) were stocked in (Food Hygiene Dept. Fac. of Vet. Med. Assiut University, Egypt). The cultures were maintained on slants of nutrient agar (Merck) at 37°C for all tested bacteria except *Y.*

enterocolitica (ast 25°C) and stocked at 4°C until used.

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATIONS (MIC,s)

The (MIC,s) of cinnamon, clove and mixtures of both oils (1 : 1 v/v) against food poisoning and spoilage bacteria were evaluated using the agar-cup diffusion technique (Garrod and O'Grady, 1972) :

15 ml Muller-Hinton agar was melted, seeded with the organism under test to give a final concentration 10^5 - 10^6 organisms/ml. Then poured in sterile Petri dishes (15 cm diameter). After allowing the agar to solidify, cups were prepared 10 mm diameter using a sterilized glass tube and a propipette. The various dilutions of both oils (5%; 2.5%; 1.25%; 0.625% and 0.312%) separately or in mixture were then added to the cups in appropriate quantities in triplicate. In each case, separate cup with pure solvent was employed as a control. The plates were incubated for 24 h at 37°C except *Y. enterocolitica* which incubated at 25°C for 24 h. After incubation, the

inhibition zones were measured in mm, and a curve determining the relation between X2 and logarithm concentration of the oil was constructed and used to estimate the MIC,

$$X = \frac{\text{diameter of inhibition zone} - \text{diameter of the cup}}{2}$$

ESTIMATION OF BACTERICIDAL ACTIVITIES

Various dilutions (5%, 2.5%, 1.25%, 0.625% and 0.312%) of both oils were inoculated by the tested bacteria (10^5 - 10^6 /ml) and incubated 48 h at 37°C for all tested bacteria and 25°C for *Y. enterocolitica*. Subculture were made from the tubes showing no growth, by transferring a loopful from each dilution into 5 ml sterile Brain Heart Infusion (BHI) broth. The results were taken after incubation period of 48 h.

DETERMINATION OF THE EXTINCTION TIMES OF 0.5% CONCENTRATION OF CINNAMON AND CLOVE OILS

The extinction time of the specified concentration of each oil against each tested organism was determined as follows: 5 ml of 0.5% concentration of the oil was inoculated with 0.5 ml of 10^6 organism/ml. Subcultures were done on (BHI) broth tubes at 10 minutes time intervals starting from 0 - 60 minutes. The inoculated tubes were then incubated at 37°C for 48 h except *Y. enterocolitica* which was incubated at 25°C for 48 h.

POULTRY MEAT PRODUCTS

Two samples of poultry meat products (raw sausage and chicken burger) were prepared. Classified into different groups and inoculated with food poisoning bacteria as illustrated in Fig. 1.

ENUMERATION PROCEDURE

To give 0.1 dilution, 20 gm from the products were homogenized in a waring blender with 180 ml sterile physiological saline, all dilutions were examined in duplicate for the different food poisoning bacteria. Samples were surface-plated on the following media:

1 - Deoxycholate hydrogen sulfide lactose agar (DHL, Merk) for *Salmonella typhimurium* and enteropathogenic *Escherichia coli*.

2 - Baird-parker media for *Staphylococcus aureus*.

3 - Cefsulodin-Trgasan Novobiocin (CIN) agar, for *Yersinia enterocolitica* (Lampert, 1975).

RESULTS AND DISCUSSION

The in vitro studies of the antibacterial activities of cinnamon and clove oils revealed that they have broad spectrum

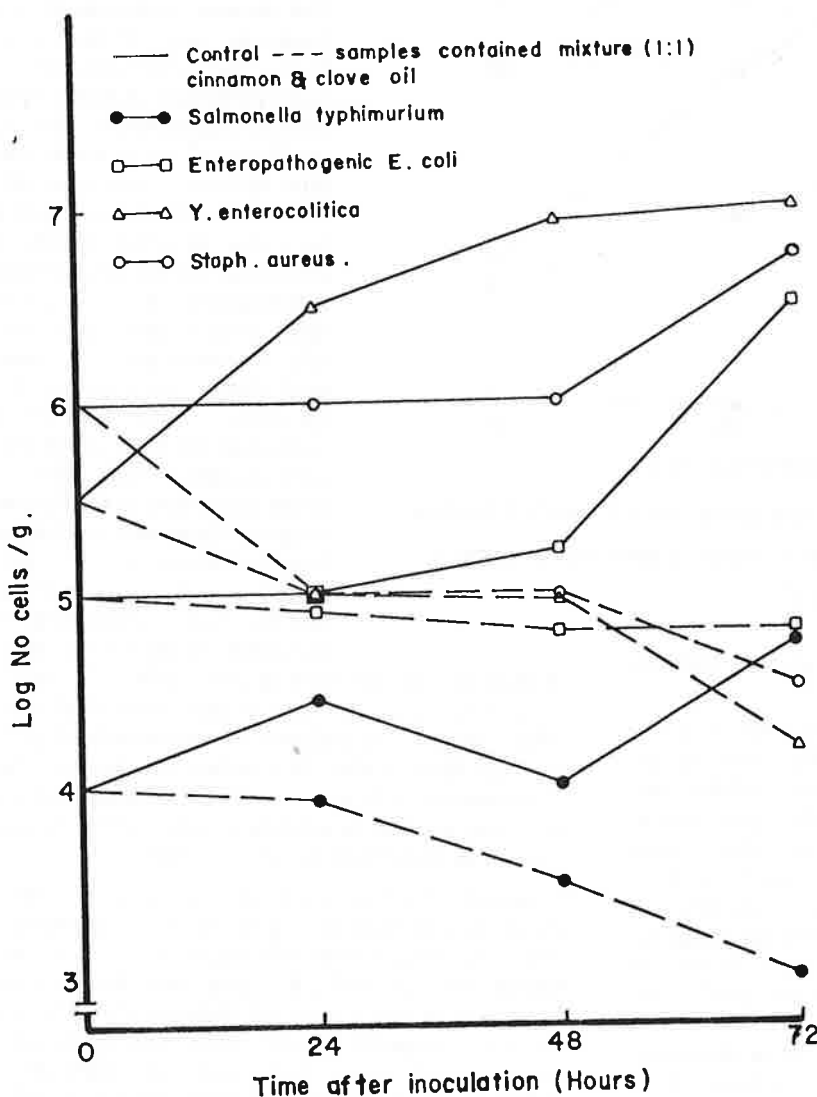


Fig.(3): Effect of 0.3 % cinnamon and clove oils (1:1 V/V) mixture on food poisoning bacteria, inoculated into poultry meat products, and stored at 10 °C

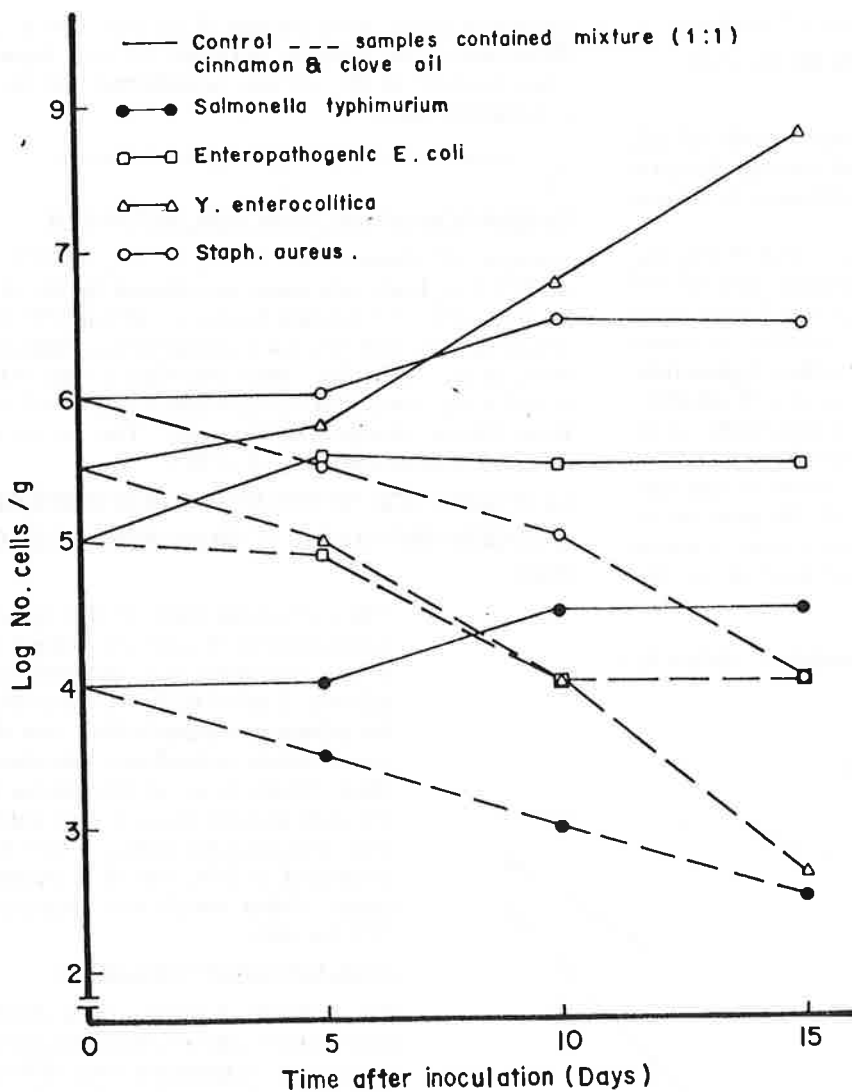


Fig. (4): Effect of 0.3% cinnamon and clove oils (1:1 V/V) mixture on food poisoning bacteria inoculated into poultry meat products and stored at 5°C.

effects against both food poisoning and spoilage bacteria with limited variable degrees of activities.

The minimum inhibitory concentration (MIC) of the tested oils on food poisoning and spoilage bacteria are listed in Table 1. The (MIC,s) of cinnamon and clove oils on food poisoning bacteria: *Salmonella typhimurium*, *Staphylococcus aureus*, *Yersinia enterocolitica* and enteropathogenic *Escherichia coli* were (0.4%, 0.45%, 0.35% and 0.12%) and (0.2%, 0.45%, 0.25% and 0.25%) respectively. While the (MIC's) of cinnamon and clove oils on spoilage bacteria: *Pseudomonas aeruginosa* and *Proteus vulgaris* were (0.3% and 0.2%) and (0.2% and 0.2%) respectively. *Sarcina lutea* was very sensitive to both oils. On the other hand the (MIC,s) of cinnamon and clove oils (1 : 1, v/v) mixture on the tested food poisoning bacteria were 0.3% for *Staph. aureus*, and 0.1% for all other tested organisms previously mentioned.

Estimating the bacteriostatic and bactericidal activities of the tested oils against all tested bacteria previously

mentioned, showed that both oils have bactericidal activities at concentration not exceeding double the (MIC).

The extension time of 0.5% cinnamon or clove oils on food poisoning and spoilage bacteria were less than 10 minutes for *Salmonella typhimurium*, *Staph. aureus* and *Yersinia enterocolitica* and less than 60 minutes for other tested bacteria in both oils as recorded in Table 2.

Poultry meat products are recently practised in Egypt by some of poultry factories. Processed poultry meat such as raw sausage and chicken-burger are recently available. Previous studies (El-Khateib et al., 1988) showed that the raw poultry meat products (sausage and chicken-burger) harbor the same bacteria and the same pathogens as chilled poultry, which represents the same problems of cross-contamination after thawing or exposure for sale. Therefore, efforts are carried out to lessen to some degree these problems during preparation and manufacture. It has been known since ancient times that essential oils of certain spices as cinnamon and clove have preservative qualities. The use of 0.5% cinnamon oil, 0.4% clove oils and 0.3% mixture of both oils (1:1, v/v) on the behaviour of food poisoning bacteria (*Salmonella typhimurium*, *Staphylococcus aureus* enteropathogenic *Escherichia coli* and *Yersinia enterocolitica*) which inoculated into sausage and

chicken-burger and held at 25°C, 10°C, 5°C and -20°C were investigated. The results revealed that the behaviour of all inoculated organisms in both products were parallelly similar. It is of interest that combination of cinnamon and clove oils (1:1 v/v) produced a more pronounced effect in inhibiting growth of food poisoning bacteria as illustrated in Fig. 2,3,4 and 5.

Concerning the behaviour of food poisoning bacteria which inoculated into poultry meat products and contained 0.3% mixture of cinnamon and clove oils and held at 25°C and 10°C, it is clear that the oils caused a decrease in the count of *Salmonella typhimurium*, *Yersinia enterocolitica* and *E. coli* of 1 log cycle, while the count of *Staph. aureus* decreased 1.5 log cycle after 48 h at 25°C and 72 h at 10°C as recorded in Fig. 2 and 3.

It is evident from the obtained results Fig. 4 that the storage temperature (5°C) retarded the growth of food poisoning bacteria except the growth of *Y. enterocolitica*

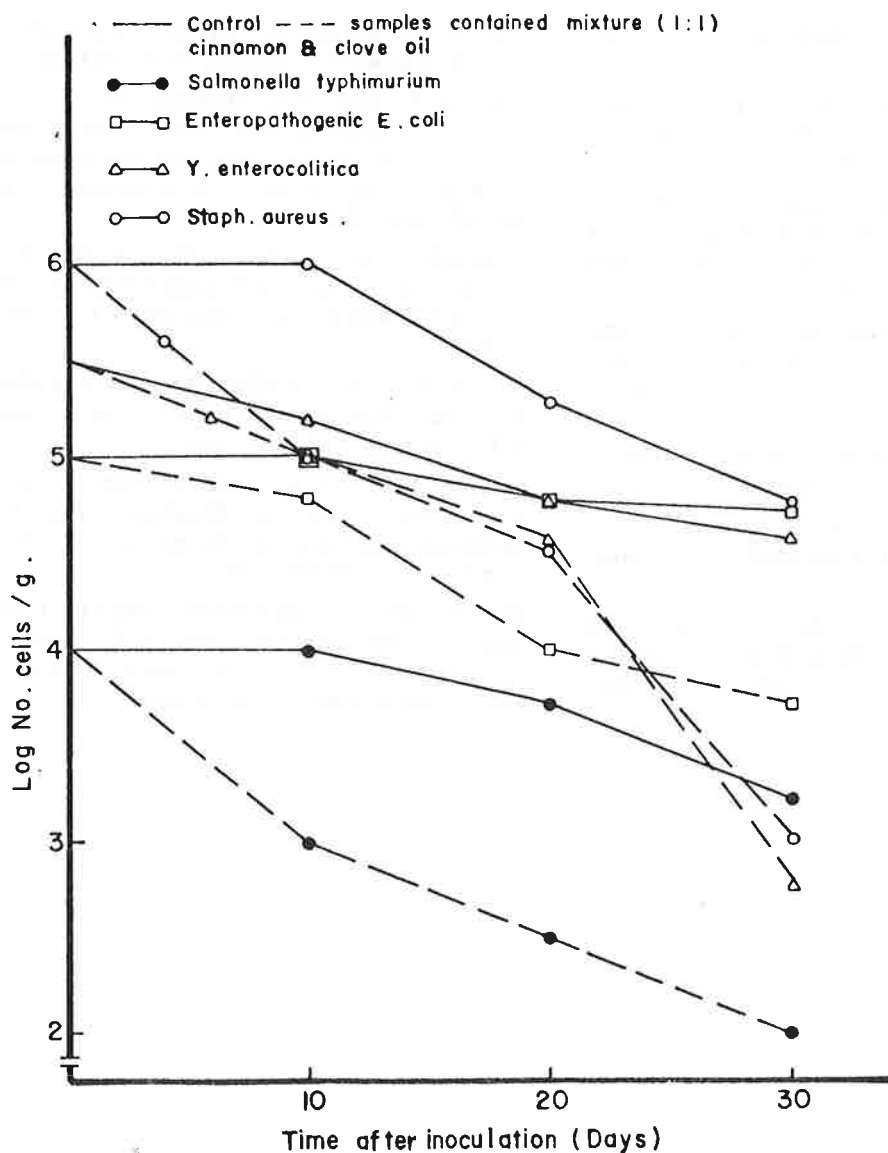


Fig. (5): Effect of 0.3 % cinnamon and clove oils (1:1 V/V) mixture on food poisoning bacteria inoculated into poultry meat products, and stored at -20°C .

which reflects the psychrotrophic capability of the organism. On the other hand, the use of oils at this temperature leads to decrease the count of *S. typhimurium*, *Staph. aureus* and *Y. enterocolitica* to 1.5, 2 and 2, log cycle, respectively. But the count in case of *E. coli* decreased to 1 log cycle.

Fig. 5 revealed that the counts of *S. typhimurium*, *Staph. aureus*, *Y. enterocolitica* and *E. coli* which inoculated in poultry meat products (control) when stored at -20°C were decreased to (0.9, 1, 0.9 and 0.2) log cycle, respectively. When the product contained cinnamon and clove oils mixture (1:1 v/v) and stored at this temperature, the count for the previously food poisoning bacteria were decreased to (2, 3, 2 and 2) log cycle, respectively.

Finally it could be concluded that the viability and survival of *S. typhimurium*, *Staph. aureus*, *Y. enterocolitica* and *E. coli* inoculated into poultry meat products affected

by storage temperature and use of cinnamon and clove oils. In respect to the effect of storage temperature on the viability and survival of the tested food poisoning bacteria except *Y. enterocolitica* inoculated into foodstuffs at 4°C reflects the psychrotrophic capability of the organism and makes it unique among enteropathogens.

This leads to the conclusion that the food poisoning bacteria do not differ greatly from non pathogens in their growth and survival at different storage temperature. The increasingly effective use of refrigeration as a part of meat production or use of special additives (as spices) which inhibit bacterial growth indicates the possibility of used combination of different spices oils as antibacterial agents. The study indicates that the combination of cinnamon and clove oils, if used in sufficient amounts can be effective inhibitors of the growth of food poisoning bacteria, without affecting the organoleptic properties (odour, taste and colour) of the final poultry meat products.

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