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Antimicrobial effects of some essential oils and honey on some pathogenic bacteria in chicken meat

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ABSTRACT

The present study was designed to investigate antimicrobial activity of garlic (*Allium sativum*), thyme (*Thymus vulgaris*) oils at concentrations (1%) and honey (10%) in chicken meat. A total of 2000g of the fresh chicken meat samples were divided into two groups (A&B) the first group was inoculated with *E. coli* (10⁶ cfu/g) and the second one with *S. aureus* (10⁶cfu /g) strains. Both of them were subdivided into untreated(control) and treated ones with garlic (1%), thyme (1%) oils and honey (10%), respectively. All samples were stored at 4°C and subjected to sensory and bacteriological evaluations after 3 hours, 1st, 2nd, 3rd, 4th and 5th day of cold storage. Sensory attributes of different treated chicken meat samples were mostly improved with honey (10%) followed by thyme oil (1%) then garlic oil (1%) when compared with control. Garlic oil (1%) with reduction % of *E.coli* & *S. aureus* (83.43, 93.43, 98.16, 99.91 and 100 %), (86.43, 98.45, 99.82 99.99 and 100%) after 1st, 2nd, 3rd, 4th & 5th days of storage, respectively. While thyme oil (1%) with reduction % of *E.coli* & *S. aureus* (90.18, 98.58, 99.90, 99.99 % and 100%), (94.35, 99.69, 99.95 %, 100% and 100%) after 1st, 2nd, 3rd, 4th & 5th days of storage, respectively. The best effectiveness of both pathogens reduction (100%) was honey (10%) at 3rd, 4th and 5th days, with a significant advantage in extend shelf-life of refrigerated chicken meat compared to all groups specially control ones. active principles in honey as bactericidal effect are hydrogen peroxide, phenolic acids, flavonoids and methylglyoxal. Generally, essential oils & honey proved to be efficient natural preservatives which is safe to human than chemical ones

1. INTRODUCTION

Poultry meat is one of the most popular food worldwide because of its easy digestibility; acceptance by the majority of people and solve the problem of the shortage in fresh meat of high price that is not within the reach of large numbers of families with limited income (Biesalski, 2005 and Sousa, 2008). Chicken meat was a common vehicle of pathogenic microorganisms such as *E. coli* and *S. aureus* that considered as the most important causes of foodborne outbreaks in people (Noori and Alwan, 2016).

Avian strains of *E. coli* show many similarities with human extra intestinal pathogenic *E. coli* (ExPEC) strains, in that most of the virulence genes they possess and they can be transferred to humans through consumption of contaminated food or food products causing a variety of infections (Moulin *et al.*, 2006; Johnson *et al.*, 2006 and Ewers *et al.*, 2007).

Staphylococcus aureus is considered the third-most important cause of food-borne disease in the world (Liu *et al.*, 2006 and Normanno *et al.*, 2007). The isolates from chicken carcasses showed proteolytic and lipolytic activity at 20°C, causing meat spoilage (Gundogan and Devren, 2010).

Pathogens difficult to be eliminated during food

processing, as many types of bacteria can adhere to food surface forming biofilms where they can survive even with sufficient methods of decontamination (Mohsenipour and Hassanshahian, 2015). Moreover, the old methods of food preservation have been changed to recent ones for example biochemical, bio preservatives and non-thermal (Zhou *et al.*, 2010).

Essential oils considered excellent natural preservatives instead of chemical ones and their use in food meets the demands of consumers for mildly processed or natural products (Burt, S., 2004).

Garlic oil has antibacterial effect, as this oil rich in organ sulfur compounds which inhibit growing of many bacteria such as *E. coli* & *S. aureus* and subsequently increase the shelf life as well as enhance the organoleptic characteristics of meat (Jolly and Menon, 2015) .

Thyme oil can be used as natural meat preservatives with antimicrobial activities against food borne pathogens, and therefore may be used in maintaining the meat quality, extending shelf life of meat products, preventing economic loss and providing the consumer with food containing natural additive (Salem *et al.* 2017)

In addition, the present study suggested the use of honey as a new natural substance for preservation of chicken meat and was selected on bases of its availability and its efficacy

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as antibacterial Sulaiman *et al.*, 2012), antifungal (Molan, 1992) and antiviral (Ghapanchi *et al.*, 2011) beside its efficiency in controlling human pathogens (Al-Waili, 2004; Mandal and Mandal, 2011 and Albaridi, 2019).

So, the aim of this study was the evaluation of the efficiency of some essential oils (thyme & garlic) and honey in control of *E. coli* and *S. aureus* growth in fresh chicken meat stored at 4°C.

2. MATERIAL AND METHODS

2.1. Bacterial strains:

Actually, *E. coli* and *S. aureus* strain were obtained from Cairo- MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain-Shams University, Egypt, with recommended dose (10^6 cfu / ml) for *E. coli* (Jagadeesh Babu *et al.*, 2013) and (10^6 cfu / ml) for *S. aureus* (Stewart *et al.*, 2003).

2.2. Antibacterial agents:

2.2.1. Natural oils:

Garlic (*Allium sativum*) and Thyme (*Thymus vulgaris*) oils (1%) were purchased from were purchased from Elgamhoria Co., Sharkia, Egypt and stored at 4°C until use.

2.2.2. Pure Egyptian honey (Baladi Honey Apiary).

2.3. Experimental application:

A total of 2000g of the fresh chicken meat was purchased from retail chicken shops in Kaliobia Governorate, Egypt. The collected samples were divided into 2 main groups (1000 g of each) (A & B) the first group was inoculated with *E. coli* (10^4 cfu/g) and the second one with *S. aureus* (10^6 cfu/g) strains. Both of them were subdivided into untreated (control) and treated ones (250 g. in each) with

garlic (1%), thyme (1%) oils and honey (10%), respectively. All samples were stored at 4°C and subjected to sensory and bacteriological evaluations after 3 hours, 1, 2, 3, 4 & 5 days of cold storage. The experiment was performed in triplicate.

2.4. Sensory examination:

The color, odor and overall acceptability were determined for each sample of meat according to (Fik and Fik, 2007).

2.5. Enumeration of *E. coli* and *S. aureus*:

The counts were determined according to FDA (2002).

2.6. Reduction percentage

$$\text{Reduction} = \frac{\text{Initial load} - \text{New Count}}{\text{Initial load}} \times 100$$

2.7. Statistical analysis

Data found were analyzed as stated by Snedecor and Cochran (1969) using the computer software program (SPSS, 2001).

3. RESULTS

Results in table (1) illustrated the effects of different concentrations of garlic, thyme oils & honey on sensory attributes & overall acceptability of artificially inoculated chicken meat samples with *E. coli* and *S. aureus*. Garlic (1%), thyme (1%) oils and honey (10%) showed overall acceptability extend to 4th and 5th days, while control (untreated subgroups) remain accepted until 2nd days of storage.

Table 1. The effects of different concentrations of garlic, thyme and honey on overall acceptability of chicken meat samples stored at 4°C (n=5).

Groups	Storage time					
	3 hrs.	1st day	2nd day	3rd day	4th day	5th day
Control	4.91±0.18 ^a	4.33±0.28 ^a	3.22±0.20 ^a	2.79±0.10 ^b	1.67±0.08 ^b	1.11±0.05 ^c
Garlic oil 1%	4.93±0.10 ^a	4.60±0.30 ^a	4.36±0.19 ^a	4.11±0.16 ^a	3.69±0.18 ^a	2.46±0.10 ^{ab}
Thyme oil 1%	4.96±0.12 ^a	4.89±0.24 ^a	4.73±0.12 ^a	4.41±0.20 ^a	3.81±0.18 ^a	3.26±0.13 ^a
Honey 10%	4.99±0.17 ^a	4.99±0.34 ^a	4.97±0.27 ^a	4.89±0.22 ^a	4.58±0.18 ^a	4.13±0.27 ^a

Score System for Sensory Evaluation: 5: Very good. 4: Good, 3: Acceptable, 2: Unacceptable, 1: Bad, S: Spoiled

As shown in table (2) results revealed the antimicrobial effects and reduction percentages of garlic, thyme oils at concentration (1%) and honey (10%) on counts of *E. coli* artificially inoculated into fresh chicken meat. Garlic oil (1%) decreased count of *E. coli* from 4×10^6 (initial load) to $3.87 \pm 0.39 \times 10^6$, $6.63 \pm 0.31 \times 10^5$, $2.63 \pm 0.57 \times 10^5$, $7.36 \pm 0.44 \times 10^4$ and $3.72 \pm 0.14 \times 10^3$ (cfu/g) with reduction percentages of 3.25, 83.43, 93.43, 98.16, 99.91 and 100% after 3 hrs. & 1st, 2nd, 3rd, 4th & 5th days of storage. Also, thyme oil (1%) decreased counts of *E. coli* to $3.41 \pm 0.16 \times 10^6$, $3.93 \pm 0.17 \times 10^5$, $5.68 \pm 0.54 \times 10^4$, $4.11 \pm 0.16 \times 10^3$ and $1.17 \pm 0.09 \times 10^2$ (cfu/g) after 3 hrs. & 1st, 2nd, 3rd, 4th & 5th days of storing time, with reduction percentages of 14.75, 90.18, 98.58, 99.90, 99.99% and 100%. Honey (10%) decreased counts of *E. coli* after 3 hrs., 1st and 2nd days of storage to $3.14 \pm 0.44 \times 10^6$, $2.89 \pm 0.54 \times 10^4$ and $6.37 \pm 0.18 \times 10^3$ (cfu/g) with reduction percentages of 21.5, 99.28 and 99.84%, with complete reduction (100%) of *E. coli* count at 3rd, 4th and 5th days of storage. Moreover, all results showed a significant $P < 0.05$ reduction (growth inhibition) of *E. coli* in chicken meat samples treated with honey 10%; thyme oil 1% and garlic oil 1% during cold storage.

Table (3) declared the antimicrobial effects and reduction percentage of garlic, thyme oils at concentration (1%) and honey (10%) on growth of *S. aureus* artificially inoculated into fresh chicken meat. Garlic oil (1%) decreased count of *S. aureus* from 4×10^6 (initial load) to $3.63 \pm 0.14 \times 10^6$, $5.43 \pm 0.24 \times 10^5$, $6.21 \pm 0.32 \times 10^4$, $7.14 \pm 0.28 \times 10^3$ and $2.14 \pm 0.28 \times 10^2$ (cfu/g) with reduction percentages of 9.25, 86.43, 98.45, 99.82, 99.99 and 100% after 3 hrs & 1st, 2nd, 3rd, 4th & 5th days of storing time, respectively. Moreover, thyme oil (1%) decreased counts of *S. aureus* to $2.89 \pm 0.61 \times 10^6$, $2.26 \pm 0.13 \times 10^5$, $1.26 \pm 0.13 \times 10^4$ and $1.92 \pm 0.04 \times 10^3$ (cfu/g) with reduction percentage 27.75, 94.35, 99.69, 99.95%, 100% and 100% of *S. aureus* count 3 hrs. & 1st, 2nd, 3rd, 4th & 5th days of storing time. At the same time, honey (10%) decreased counts of *S. aureus* to $2.55 \pm 0.21 \times 10^6$, $1.79 \pm 0.65 \times 10^4$ and $3.92 \pm 0.37 \times 10^3$ (cfu/g) with reduction percentages of 36.25, 99.55, 99.90%, 100%, 100% and 100% of *S. aureus* count after 3 hrs. & 1st and 2nd, 3rd, 4th & 5th days of time. Moreover, there was a significant difference at ($P < 0.05$).

Table 2 The effects and Reduction percentage of various concentrations of garlic, thyme and honey on *E. coli* counts (cfu/g) inoculated into chicken meat samples.

Storage time	Control		Garlic oil 1%		Thyme oil 1%		Honey 10%	
	Count	Reduction %	Count	Reduction %	Count	Reduction %	Count	Reduction %
Initial load	4.0x10 ⁶ ± 0.1x10 ⁶	-	4.0x10 ⁶ ± 0.1x10 ⁶	-	4.0x10 ⁶ ± 0.1x10 ⁶	-	4.0x10 ⁶ ± 0.1x10 ⁶	-
3 hrs	4.83±0.27 X10 ⁶	-	3.87±0.39 X10 ⁶	3.25	3.41±0.16 X10 ⁶	14.75	3.14±0.44 X10 ⁶	21.5
1 day	4.28±0.26 X10 ⁶	-	6.63±0.31 X10 ⁵	83.43	3.93±0.17 X10 ⁵	90.18	2.89±0.54 X10 ⁴	99.28
2nd day	1.59±0.12 X10 ⁷	-	2.63±0.57 X10 ⁵	93.43	5.68±0.54 X10 ⁴	98.58	6.37±0.18 X10 ³	99.84
3rd day	2.97±0.19 X10 ⁷	-	7.36±0.44 X10 ⁴	98.16	4.11±0.16 X10 ³	99.9	Not Detected	100
4th day	8.33±0.46 X10 ⁷	-	3.72±0.14 X10 ³	99.91	1.17±0.09 X10 ²	99.99	Not Detected	100
5th day	Spoiled	-	Not Detected	100	Not Detected	100	Not Detected	100

Table (3) The effects and Reduction percentage of various concentrations of garlic, thyme and honey on *S. aureus* counts (cfu/g) inoculated into chicken meat samples.

Storage time	Control		Garlic oil 1%		Thyme oil 1%		Honey 10%	
	Count	R%	Count	R%	Count	R%	Count	R%
Initial load	4.0x10 ⁶ ± 0.1x10 ⁶	-	4.0x10 ⁶ ± 0.1x10 ⁶	-	4.0x10 ⁶ ± 0.1x10 ⁶	-	4.0x10 ⁶ ± 0.1x10 ⁶	-
3 hrs	4.15±0.10 X10 ⁶	-	3.63±0.14 X10 ⁶	9.25	2.89±0.61 X10 ⁶	27.75	2.55±0.21 X10 ⁶	21.5
1 day	9.64±0.36 X10 ⁶	-	5.43±0.24 X10 ⁵	86.43	2.26±0.13 X10 ⁵	94.35	1.79±0.65 X10 ⁴	99.28
2nd day	5.75±0.19 X10 ⁷	-	6.21±0.32 X10 ⁴	98.45	1.26±0.13 X10 ⁴	99.69	3.92±0.37 X10 ³	99.84
3rd day	4.82±0.36 X10 ⁸	-	7.14±0.28 X10 ³	99.82	1.92±0.04 X10 ³	99.95	Not Detected	100
4th day	Spoiled	-	2.14±0.28 X10 ²	99.99	Not Detected	100	Not Detected	100
5th day	Spoiled	-	Not Detected	100	Not Detected	100	Not Detected	100

4. DISCUSSION

The presence of *E. coli* in food is considered as indicator of faults during preparation, handling or storage. It is also, considered as indicator of fecal contamination, beside. It may induce severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults (Hassan, 2007). Further, *S. aureus* is an important reason of food poisoning because of eating food containing staphylococcal enterotoxins. Many types of foods are considered an excellent medium for *S. aureus* especially meat products (Güven et al., 2010).

Essential oils inhibit DNA, RNA, proteins and polysaccharides synthesis in the bacterial cells (Akthar et al., 2014). Likewise, they affect membrane integrated and associated enzyme-proteins, inhibit their production.

Tables (2 & 3) indicated that considered essential oils have a significant uncontaminated effect on *E. coli* and *S. aureus* growth, where, thyme (1%) had higher inhibitory effect than that obtained by garlic (1%). Used essential oils can be chosen for use as potential antibacterial efficient and extend shelf life of fresh chicken meat. These findings were similar with those of Jolly and Menon (2015), who reported that garlic possess a good potential to act as natural preservative against *E. coli*.

The tested essential oils were more effective on Gram +ve *S. aureus* than Gram -ve *E. coli* this may be because of that volatile action of essential oils and absence of lipopolysaccharide layer in Gram positive bacteria which consider a good barrier against any external biomolecule. Also, it may be due to that essential oils can perfectly prevent bacterial respiration and rise the plasma membrane permeability, this resulted in bacterial cells death (Burt, 2004; Wolde et al., 2018). Also, these results may be attributed to the antibacterial effect of thyme because it contains high thymol content which altering the permeability features of *E. coli* and *S. aureus* membrane. Thymol is more inhibitive at pH 5.5 than 6.5. At low pH its molecule would be un-dissociated and more hydrophobic. So, it can bind better to the hydrophobic parts of proteins and liquify better in the lipid phase (Helmy, Shahinaz,

2012; Küçükbay et al., 2014; Zengin and Baysal, 2015).

Garlic oil has antibacterial effects, as it contain high amount of organo-sulfur compounds and their precursors which inhibit growing of most microbes like *E. coli* and *S. aureus*, increase shelf life of food, therefore it consider effective as natural preservative to chicken meat products. Addition of garlic extracts improves color, phospholipid and bacterial safety of foods (Chand, 2013; Jolly and Menon, 2015; Wolde et al., 2018). On the other hand, honey (10%) showed the higher antibacterial effect than thyme and garlic oil (1%). The chicken meat samples treated with honey 10% had the highest reduction rate of *E. coli* & *S. aureus* counts.

The results of Honey treated group was similar to those of Mundo et al. (2004) and Istrati et al. (2011) as they recorded that, marination of meat with honey resulting in inhibition the growth of foodborne pathogens and maintain the shelf-life of the meat. In addition, conceded the results of Kwakman and Zaat (2011) and Matzen et al. (2018) that honey had inhibitory effects against most Gram-positive and negative bacteria specially *S. aureus* and *E. coli*, but it had more effects on Gram-positive bacteria than Gram-ve ones. The antibacterial activity of honey may be attributed to the hydrogen peroxide produced and accumulated in diluted honey either with water or tissue fluids by honey enzyme glucose oxidase during its action on honey glucose to form gluconolactone (Molan, 1996; Cooper et al., 2002; Ka ániová et al., 2011). The high osmotic pressure and potassium content of honey that leads to withdrawal of water and plasmolysis of the organisms (Cooper et al., 2002; Sulaiman et al., 2012).

5. CONCLUSION

Finally, the present study allowed to conclude that honey (10%) proved to be more efficient than garlic and thyme oils (1%) in suppression of both of *E. coli* and *S. aureus* growth in chicken meat. So, the use of honey (10%), as it is safe antibacterial agent, is therefore recommended to improve safety of chicken meat products

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