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Use of oregano (*Origanum onites* L.) essential oil as hatching egg disinfectant

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This study was carried out to determine whether oregano (*Origanum onites*) essential oil works as a disinfectant for hatching egg obtained from broiler breeder flock. Oregano essential oil was applied at two doses 0.55 and 0.75 $\mu\text{l}/\text{cm}^3$ and two exposure times, 3 and 6 h. The formaldehyde treated eggs were used as positive control and untreated eggs used as negative control. After chemical analysis, the main constituents of oregano essential oil were carvacrol, linalool, para-cymene and γ -terpinene. The lowest microbial counts on eggs were obtained from oregano essential oil. Microbial inhibition increased with the increasing essential oil concentrations. Essential oil exposure times had no significant effects on microbial counts. Essential oil fumigation lowered middle embryonic mortality and discarded chick rate, but increased early and late embryonic mortalities compared to formaldehyde treatment. Essential oil doses significantly affected late embryonic mortality, discarded chicks rate, contamination rate, hatchability of fertile egg, body weight at 21 and 42 days, body weight gain and total feed consumption. But, early and middle embryonic mortality were not significantly affected by treatments. These results imply that oregano essential oil had great potential for hatching egg disinfectant and it could be used as natural egg disinfectant.

Key words: Egg disinfectant, bio-fumigation, hatching egg, *Origanum onites*.

INTRODUCTION

There are numerous infectious organisms that can infect an egg before and after laying. *Proteus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Klebsiella* sp., *Staphylococcus* sp., *Streptococcus* sp., *Clostridium* sp., *Bacillus cereus*, *Salmonella typhimurium* and *Enterococcus* have been isolated from hatching eggs. However, the most common isolated bacterium is *Escherichia coli* (Sarma et al., 1985; Cason et al., 1994). It can enter the egg from an infected reproductive tract of a hen. Also *E. coli* can penetrate through the eggshell if the egg is contaminated with fecal material. *E. coli* commonly causes yolk sac infection, leading to a watery and yellow-green or yellow-brown content. Dirty nests and cages can serve as sources of

contamination to eggs (Harry, 1963; Board et al., 1964; Williams et al., 1968). In addition to surface contamination, egg became wet and warm after penetration by microorganisms (Board, 1966; Williams et al. 1968; Mayes and Takeballi, 1983). The infectious organisms can be transferred from the infected hen to the egg during fertilization, egg development in the oviduct of the hen or immediately after oviposition or a laid egg could be contaminated with some infectious organisms passing through the eggshell upon contact with contaminated faeces or bedding. Therefore, sanitation is essential in successful healthy hatchlings. Several methods are available for sanitizing hatching eggs. Fumigation, spray application, UV light and washing with appropriate sanitizer are the common applied practices for sanitation (Alder et al., 1979; Arhienbuwa et al., 1980; Proudfoot et al., 1985; Kuhl, 1989; Sacco et al., 1989; Whistler and Sheldon, 1989; Coufal et al., 2003). The applied sanitation

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practices depend on the size of operation, history of contamination at the site and the capacity of the equipments. Many embryos infected with *E. coli* die late in incubation or shortly after hatching. If hatching eggs are not sanitized prior to incubation, excessive bacterial contamination and subsequent growth of bacterial population can lead to decreased hatchability, poor chick quality, poor growth and performance (Scott and Swetnam, 1993) and increased mortality (Reid et al., 1961). If an *E. coli* infection is acquired during incubation, the hatchling may develop an umbilical and yolk sac infection (omphalitis) and they may have poor weight gain.

Essential oils represent a rich potential source of alternative and environmentally acceptable control agents for infectious organisms due to their antimicrobial properties. Plants in Labiate family possess essential oils, which could be utilized for killing microorganisms (Mishra and Dubey, 1994; Montes-Belmont and Carvajal, 1998; Vazquez et al., 2001; Kalemba and Kunicka, 2003). Most of these substances are volatile and can act as fumigants, thus offering the prospect of use against numerous infectious organisms (Goi et al., 1985; Sekiyama et al., 1996; Arras and Usai, 2001). Oregano, one kind of labiate has been known for a long time as a popular remedy. The essential oil of oregano has anti-bacterial (Aureli et al., 1992; Biondi et al., 1993; Baydar et al., 2004; Vagi et al., 2005), anti-oxidant (Gouladis et al., 2003; Tepe et al., 2004), anti-fungal (Muller et al., 1995; Bouchra et al., 2003), cytotoxic (Sivropoulou et al., 1996; Wilson et al., 1997), insecticidal (Traboulsi et al., 2002) and nematocidal (Oka et al., 2000) properties mainly due to the basic constituents including carvacrol, thymol, γ -terpinene and *p*-cymene.

Human health, environmental concern and consumers' demand for residue-free food necessitate the evaluation of alternative, reduced-risk control methods. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents. Understanding of plant biochemistry, physiology and chemistry of natural products have shown that the secondary metabolites may be used to control infectious organisms to overcome the above problems associated with synthetic chemicals (Delaquis and Mazza, 1995; Hammer et al., 1999). As a result, increased interest is being shown in developing alternative methods for microbial contamination control to reduce or eliminate reliance on synthetic pesticides. One such method involves the use of plant-derived-products, such as plant essential oils, having antimicrobial effect.

Keeping all these points in consideration, an attempt has been made in the present piece of work to find out the practical applicability of oregano essential oil to control microbial activity on eggshell and to determine its effects on hatchability of fertile egg, growth and development of chicks after hatching.

MATERIALS AND METHODS

Oregano (*Origanum onites* L.) plants were collected in Telkalis Research Farm of Mustafa Kemal University, Turkey in 2007 growing season. The plant parts (stems, leaves and flowers) were air-dried in shadow. Essential oil was extracted by water distillation for 3 h from air-dried leaves, by using a Clevenger-type apparatus.

The essential oils obtained from extraction were dried over anhydrous sodium sulfate and stored at 4°C in a refrigerator until analysis. The GC analyses were carried out using Hewlett-Packard 6890 GC with FID. A HP-5 MS capillary column (30 m x 0.25 mm i.d. 0.25 μ m film thickness) was used. Helium was used as a carrier gas (1.4 ml/min). The column was temperature programmed as follows: 5 min at 45°C; then at 3°C/min to 220°C and held for 10 min. The injector and detector temperatures were 220 and 250°C, respectively. Injection was applied with automatic mode. Samples (0.5 μ l of the oil solution in hexane (1:100) was injected using the splitless technique into Helium carrier gas. Peak areas and retention times were measured by electronic integration.

GC/MS analysis of the essential oil was carried out on Hewlett Packard 5970A mass selective detector (MSD), directly coupled to a HP 6890 GC. The column, temperature program and injection were performed as described above. Injection was applied with automatic mode. Library search was carried out using "Wiley Library, WILEY275, NBS75K, NIST98, FLAVOR". EI mass spectra were measured at 70 eV ionizations voltage over the mass range 10 - 400 u. Identification of the compounds was achieved by comparing retention times and mass spectra with those of the standards in the library (Stenhagen et al., 1974; Adams, 1995).

The daily collected freshly laid eggs of 54 weeks old broiler genotype Ross-308 were obtained from a commercial company located in Antakya, Turkey. The eggs were collected twice a day (early in the morning, 9 a.m. and late in the afternoon 4 p.m.) from this commercial company farm. The eggs were inspected for any shell crack and faecal contamination and eggs having visible crack or faecal contamination were discarded. After collection, the eggs were stored for 1 day at around 15 - 18°C and 75% relative humidity (RH) prior to initiation of the experiment.

In the present study, a total of 1800 eggs were used. The eggs were divided into six groups to investigate the effects of sanitizers on hatching results. The first group consisted of non-treated eggs (negative control). The second group was treated with formaldehyde (triple strength formaldehyde gas 3X = 119.8 ml formalin: 59.9 g potassium permanganate/m³) for 20 min at 24°C (positive control). Triple strength formaldehyde was used commercially on hatching eggs (USDA, 1985). The third, fourth, fifth and sixth groups were treated with oregano (*O. onites*) essential oil at two doses, 0.55 and 0.75 μ l/cm³ and two exposure times, 3 and 6 h with 300 eggs in each group, in a fumigation cabinet at 24°C in a temperature controlled room. An ambient room temperature of 24°C was selected, since these disinfectants do not need to penetrate the eggshell and contains no temperature-dependent ingredients. After disinfection, five eggs from each treatment group were immediately placed on sterile plastic bags. Following transport to the laboratory, all eggs were handled aseptically with new disposable gloves for each egg. A whole egg washing technique was used to recover the shell associated micro-organisms for estimating the total bacteria count, coliforms and fungi and molds counts of five eggs per treatment. Dilutions were prepared (10^{-1} - 10^{-3}) and then were inoculated into sterile petri plate. The total bacteria, coliforms and fungi were incubated at 37°C for 48 h. Total aerobic bacteria was counted using plate count agar (Merck) while coliforms and *E. coli* were counted according to FDA (1998). Potato dextrose agar (Merck) was used to count fungi and molds. Colonies were measured as cfu/ml.

Eggs were incubated at 37.8°C and 55 - 60% RH until 18th day of incubation when incubator conditions were changed to 37.2°C and 70 - 80% RH for the actual hatching process. After hatching all

Table 1. Chemical component, retention time and total peak area of oregano essential oil.

| Component | Retention time (min) | Total peak area (%) |
|-------------------|----------------------|---------------------|
| β-pinene | 5.24 | 0.50 |
| α-phellandrene | 7.23 | 0.45 |
| Myrcene | 7.41 | 2.48 |
| α-terpinen | 7.83 | 2.87 |
| Limonene | 8.54 | 0.46 |
| Sabinene | 8.87 | 0.47 |
| P-cymene | 12.07 | 7.86 |
| Linalool | 27.79 | 8.39 |
| β-caryophyllene | 28.74 | 1.27 |
| Terpinen-4-ol | 29.32 | 2.09 |
| β-bisabolene | 33.33 | 0.94 |
| Endo-borneol | 33.69 | 1.70 |
| Carvacryl acetate | 37.05 | 0.42 |
| Thymol | 43.58 | 0.86 |
| Germacrene | 43.72 | 0.33 |
| Carvacrol | 44.30 | 57.01 |

death for the non hatched fertile eggs (%). Hatchability of fertile eggs was determined by discounting all truly infertile eggs and dividing the number of chicks hatched by the total number of fertile eggs. In addition to these parameters, early embryonic mortality (%), middle embryonic mortality (%), late embryonic mortality (%), discarded chicks rate (%), pipped (%) and contamination rate (%) were determined.

After hatching, the chicks in the different treatment groups were separately raised in a poultry house. Each of the six treatments had 13 chicks and each treatment was replicated 3 times. The chicks were fed by starter feed (24% CP and 3000 kcal ME/kg) and chick diets (22% CP and 3100 kcal ME/kg), between 0 and 10 days and between 10 and 28 days, respectively. The chickens were fed by chicken diets (20% CP and 3200 kcal ME/kg) and finisher diet (19% CP and 3200 kcal ME/kg) between 28 and 35 days and between 35 and 42 days, respectively. Water was provided *ad libitum*. 42 days old chickens were slaughtered in each treatment. Initial body weight (g), body weight (g), body weight gain (g), total feed consumption (g) and feed conversion ratio (%) (g) were determined.

The obtained data from the experiment were subjected to analysis of variance as a 3 x 2 factorial design, using the general linear models procedure in the Statistical Analysis System (SAS Institute, 1996). The factors evaluated were essential oil doses (0.00, 0.55 and 0.75 µl/cm³) and essential oil treatment times (3 and 6 h). Each treatment was replicated 3 times with 300 eggs. For statistical analyses, microbial counts were transformed to Log₁₀ prior to statistical analysis. Means of measured plant parameters were compared using Fisher's protected least significance difference (LSD) at P < 0.05.

RESULTS

More than 30 components of oregano essential oil were identified. However, only 16 components detected in the essential oil of oregano in concentrations greater than 0.30% were β-pinene, α-phellandrene, myrcene, α-terpinen, limonene, sabinene, p-cymene, Linalool, β-caryophyllene, terpinen- 4 -ol, β-bisabolene, endo-

borneol, carvacryl acetate, thymol, germacrene and carvacrol (Table 1).

Application of oregano essential oil significantly reduced microbial activities on eggshell surface. The lowest bacteria and yeast-mold counts were obtained from oregano essential oil (Table 2). The highest bacteria and yeast-mold counts were obtained from both negative and positive control treatments.

Although the lowest bacteria and yeast-mold counts were obtained from 6 h essential oil treatment, the difference between treatment times was not significant (Table 3). Essential oil had significant effects on bacteria, yeast and mold count. The bacteria, yeast and mold count decreased with increasing doses of oregano essential oil. The lowest bacteria and yeast-mold counts were obtained from 0.75 µl/cm³ treatments with 1.14 and 1.02 cfu/egg, respectively (Table 3).

Early embryonic mortality, middle embryonic mortality, late embryonic mortality, discarded chicks rate, pipped, contamination rate, hatchability of fertile egg significantly varied among treatments (Table 4). The early embryonic mortality values varied between 2.81 and 3.87%. The lowest and the highest early embryonic mortality were obtained from formaldehyde and control treatment, respectively. Late embryonic mortality of oregano essential oil was slightly higher than the formaldehyde treatment. Discarded chick rate values varied between 0.62 and 1.41%. The lowest and the highest values were obtained from oregano essential oil and control treatments, respectively. The pipped values varied between 0.35 and 1.05%, the highest and the lowest values were obtained from the control and formaldehyde treatments, respectively. However, oregano essential oil treatment had a moderate pipped value (0.70%) between positive control formaldehyde and the negative control.

Table 2. Anti-microbial effect of oregano essential oil and formaldehyde on the microbial count of hatching egg surface.

| Treatment | Microbiological determinations (cfu/egg, geometric mean Log ₁₀)* | |
|-------------------------|--|----------------|
| | Bacteria | Yeast and mold |
| Control | 1.83 a | 1.37 a |
| Formaldehyde | 1.77 a | 1.30 a |
| Oregano essential oil** | 1.36 b | 1.13 b |
| LSD (0.05) | 0.12 | 0.18 |

* = Counts expressed as logarithms (base 10) of number per egg; ** = Microbial count of oregano essential oil is the mean of 2 treatment doses.

Table 3. Anti-microbial effect of oregano essential oil dose and exposure time on the microbial count of hatching egg surface.

| Treatment dose ($\mu\text{l}/\text{cm}^3$) | Microbiological counts (cfu/egg, geometric mean Log ₁₀)* | |
|--|--|----------------|
| | Bacteria | Yeast and mold |
| Control | 1.83 | 1.37 |
| 0.55 | 1.58 | 1.24 |
| 0.75 | 1.14 | 1.02 |
| LSD (0.05) | NS | NS |
| Exposure times (h) | | |
| 3 | 1.58 | 1.20 |
| 6 | 1.45 | 1.21 |
| LSD (0.05) | 0.15 | 0.16 |

*Counts expressed as logarithms (base 10) of number per egg; NS = not significant.

Table 4. Effect of essential oil treatment and formaldehyde on the hatchability and embryonic mortality stages of fertile egg.

| Treatment | EEM (%) | MEM (%) | LEM (%) | DCR (%) | Pipped (%) | CR (%) | HFE (%) |
|--------------|---------|---------|---------|---------|------------|--------|---------|
| Control | 3.87 | 0.35 | 3.52 | 1.41 | 1.05 | 1.76 | 88.02 |
| Formaldehyde | 2.81 | 1.40 | 4.21 | 1.40 | 0.35 | 0.35 | 89.91 |
| Oregano oil | 3.10 | 0.62 | 4.58 | 0.62 | 0.70 | 0.34 | 90.00 |
| LSD 0.05 | 0.53 | 0.46 | 0.33 | 0.46 | 0.49 | 0.54 | 1.23 |

EEM = early embryonic mortality; MEM = middle embryonic mortality; LEM = late embryonic mortality; DCR = discarded chicks rate; CR = contamination rates HFE: hatchability of fertile egg; NS = not significant.

When contamination rate was considered, oregano essential oil had the lowest value followed by formaldehyde treatment. The hatchability of fertile egg varied between 90.00 and 88.02%. Oregano essential oil had the highest hatchability value (90.00%) compared to formaldehyde (89.91%) treatment, but the difference between essential oil and formaldehyde treatments was not statistically significant.

Application of different oregano essential oil doses significantly affected late embryonic mortality, discarded chick rate, contamination rate and hatchability of fertile eggs (Table 5). However, early and middle embryonic mortality and pipped were not significantly affected from the essential oil doses. The highest and the lowest hatchability values of fertile eggs were obtained from 0.75

$\mu\text{l}/\text{cm}^3$ oregano essential oil and negative control treatments, respectively (Table 5).

Exposure time of oregano essential oil had no significant affect on the early embryonic mortality, middle embryonic mortality; late embryonic mortality, discarded chick rate, pipped, contamination and hatchability of fertile egg (Table 5). The exposure of 3 and 6 h of oregano essential oil had similar effects on all measured hatching egg parameters.

The effect of essential oil treatments on total feed consumption was significant, while body weight gain, feed conversion ratio and body weight were not significant (Table 6). The highest and the lowest feed conversion ratio were obtained from negative control and oregano essential oil treatments, respectively. Oregano

Table 5. Effect of essential oil doses and exposure time on the hatchability and embryonic mortality stages of fertile egg.

| Treatment dose ($\mu\text{l}/\text{cm}^3$) | EEM (%) | MEM (%) | LEM (%) | DCR (%) | Pipped (%) | CR (%) | HFE (%) |
|--|---------|---------|---------|---------|------------|--------|---------|
| Control | 3.87 | 0.35 | 3.52 | 1.40 | 1.05 | 1.75 | 88.02 |
| 0.55 | 3.00 | 0.52 | 4.94 | 0.87 | 0.87 | 0.35 | 88.87 |
| 0.75 | 3.18 | 0.18 | 4.23 | 0.35 | 0.52 | 0.35 | 91.13 |
| LSD 0.05 | NS | NS | 0.74 | 0.78 | NS | 0.86 | 2.45 |
| Exposure time (h) | | | | | | | |
| 3 | 3.29 | 0.59 | 3.99 | 1.05 | 0.70 | 0.82 | 89.52 |
| 6 | 3.40 | 0.47 | 4.46 | 0.70 | 0.93 | 0.82 | 89.17 |
| LSD 0.05 | NS | NS | NS | NS | NS | NS | NS |

EEM = Early embryonic mortality; MEM = middle embryonic mortality; LEM = late embryonic mortality; DCR = discarded chicks rate; CR = contamination rate HFE = hatchability of fertile egg; NS = not significant.

Table 6. Effect of essential oil and formaldehyde treatments on growth performance and feed consumption of chicks.

| Treatment | Initial body weight (g) | Total feed consumption (g) | Body weight gain (g) | Feed conversion ratio (%) | Body weight (g) |
|--------------|-------------------------|----------------------------|----------------------|---------------------------|-----------------|
| Control | 46.00 | 3781.33 | 2407.00 | 1.57 | 2441.33 |
| Formaldehyde | 46.00 | 3853.33 | 2430.33 | 1.55 | 2480.00 |
| Oregano oil | 46.08 | 3742.42 | 2416.67 | 1.52 | 2463.08 |
| LSD 0.05 | NS | 105.2 | NS | NS | NS |

NS = not significant.

Table 7. Effect of essential oil dose and exposure time on growth performance and feed consumption of chicks.

| Treatment dose ($\mu\text{l}/\text{cm}^3$) | Initial body weight (g) | Total feed consumption (g) | Body weight gain (g) | Feed conversion ratio (%) | Body weight (g) |
|--|-------------------------|----------------------------|----------------------|---------------------------|-----------------|
| Control | 46.00 | 3781.33 | 2448.67 | 1.57 | 2411.33 |
| 0.55 | 46.17 | 3565.17 | 2330.17 | 1.50 | 2377.00 |
| 0.75 | 46.00 | 3919.67 | 2503.17 | 1.54 | 2549.17 |
| LSD 0.05 | NS | 194.66 | 224.70 | NS | 163.69 |
| Exposure time (h) | | | | | |
| 3 | 46.1 | 3747.44 | 2383.78 | 1.54 | 2461.78 |
| 6 | 46.0 | 3763.33 | 2470.89 | 1.53 | 2429.89 |
| LSD 0.05 | NS | NS | NS | NS | NS |

NS: not significant.

essential oil treatment had the lowest feed conversion ratio values compared with negative and positive control treatments. The differences of feed conversion rate among treatments were not significant.

The highest body weight was obtained from positive control formaldehyde treatment (2480.33); however, the differences among treatments were not significant.

Application of 0.55 and 0.75 $\mu\text{l}/\text{cm}^3$ oregano essential oil doses did not significantly affect feed conversion ratio (Table 7). However, total feed consumption, body weight gain and body weight were significantly affected from essential oil doses. Essential oil doses significantly affected body weights. The highest body weight at 42

days was obtained from 0.75 $\mu\text{l}/\text{cm}^3$ with 2549.17 g and the lowest was obtained from 0.55 $\mu\text{l}/\text{cm}^3$ with 2377.00 g.

When essential oil exposure time was in consideration, exposure time had no significant effect on initial body weight, total feed consumption, body weight gain, feed conversion ratio, body weight at 21 and 42 days (Table 7).

Essential oil and formaldehyde treatments had no significant effect on slaughter weight, carcass weight, leg weight and breast weight (Table 8). Leg weight and abdominal fat weight were the only parameters that were significantly affected from the treatments. The highest and the lowest values were obtained from the oregano

Table 8. Effect of essential oil and formaldehyde treatments on slaughter weight and carcass parts of chicks.

| Treatment | Slaughter weight (g) | Carcas (g) | Legs (g) | Wing (g) | Breast (g) | Abdominal fat (g) |
|--------------|----------------------|------------|----------|----------|------------|-------------------|
| Control | 2429.00 | 1864.33 | 492.67 | 199.00 | 578.33 | 49.67 |
| Formaldehyde | 2404.33 | 1830.33 | 500.00 | 226.00 | 552.00 | 36.00 |
| Oregano oil | 2416.25 | 1862.58 | 502.25 | 210.08 | 581.33 | 51.76 |
| LSD 0.05 | NS | NS | NS | 30.73 | NS | 5.3 |

NS = not significant.

Table 9. Effect of essential oil treatment dose and exposure time on slaughter weight and carcass parts of chicks.

| Treatment dose ($\mu\text{l}/\text{cm}^3$) | Slaughter weight (g) | Carcass (g) | Legs (g) | Wing (g) | Breast (g) | Abdominal fat (g) |
|--|----------------------|-------------|----------|----------|------------|-------------------|
| Control | 2429.00 | 1864.33 | 492.67 | 199.00 | 578.33 | 49.67 |
| 0.55 | 2349.67 | 1808.50 | 490.83 | 206.17 | 567.50 | 46.13 |
| 0.75 | 2467.33 | 1897.83 | 525.67 | 214.00 | 595.17 | 54.83 |
| LSD 0.05 | NS | NS | NS | NS | NS | NS |
| Exposure time (h) | | | | | | |
| 3 | 2395.70 | 1850.10 | 499.50 | 206.00 | 578.60 | 47.48 |
| 6 | 2439.88 | 1865.38 | 507.50 | 206.87 | 582.50 | 54.50 |
| LSD 0.05 | NS | NS | NS | NS | NS | NS |

NS: Not significant.

essential oil and formaldehyde treatment, respectively. Application of oregano essential oil had slightly higher leg weight and breast weight than formaldehyde treatment, while formaldehyde had slightly higher wing weight than oregano essential oil treatment (but not significant).

Essential oil doses and exposure times had no significant effect on slaughter weight, carcass weight, legs weight, wing weight, breast weight and abdominal fat weight (Table 9). The highest values for on slaughter weight, carcass weight, legs weight, wing weight, breast weight, abdominal fat weight were obtained from 6 h exposure, however the difference between 3 and 6 h exposures were not statistically significant (Table 9).

DISCUSSION

The number of total bacteria, yeast and mold on the surface of the egg shell were significantly reduced by the essential oil fumigation. Oregano essential oil fumigation produced good microbial reduction compared to formaldehyde treatment. Microbial activities of the essential oil were attributed to its interaction with the microbial cell membranes by means of their physiochemical properties and molecular shapes that influence their enzymes, carriers, ion channels and receptors. The results of this study agree with the findings of Yildirim et al. (2003) that oregano essential oil eliminates microbial populations naturally occurring on the egg shell surface.

Similarly, Arhienbuwa et al. (1980) demonstrated that eggs treated with quaternary ammonium had lower microbial counts than eggs treated with formaldehyde. However, Sacco et al. (1989) observed that turkey eggs treated with formaldehyde or a quaternary ammonium still supported significant bacterial populations.

E. coli was the predominant bacteria on the surface of the hatching eggs. *E. coli* was reported in other studies as the predominant bacteria on the surface of the hatching eggs (Sarma et al., 1985; Cortes et al., 2004). Eggshells of hens are perforated with many pores of diameters from 9 to 35 μm (Smeltzer et al., 1979). It is known that pathogenic bacteria present on the surface of egg may contaminate the egg shell and penetrate the egg through shell pores (Barbour et al., 1985). When microorganisms get past the membranes of hatching eggs, there is no effective way to eliminate them or prevent their further invasion to the egg contents or developing embryo. Therefore, harmful microorganisms must be removed or destroyed as rapidly as possible on the surface of the hatching egg.

The present study showed that oregano essential oil fumigation had no detrimental impact on the cuticle of the egg or the developing embryo, which agrees with the findings of previous work (Yildirim et al., 2003). In addition, oregano oil fumigation improves hatchability and reduces internal contamination of eggs. Improved hatchability may be as a direct result of decreased microbial contamination of the egg. Although hatching egg

disinfection is often helpful to reduce contamination on egg shell surface, it is not the only solution and special attention should be taken to produce microbial free egg that does not need to be disinfected. Less microbial contamination could also aid in the production of cleaner and healthier chicks (Harry and Gordon, 1966).

It is obvious that the results of disinfection are greatly influenced by the timing of treatment and the type of disinfectant. Duration of the treatment will likely have a significant influence on the success of the disinfection. In the current study, however, the microbial count between 3 and 6 h essential oil treatment did not vary significantly. Three hours essential oil fumigation was as effective as 6 h fumigation. Further studies are needed to determine the minimum required time for the highest microbial elimination with the oregano essential oil. Exposure time requirement for oregano essential oil was remarkably higher than that of synthetic chemicals. Cox and Bailey (1991a, b) treated hatching eggs with various chemicals to eliminate *Salmonella*. They reported that there was a 77% reduction of the incidence of contaminated eggs when treatment was within 1 min, 64% reduction for treatment within 5 min, 45% reduction for treatment within 4 h and less than 10% reduction for treatment within 24 h. Consequently, the time lapsed from contamination to treatment with a disinfectant is crucial to the success of the disinfection.

Oregano essential oil slightly increased early embryonic and late embryonic mortalities of the hatching eggs. This could not probably be resulted from the harmful effect of oregano essential oil, since control treatment had higher early embryonic mortality than the oregano essential oil.

Significant improvement in hatchability of oregano essential oil treated eggs has also being observed by other researchers (Yildirim and Ozcan, 2001; Yildirim et al., 2003). This study showed that fumigating hatching eggs with oregano essential oil can be an effective way to reduce the number of microorganisms on the hatching eggs.

Field studies showed that feed conversion ratios did not change with increasing oregano essential oil concentrations. However, body weights at 21 and 42 days, body weight gains and total feed consumptions increased with the increasing oregano essential oil concentrations. The results indicated that oregano essential oil can be used as natural hatching egg disinfectant.

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