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USE of OREGANO and CUMIN ESSENTIAL OILS as DISINFECTANT on HATCHING QUAIL EGGS

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Kekik ve kimyon uçucu yağlarının kuluçkalık bıldırcın yumurtalarında dezenfektan olarak kullanımı

SUMMARY

Oregano *(Origanum vulgare* L.*)* and cumin *(Cuminum cyminum* L.*)* essential oils, ethanol and formaldehyde as disinfectans were evaluated on hatchability, embriyonic mortality and shell bacterial counts of hatching quail *(Coturnix japonica)* eggs.

No significant difference were found in hatchability of fertile eggs and embryonic mortality between the disinfectants and control. Total bacteria were significantly diminished in all treatments. Coliforms were markedly reduced by essential oils and formaldehyde. Salmonella counts remarkably were decreased by oregano alcohol and FF. The essential oils could be used as alternative natural disinfectans on hatching quail eggs.

KEY WORDS: Quail *(Coturnix japonica),* oregano *(Origanum vulgare* L.*),* cumin *(Cuminum cyminum* L.*),* essential oils, formaldehyde fumigation, hatching egg disinfectant

ÖZET

Kekik ve kimyon uçucu yağları ile etanol ve formaldehit (FF) dezenfektanlarının kuluçkalık japon bıldırcını *(Coturnix japonica)*, yumurtalarında çıkış gücü, embriyonik ölüm ve kabuki bakteri sayısı üzerine etkileri incelenmiştir.

Embriyonik ölümler ve çıkış gücü bakımından grup ortalamarı farksızdır. Tüm muameleler toplam bakteri sayısını azaltmıştır. Koliform bakteri sayısı uçucu yağlar ve formaldehit uygulanan gruplarda azalmıştır. Salmonella populasyonu kekik uçucu yağı , etanol ve formaldehit uygulanan gruplarda önemli derecede azalmış, kimyon uçucu yağlı ve kontrol grupları arasındaki farklılık önemsiz bulunmuştur. Uçucu yağların kuluçkalık bıldırcın yumurtalarında alternatif bir dezenfektan olarak kullanılabileceği sonucuna varılmıştır.

ANAHTAR KELIMELER: Bıldırcın *(Coturnix japonica),* kekik *(Origanum vulgare* L.*),* kimyon *(Cuminum cyminum* L.*),* uçucu yağlar, kuluçkalık yumurta dezenfektanı

INTRODUCTION

Several investigations have been conducted on the antimicrobial effect of various spices and their derivatives (Shelef 1983). Antimicrobial properties of certain spices have been reported in meats and meat products, e.g. poultry meat, turkey breast and beef (Farbood *et al.* 1976), various meats (Nkanga and Uraih, 1981), broth and foods (Shelef *et al.* 1984) and turkey frankfurter slurries (Hall and Maurer 1986). The effect has been mainly attributed to their essential oil content. Antimicrobial activities of essential oils have been also reported by some workers (Yousef and Tawil 1990, Deans and Svoboda 1990). Oregano and cumin species have been used in medicine and as a spice since antiquity, mainly because of their content of essential oils.

In this study, Oregano *(Origanum vulgare* L.*)* and cumin *(Cuminum cyminum* L.*)* essential oils, ethanol and formaldehyde as disinfectans were evaluated on hatchability, embriyonic mortality and shell bacterial counts of hatching quail *(Coturnix japonica)* eggs.

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MATERIALS and METHODS

Essential oils: Spice plants, used in this study were *Origanum vulgare* L. and *Cuminum cyminum* L. The herbs were identified and voucher specimens were deposited in the herbarium of Botany Department, Selçuk University. The air-dried plants were subjected to hydrodistillation for 3 h using a Clevenger- type apparatus. The essential oils were dried with anhydrous sodium sulphate, and kept in sealed dark bottles under cool conditions.

Egg material: All eggs used in the present study were from a non-commercial strain of quail (*Coturnix japonica*) breeder hens at 12-wk of age, fed with standard ration (20 % CP, 3000 kcal ME/kg). Eggs freshly laid onto cages were collected twice a daily (early in the morning, 9 a.m.; and late in the afternoon, 4.0 p.m.). Faecal-contaminated eggshells and with visible checks were discarded. Eggs were stored for no longer 3 days at 18 ^{0}C and 65 % relative humidity (RH) prior to initiation of an experiments. Incubation was done in a common Minisetter/ Hatcher incubator set at 37.8 ^{0}C and %55 RH. A total of 1520 eggs were used in this study.

Embryonic mortality and hatch of surviving embryos were examined in experiment. Post-sanitation levels of bacteria on the eggshell surface were determined in experiment *in vivo*.

Experiment 1

The eggs were divided into five groups, each containing 300 eggs. The first three groups were sprayed a drip state with aqueous ethanol solutions using a hand sprayer. The first one were treated with cumin essential oil (CEO) (125 ml alcohol plus 0.1 ml CEO per L solution). Eggs from the second group were treated with Oregano essential oil (OEO) (125 mL Alcohol plus 0.1 mL OEO per L solution). Eggs from the third group were treated with alcohol[1] (125 mL alcohol per L solution). Eggs from the fourth group were treated with FF (triple strength formaldehyde gas: 3X= 119.8 ml formalin; 59.9 g potassium permanganate/ 2.83 m^3) recommended commercially (USDA, 1985). FF was produced inside the setter at ambient temperature (15 ^{0}C) and 90% RH. Eggs from the fifth group served as the control which no treatment was applied. For first three groups, temperature of 24 ^{0}C was selected, since this disinfectants does not need to penetrate the eggshell and contains no temperature-dependent ingredients. All eggs were completely wetted with the disinfectants and were allowed to air dry at room temperature (24 ^{0}C), for 30 min. Then, all egg flats were randomly distributed the incubator.

At hatch, all of the unhatched eggs were opened and examined for evidence of embryonic development. Eggs were characterised as infertile, early dead (1-7 days) and late dead (8-17 days).

Experiment 2

Twenty eggs were immediately placed on plastic egg flats. A whole egg washing technique was used to recover the shell associated microorganisms for estimating the total bacteria count, Coliforms and Salmonella counts of four eggs per treatment. Dilutions were prepared ($*10^2$) and colonies were measured as cfu/mL (Özçelik 1992).

The total bacteria, Coliforms and Salmonella (in aerobic conditions) were incubated (37 ^{0}C, 48 h), enumerated using Nutrient Agar (Oxoid) and Violed Red Bile Agar (Merck) and Salmonella Shigella-SS Agar (Oxoid), respectively.

Statistical Analyses

The data from experiment, 1 and 2 were subjected to a one-way ANOVA using a randomised complete block design with the Statistical Analysis System-ANOVA procedure (Minitab 1995). Microbial counts were transformed to Log_{10} prior to statistical analysis. Differences among means were partitioned using the Duncan option. Statement of statically significance were based on $p<0.05$ unless otherwise noted.

[1] Ethanol % 96

RESULTS and DISCUSSION

Experiment 1

The effects of treatments on hatchability of fertile eggs and on embryonic mortality are summarised in Table 1. Applications did not have a significant effect on hatchability of fertile eggs and on embryonic mortality in all groups.

Experiment 2

The effects of treatments on total bacteria, Coliforms and Salmonella counts are given in Table 2. Total bacteria population of eggshell was reduced by HES in all disinfectant, ($p<0.01$) by comparison with control. Coliforms on the eggshell surface were reduced with OEO, CEO and FF ($p<0.01$), but no significant differences were found between control and alcohol groups. There were found significant differences between CEO and alcohol groups ($p<0.01$). This result indicate that the interaction between CEO and alcohol was effective to reduction coliforms. The use of Alcohol without CEO did not have effect in this group according to these dates.

Salmonella population were reduced with OEO, alcohol and FF ($p<0.01$). The effect of OEO on total bacteria Salmonella may be masked by alcohol, and the interaction effects between them may be considered. Including of some fungi and bacteria by spice essential oils including cumin and oregano was reported, however CEO was partly active against bacteria and it had completely inhibited mycelial growth of some moulds (Yousef and Tawil 1990).

The findings indicated that oregano and cumin have an inhibitory effect against bacteria at the surface of egg. In the previous studies, the composition of OEO has been studied. Though carvacrol was the major constituent and considerable amounts of thymol may also be present. It was reported that carvacrol and thymol exhibited strong antibacterial activity (Zaika and Kissinger 1981, Akgül and Kıvanç 1988a, Akgül and Kıvanç 1988b).

In conclusion, spraying hatching eggs with OEO and CEO significantly reduced total bacteria and coliforms contamination, but CEO was not effective against Salmonella. However, all spice oils were not effect on tested hatching results. This situation can be explained as loss of activity by volatilisation of the spice active components on the eggshell surface. There were found some contradictions between bactericidal challenge and hatching results. From microbial counts, low hatchability of fertile eggs and high embryonic mortality were expected in control and CEO groups. On the contrary, there were found no significant difference among groups by hatchery results. This may be due to the age of our parent stock. In this age stage, the egg components such as cuticle, albumen, avidin *etc* may protect to the developing embryo from microbial invasion. As a result, it seems that OEO and CEO could be used as alternative hatching disinfectants in quail hatching eggs, but this requires further clarification by other comprehensive studies.

Table 1. Effect of Treatments on the Hatchability of Fertile Eggs and Embryonic Mortality (%).

Treatment	Hatch of fertile eggs $X \pm S_x$	Early dead $X \pm S_x$	Late dead $X \pm S_x$
CEO	84.41±3.62	6.42±1.24	9.14±4.31
OEO	83.04±3.74	5.39±3.19	11.54±2.56
Alcohol	82.59±4.04	6.20±3.89	11.19±3.15
FF	78.90±3.15	8.08±4.08	12.99±2.79
Control	89.03±2.18	3.76±1.56	7.19±1.81

CEO: Cumin Essential Oil, OEO: Origanum Essential Oil, FF: Formaldehyde Fumigation.

Table 2. Bactericidal Activity (cfu per egg)*10^2 of Treatments on the Microflora of Hatching Eggs (geometric means $\log_{10} \pm$ SD, n=4).

HES Treatment	Total Bacteria $X \pm S_x$	Coliforms $X \pm S_x$	Salmonella $X \pm S_x$
CEO	1.08 ± 0.03^{b}	1.04 ± 0.03^{c}	1.30 ± 0.10^{a}
OEO	1.09 ± 0.04^{b}	1.10 ± 0.07^{bc}	1.04 ± 0.03^{b}
Alcohol	1.06 ± 0.02^{b}	1.18 ± 0.09^{ab}	1.07 ± 0.04^{b}
FF	1.04 ± 0.04^{b}	1.04 ± 0.03^{c}	1.04 ± 0.06^{b}
Control	1.30 ± 0.02^{a}	1.24 ± 0.02^{a}	1.38 ± 0.01^{a}

a, b: Different superscripts in the same column indicate significant differences ($p<0.01$) among mean values.

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