Effects of clove essential oil on eggshell bacterial load, antibacterial sensitivity, and hatchability

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Background and objective
Clove essential oil's (CEO) physical and chemical properties allow for its potent antibacterial action. This study sought to determine the impact of various CEO disinfectant concentrations on the eggshell bacterial load, embryonic mortality, hatchability, and chick quality.
Materials and methods
A total of 1500 fertile chicken eggs were randomly divided into five treatment groups. Group one was sprayed with a commercial disinfectant (BioSentry 904), the second group was sprayed with ethyl alcohol 70%, and the last three groups were sprayed with 0.5, 1, or 2% of CEO. After spraying, eggs in each group were used for the determination of total bacterial load on the eggshell, hatchability, embryonic mortality, and chick quality.
Results and conclusion
Results showed that the bacterial load on the eggshell declined with the increase in the concentration of CEO. The use of CEO at 1, 2%, or BioSentry 904 resulted in the lowest bacterial load. However, hatchability of set and fertile eggs for the group treated with CEO at 0.5% was numerically greater than other groups, while chick quality grade (A) was numerically greater by CEO at 1% concentration. The chick yield of the egg group treated with CEO at 1% was significantly higher than in the control group. Embryonic mortalities for all groups were statistically similar. In conclusion, using CEO at different concentrations 1, 2%, or BioSentry 904 in disinfecting broiler breeder eggs can effectively reduce the bacterial load on eggshells. In addition, using CEO improved chick quality and chick yield without any adverse effect on hatchability. Consequently, it is considered a strong competitor to BioSentry 904 and a less hazardous disinfectant for hatching egg disinfection on a commercial scale.

Keywords:
bacterial load, broiler breeder, clove essential oil, hatching eggs and sanitation

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Introduction

Contamination of eggs could happen both before and after oviposition. Typical contaminants on eggshell surfaces include yeast, molds, coliforms, *Salmonella spp.*, and *E. coli.* [1–4]. Some of these microorganisms may pass inside the egg, while the eggshell contacts the feces and bedding. It leads in turn to lower the hatchability with poor chick quality. Thus, the use of disinfectants in hatcheries is routine to have healthy and high-quality chicks.

When an egg is laid, the total number of bacteria on the eggshell surface is estimated to range from 300 to 500 cells. This number increases rapidly to 20 000–30 000 bacterial cells in 1 h after the eggs are laid Aygun and colleagues, North and Bell [3,5]. Therefore, sanitation of hatching eggs is essential in different stages of poultry production mainly in hatcheries. Several hatching egg disinfection methods are available such as fumigation, eggshell spraying, UV light, washing with appropriate disinfectant, and using silver nanoparticles Aygun and colleagues, Ibrahim and colleagues, Adler and colleagues, Oliveira and colleagues [3,4,6–12].

Numerous endeavors have been undertaken to enhance hatchability, as well as the quality and performance of chicks. However, it is imperative to take into account human health and environmental considerations. In this regard, various strategies have been used to achieve both high production and consumer safety. As a result, there is a need to reassess the use of antibacterial synthetic compounds and explore new applications to ensure their safety and efficacy Aygun and colleagues [3].

Plant-derived compounds are often preferred over synthetic counterparts due to their perceived acceptability and lower potential hazards. These compounds serve as a valuable and abundant source of potential antibacterial agents, as highlighted in the study by Aygun and colleagues [3]. Among these, plant essential oils have been recognized for their microbicidal effects and are commonly used in such applications [3].

The use of essential oils has shown potential in improving carcass hygiene by effectively reducing pathogen loads as highlighted in the study by Zhai and colleagues [13]. Alali and colleagues [14] concluded that a combination of carvacrol, thymol, eucalyptol, and lemon could contribute to reducing *Salmonella heidelberg* contamination in crops, thereby minimizing cross-contamination during carcass processing. In addition, Witkowska and Sowinska [15] demonstrated that thyme and peppermint oils, when used as primary ingredients in air disinfectants, enhanced hygiene conditions in poultry houses.

According to Oliveira and colleagues [16], the use of clove essential oil as a disinfecting agent at a concentration of 0.6 mg/ml on hatching eggs did not show significant differences compared with paraformaldehyde concerning hatchery performance parameters.

In the study conducted by Oliveira and colleagues [17], it was found that using clove essential oil at a concentration of 0.39% was both effective and safe for sanitizing hatching eggs. The research strongly recommends its use as an alternative to paraformaldehyde, as it successfully reduced the microbial load on the eggshell, resulted in favorable incubation parameters, and improved chick quality. Furthermore, the data indirectly suggested that disinfecting hatching eggs with clove essential oil had no negative impact on the cuticle structure or embryonic development.

In a study conducted by Bauer and colleagues [18], it was demonstrated that clove essential oil exhibited inhibitory effects on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* when tested in vitro using the disk diffusion method.

The antibacterial activity of certain compounds is attributed to their hydrophobicity, which disrupts the permeability of cell membranes, leading to cellular imbalance, loss of cellular components, influx of foreign substances, and, ultimately, cell death. This mechanism has been discussed in studies by Zhai and colleagues, Brenes and Roura, OBryan and colleagues [13,19–22].

The objectives of the current study were to evaluate the disinfectant properties of clove essential oil on eggshell bacterial load, hatching traits, embryonic mortality, and chick quality in hatching eggs of broiler breeders.

Materials and methods

Ethics approval

Institutional Animal Care and Use authorized this research project. IACUC, Cairo University, under approval Protocol number CU-II-F-38-22, is Cairo University's ethics committee for the care and use of experimental animals in teaching and research.

Experimental procedure

A total of 1500 fresh, clean, and fertile eggs with a mean weight of 61.89 g ($P=0.0853$) were obtained from a commercial broiler breeder chicken flock (Ross 308), aged 35 weeks. Eggs were incubated in a commercial hatchery. The embryonated eggs were equally distributed into five treatment groups (25 eggs/replicate). Group one was sprayed with a commercial disinfectant containing quaternary ammonium chlorides and tributyltin oxide (BioSentry 904) and served as the control group; the second group was sprayed with ethyl alcohol at 70%. The other three groups were sprayed with three solutions containing clove essential oil at 0.5, 1, or 2%, diluted in ethyl alcohol at 70%.

Preparation of clove essential oil-based disinfecting agents

Dried clove flower buds were obtained from a commercial market in Cairo, Egypt. The clove essential oil was extracted in the Laboratory of Food Sciences, Faculty of Agriculture, Cairo University. The extraction was performed according to Ascençao and Filho [23], involving the hydro-distillation method with the Clevenger extraction system. To prepare the disinfectant, Clove essential oil (CEO) was diluted in 70% ethyl alcohol to concentrations of 0.5, 1, or 2%.

Bacteriological examination

Eggshell bacterial count

The eggshell bacterial count was performed before and after application of different treatments. One hour after sanitation application, 15 eggs from each treatment (5 eggs/replicate) were subjected to surface bacterial count, which was performed according to Loongyai

and colleagues [24]. A sterile cotton swab dipped in sterile phosphate buffer saline (PBS) was used to aseptically swab the surface of the entire egg. The samples were serially diluted in PBS, and 1 ml of the appropriate dilutions was then applied to the standard plate count agar (Oxoid). The agar plates were incubated at 37°C for 24–48 h Gentry and Quarles, Jones and colleagues [25,26]. A pooled sample of 5 eggs yielded data that are expressed in $\log_{10}$ CFU/ml.

Bacterial isolation and identification

Bacterial isolation from eggshells was carried out on different bacteriological media. Briefly, samples were inoculated in buffered peptone water and nutrient broth and then incubated at 37°C for 16–18 h. After 16 h of incubation, a loopful of positive buffered peptone water was then inoculated into Rappaport-Vassiliadis broth (Himedia) and incubated at 37°C for 24 h. A loopful of positive incubated nutrient broth was inoculated onto the nutrient agar (Lab M) and MacConkey's agar media (Lab M) and incubated at 37°C for 24 h. The positive Rappaport-Vassiliadis broth was streaked onto XLD (Himedia). The isolated bacterial organisms were subjected to complete identification Andrews and colleagues [27].

Antibacterial sensitivity test (disk diffusion method)

Using the disk diffusion method, the antibacterial activity of different concentrations of clove oil was assessed against isolated microorganisms at a concentration of 1.5×10^8 cfu/ml Bauer and colleagues, Abdulwahab and colleagues [18,28]. Succinctly, sterile Petri plates were filled with 20 ml of Mueller Hinton agar (MHA) and left to set. Then, to create a full lawn, 0.2 ml of each microorganism's overnight broth culture was streaked on MHA (Oxoid). The clove oil was soaked with various concentrations for 30 min in sterile 4 mm filter paper disks (Whatman No. 3). The disks were plated on MHA after drying completely. Bacterial isolates were classified into three groups, namely resistant (>7 mm), intermediate (>12 mm), and susceptible (>18 mm), based on the size of the growth inhibition zones Upadhyay and colleagues [29].

Incubation and hatching

After egg spraying, 300 eggs from each group (5 groups) were divided into 12 replicates (25 eggs each); eggs of all treatments received the standard temperature and humidity levels of 37.7°C and 55–60% relative humidity (RH) in the setter for 18 days. Thereafter, the eggs were then transferred to the hatchery, and at 36.5°C and 75% RH for 3 days. Within 45 min of the hatch, every chick was weighed. The percentage of eggs that have hatched overall was used to calculate hatchability. Infertile, early-dead (0–7th day of incubation), middle-dead (8th–18th day of incubation), and late-dead (18th day to hatch) embryos were counted among the non-hatched eggs after they were separated. Chicks were graded according to their quality of two classes, where chicks that looked healthy, clean, dry, deformity-free, and alert were graded as A class Tona and colleagues [30], and the chicks that had an unhealed navel, leg abnormalities, or difficulty standing were graded as B class.

Statistical analysis

The one-way linear model approach with probability P 0.05 as given in the SAS user guide was used to statistically analyze the data [31]. Duncan's multiple range test [32] was used to examine differences between means. The one-way analysis model applied was

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the observation j^{th} of i^{th} treatment, μ is the overall mean, T_i is the effect of i^{th} treatment, and e_{ij} is the experimental error.

Results and discussion

The chemical analysis of the clove essential oil

Three components were identified through the chemical analysis of the clove essential oil using gas chromatography-mass spectrometry (GC-MS) analysis, Eugenol comprised about 62.69% of the chemical analysis of the CEO (Table 1).

Eggshell bacterial count

The air of the chicken house, the feed, or the hatchery are a few potential sources of microbial contamination on eggshells. One of the most common pathogenic contaminants are yeast and molds, along with *Salmonella* spp., coliforms, and *E. coli*. Ibrahim and colleagues Smith and colleagues, Kim and colleagues [4,33–36]. De Reu and colleagues [37] reported that a high microbial load on eggshells increases the possibility of the contamination of egg contents which, in turn, results in lowering hatchability. The

Table 1 Chemical compounds identified for the clove essential oil

Peak	CRT (min)	Area (%)	Compound
1	18.07	62.69	Eugenol
2	19.51	8.08	β-Caryophyllene
3	22.16	25.30	Acetyleugenol

CRT, compound retention time.

transmission of diseases through eggs infected with pathogenic bacteria is widely established. These bacteria result in embryonic death, reduced hatchability, and increased early chick mortality Ibrahim and colleagues [4].

In this study, the count of total aerobic mesophilic bacteria was significantly different among all treated groups, compared with that recorded before application ($P = 0.0001$, Table 2). The results showed that the lowest total bacterial count was found when the eggs were treated with 2% CEO (5.08 $\log_{10}$ CFU/ml), followed by 1% CEO (5.36 $\log_{10}$ CFU/ml), and then the commercial disinfectant treatment (5.49 log10 CFU/ml), 0.5% CEO (5.99 $\log_{10}$ CFU/ml), and ethyl alcohol 70% (6.44 $\log_{10}$ CFU/ml), compared with those recorded before application (7.67 $\log_{10}$ CFU/ml). Similarly, the results of Oliveira and colleagues [17] recorded a significant reduction in the total bacterial count when eggs were treated by CEO (0.39%) compared with before application. However, the differences between CEO and paraformaldehyde treatments were insignificant.

The application of essential oils as a disinfectant demonstrated efficacy in reducing microbial contamination on eggshells designated for incubation Oliveira and colleagues, Copur and colleagues, Oliveira and colleagues [17,38–40]. In the current study, the results denoted that using CEO at different concentrations for sanitizing hatching eggs had successfully reduced the total bacterial count, without significant differences with the commercial disinfectant. The percentage of reduction due to CEO spraying ranged from 22 to 33% compared with before application. Accordingly, spraying hatching eggs with clove essential oil did reduce the total bacterial count on the eggshell surface and can be considered a good treatment and comparable to commercial disinfectants for sanitizing hatching eggs.

Table 2 Total bacterial count (TC) on hatching eggshell surface in different disinfection groups

Group	TC (Log_{10} CFU/ml)
Before application	7.67 [a]
BioSentry 904 (Control)	5.49 [b]
Ethyl Alcohol 70%	6.44 [b]
0.5% CEO	5.99 [b]
1% CEO	5.36 [b]
2% CEO	5.08 [b]
± SEM	3.95
P value	0.0001

[a, b]Means with different superscripts differ significantly ($P \leq 0.05$).

Antibacterial sensitivity test (disk diffusion method)

The results of the isolated bacteria and antibacterial sensitivity test are presented in Table 3. There were significant differences between all treatments on both *E. coli* O86, *Salmonella enteriditis, Enterococcus fecalis, Klebsiella pneumoniae, Shigella sp., and Staphylococcus aureus*.

For *E. coli* O86, the results showed the differences among 2% CEO, and the control groups (10.67 mm, and10.33 mm, respectively), which were insignificant ($P = 0.0079$). However, the 0.5% CEO and ethyl alcohol 70% treatments recorded the lowest significant effect on *E. coli* O86 compared with the control group.

For *Salmonella enteriditis*, the differences between the treatment with different levels of CEO and the control group were insufficient. Nevertheless, spraying eggs with 2% CEO had a numerically high effect (11.67 mm) than the control group (10.67 mm). The ethyl alcohol 70% group had significantly the lowest effect (4.33 mm) compared with the other groups.

For *Enterococcus fecalis,* the highest significant values were obtained for the control (14.33 mm) and the 2% CEO (13.67 mm) groups, without significant

Table 3 Antibacterial sensitivity test (disk diffusion method) against different isolated bacteria in different disinfectant groups

Group	E coli O86 (mm)	Salmonella entereditis (mm)	Enterococcus fecalis (mm)	Klebsiella pneumoniae (mm)	Shigella sp. (mm)	Staphylococcus aureus (mm)
BioSentry 904 (control)	10.33 [a]	10.67 [a]	14.33 [a]	11.67 [ab]	20.00 [a]	15.33 [a]
Ethyl alcohol 70%	7.00 [b]	4.33 [b]	8.00 [c]	7.00 d	10.00 [c]	7.33 d
0.5% CEO	7.67 [b]	10.33 [a]	8.67 [bc]	8.67 [cd]	14.33 [b]	9.67 [c]
1% CEO	9.00 [ab]	10.67 [a]	10.33 [b]	10.67 [bc]	16.67 [ab]	13.00 [b]
2% CEO	10.67 [a]	11.67 [a]	13.67 [a]	13.33 [a]	19.00 [a]	14.33 [ab]
±SEM	0.63	1.17	0.70	0.75	1.12	0.65
P value	0.0079	0.0084	0.0002	0.0011	0.0006	0.0001

[a, b, c,d] Means with different superscripts differ significantly ($P \leq 0.05$).

differences between them. In addition, 0.5% CEO and ethyl alcohol 70% groups showed the lowest effect on *Enterococcus fecalis* compared with the other groups.

For *Klebsiella pneumoniae*, the 2% CEO treatment had a highly significant effect (13.33 mm) similar to the control group (11.67 mm), while ethyl alcohol 70% and 0.5 CEO groups had the lowest effect on *Klebsiella pneumoniae* (7 mm).

For *Shigella sp*, the control (20 mm), 2% CEO (19 mm), and 1% CEO had the highest significant effect on *Shigella sp.*, while the lowest effect on *Shigella sp. w*as recorded for ethyl alcohol 70% group (10 mm).

For *Staphylococcus aureus*, the control (BioSentry 904) and 2% CEO groups had a highly significant effect compared with the other groups. Also, the ethyl alcohol 70% group had the lowest significant effect on *Staphylococcus aureus*.

Bauer and colleagues [18] demonstrated that the clove oil concentration tested in vitro using the disk diffusion method demonstrated the lowest inhibitory effect among all concentrations tested, measuring at a concentration of 0.39%, This concentration exhibited inhibitory effects against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. According to the findings of Prabuseenivasan and colleagues [41], clove essential oil demonstrated notable bioactivity primarily against Gram-negative bacteria. These results were further supported by the findings of Oliveira and colleagues [17]. The low presence of Enterobacteriaceae generally observed indicates that the farm supplying the eggs maintained good hygienic conditions as noted by Oliveira and colleagues [17]. The strong antimicrobial activity of clove essential oil is primarily due to its high content of phenolic compounds Oliveira and colleagues, Chaieb and colleagues [17,42].

The mode of action of essential oils depends on their chemical structure, and their antibacterial activity is not due to a single mechanism but rather a series of reactions that affect the entire bacterial cell. However, it is generally acknowledged that the components' lipophilicity affects the antibacterial action. The elements penetrate the mitochondria and cell membranes of the microorganisms and hinder, among other things, the flow of electrons bound to the membrane, and consequently energy metabolism. The ATP pool is drained as a result, causing the proton pump to fail. In addition, cytoplasmic proteins may get denaturized and cell membranes may lyse at high quantities Abdulwahab and colleagues [28].

Hatching results

As expected, there were no significant differences between the different treatments ($P = 0.0807$) in the percentage of visual fertility (Table 4). This result was recorded because the hatching eggs were obtained from the same breeder hens that received the same management factors at the poultry house; similar results were noted by Oliveira and colleagues [17]. Consistently, there were no significant differences ($P = 0.1105$) in the percentage of hatchability of set eggs between the different treatments (Table 4). However, the percentage of hatchability of set eggs is numerically increased in concentrations of CEO 0.5% and 1% (93.67%, and 91.33%, respectively) compared with the control group (90.33%). This result may be attributed to the effects of the treatment, as there was no significant difference in visual fertility. Oliveira and colleagues [17] observed no significant difference in the hatchability of set eggs for CEO treatment at a concentration of 0.39% compared with paraformaldehyde.

The percentage of hatchability of fertile eggs was higher numerically when eggs were disinfected by 0.5% CEO (95.86%), compared with the control group (94.43%), while the hatchability of fertile eggs of CEO 1% and 2% were similar (94.14%, and 94.39%, respectively) compared with the control group (94.43%). However, the differences in the hatchability of fertile eggs were insignificant ($P = 0.6337$) among the different treatments (Table 4). Oliveira and colleagues [16] compared the hatchability of fertile eggs between the CEO group at a concentration of 0.6 mg/ml (92.37%) and the paraformaldehyde group (94.44%) and did not

Table 4 Visual fertility and hatchability of hatching eggs in different disinfection groups

Group	Visual fertility%	Hatchability of set eggs%	Hatchability of fertile eggs%
BioSentry 904 (Control)	95.67	90.33	94.43
Ethyl alcohol 70%	96.33	90.33	93.84
0.5% CEO	97.67	93.67	95.86
1% CEO	97.00	91.33	94.14
2% CEO	94.99	89.64	94.39
± SEM	0.72	1.14	0.97
P value	0.0807	0.1105	0.6337

*No significant differences between treatments.

record any significant differences. However, Oliveira and colleagues [17] cleared that a significant difference was observed in the hatchability of fertile eggs for CEO treatment at a concentration of 0.39% compared with the paraformaldehyde and all treated groups. Hence, disinfection with CEO 0.5% increased hatchability numerically compared with the control group, possibly because of decreased bacterial load on the eggshell surface.

Table 5 shows that no significant differences in embryonic mortality were found between all treatments during the early ($P = 0.7796$), middle ($P = 0.0592$), and late ($P = 0.7098$) embryonic mortality. Oliveira and colleagues [17] concluded that no significant differences in embryonic mortality were found between the disinfectant groups during the early or middle embryonic mortality. However, there was a significant reduction in the late embryonic mortality in the eggs sprayed with CEO 0.39% compared with the disinfectant group sprayed with grain alcohol. Besides, Copur and colleagues [43] and Baylan and colleagues [44] observed a decrease in early and late embryonic mortality possibly because of decreased eggshell contamination.

Results in Table 5 show significant differences in egg contamination rate between different treatments ($P = 0.0040$). The largest percentage of contaminated eggs was observed in the ethyl alcohol 70% group (1.33%), followed by BioSentry 904 (0.33%), CEO 0.5% (0.33%), CEO 2% (0.33%), and CEO 1% (0.00%) groups. Similarly, Oliveira and colleagues [17] reported that the lowest percentage of contaminated eggs was recorded in the CEO group compared with all other treated groups. These results indicate that the CEO as a disinfectant for hatching eggs has antimicrobial activities on the eggshell surface during the incubation period. Moreover, Magwood [45] observed a growth of microorganisms on the eggshell surface that was noticeably larger than usual while it was being incubated.

Egg weight loss (%) differed significantly between the different treatments ($P = 0.0001$), where the lowest percentage of egg weight loss was observed in the CEO 1% group (10.86%), and the largest percentage of egg weight loss was observed in the ethyl alcohol 70% group (11.49%) as shown in Table 6. The averages of egg weight loss were similar ($P = 0.0001$) for the eggs treated with CEO 0.5% (11.06%), BioSentry 904 (11.21%), and CEO 2% (11.38%). However, Oliveira and colleagues [17] found that the percentage of egg weight loss did not differ between CEO 0.39% and all other treatments. Likewise, Oliveira and colleagues [16] observed that the egg weight loss averages

Table 5 Embryonic mortalities and contamination eggs in different disinfection groups

Group	Early embryonic mortality%	Middle embryonic mortality%	Late embryonic mortality%	Contamination eggs%
BioSentry 904 (Control)	1.00	1.00	1.67	0.33 [b]
Ethyl alcohol 70%	1.67	1.67	2.00	1.33 [a]
0.5% CEO	1.67	0.67	1.33	0.33 [b]
1% CEO	1.33	0.67	2.33	0.00 [b]
2% CEO	1.68	0.33	2.33	0.33 [b]
±SEM	0.45	0.33	0.59	0.25
P value	0.7796	0.0592	0.7098	0.0040

[a, b]Means with different superscripts differ significantly ($P \leq 0.05$).

Table 6 Egg weight loss and chick yield in different disinfection groups

Group	Egg weight 0 day (g)	Egg weight 18 day (g)	Egg weight loss%	Chick yield%
BioSentry 904 (control)	61.83	54.90	11.21 [ab]	67.73 [bc]
Ethyl alcohol 70%	62.67	55.47	11.49 [a]	67.19 [c]
0.5% CEO	61.80	54.97	11.06 [bc]	67.56 [bc]
1% CEO	61.73	55.03	10.86 [c]	68.70 [a]
2% CEO	61.40	54.42	11.38 [a]	68.07 [b]
±SEM	0.32	0.30	0.10	0.20
P value	0.0853	0.1968	0.0001	0.0001

[a, b, c]Means with different superscripts differ significantly ($P \leq 0.05$).

showed similarities ($P > 0.05$) among the eggs treated with grain alcohol, clove essential oil, and paraformaldehyde. Temperature and relative humidity are two physical variables that have a greater impact on this parameter throughout the incubation phase Barott, Meijerhof and van Beek [46–48] as the eggs in this experiment were incubated under the same circumstances. Furthermore, the evaluation of egg weight loss during the incubation period allowed us to estimate the level of disinfectant damage to the cuticle indirectly, as well as embryonic development Brake and Sheldon, Peebles and colleagues [49,50].

The process of water diffusion through the eggshell accounts for egg weight loss during incubation as stated by Tona and colleagues [51]. Various studies have indicated that achieving a weight loss percentage of between 10 and 15% yields favorable hatching outcomes Rosa and de Avila, Molenaar and colleagues [52–54]. In this particular experiment, where all eggs experienced similar temperature and humidity conditions, the eggs treated with CEO 1% exhibited limited weight loss compared with all treatments. This result can be attributed to the creation of a protective coating on the eggshell by the sanitizer especially at a concentration of 1%, which minimizes water loss through the pores of the eggshell.

Chick yield (%) differed significantly between the treatments ($P = 0.0001$) (Table 6). The CEO 1% group presented a higher chick yield value (68.70%) than those of the CEO 2% (68.07%), followed by BioSentry 904 (67.73%), CEO 0.5% (67.56), and ethyl alcohol 70% (67.19%). Oliveira and colleagues [16,17] reported that chick yield (%) averages showed similarities among the eggs treated with grain alcohol, clove essential oil, and paraformaldehyde. According to a study by Aviagen [55], attaining the ideal chick yield, usually ranging between 67% and 68%, requires careful management of incubation time and parameters. In addition, enhancing the quality of the chicks, as highlighted in separate research conducted by Boleli and colleagues [56], is also crucial for achieving optimal results. By ensuring appropriate incubation conditions and focusing on improving chick quality, poultry producers can strive for higher chick yield rates.

Chick quality

Chick weight and chick length are good indicators of chick performance throughout the growing phase. Monitoring these parameters provides valuable visions into the overall health and development of the chicks with their progress Ibrahim and colleagues, Willemsen and colleagues [4,57]. In the current study (Table 7), the differences in 1-day-old body weight were insignificant among all treatments ($P = 0.1359$). However, the differences in 1-day-old chick length were significant among treatments ($P = 0.0379$), as the control group (BioSentry 904) and CEO 1% had higher significant values compared with the other treatments. Although the count and categorization of chicks as either category A or B in all treated groups showed insignificant differences, the percentages of grade A chicks of the CEO 1% group were numerically greater (99.17%) compared with other treated groups as followed: BioSentry 904 (98.90%), CEO 2% (98.89%), CEO 0.5% (97.83%), and ethyl alcohol 70% (97.79%) (Table 7). In this context, Oliveira and colleagues [58] cleared that the utilization of clove essential oil treatment demonstrated no detrimental impact on the quality of embryos and one-day-old chicks. The results may be in favor of using CEO as an alternative disinfectant for untraditional disinfectants. Similarly, the results indicate that the concentrations of CEO resulted in decreasing the bacterial load on the eggshell surface, and consequently, hatchability was numerically increased but not significantly.

Conclusion

Clove essential oil, when compared with the commercial disinfectant, proved to be a strong contender for sanitizing fertile eggs and ensuring

Table 7 Chick weight, chick length, and chick quality of hatching eggs in different disinfection groups

Group	Chick weight (g)	Chick length (cm)	Chick quality grade% (A)	Chick quality grade% (B)
BioSentry 904 (Control)	41.88	18.41 [a]	98.90	1.12
Ethyl alcohol 70%	42.11	18.31[ab]	97.79	2.21
0.5% CEO	41.75	18.31 [ab]	97.83	2.17
1% CEO	42.41	18.38 [a]	99.17	0.84
2% CEO	41.79	18.14 [b]	98.90	1.11
±SEM	0.20	0.06	0.52	0.52
P value	0.1359	0.0379	0.1787	0.1787

[a, b]Means with different superscripts differ significantly ($P \leq 0.05$).

their safe incubation. Besides its ability to significantly reduce the bacterial load on the eggshell, it offers an effective, natural, and safe alternative. This reduction in total bacterial count not only leads to optimal incubation parameters but also maintains the quality of the developing chicks. Besides, the present results suggest that the use of clove essential oil does not have any negative impact on the eggshell's protective cuticle or the embryo's development, making it a highly recommended natural choice and a strong competitor to a commonly used commercial disinfectant (BioSentry 904) for hatching egg disinfection on a commercial scale.

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Conflicts of interest
There is no conflict of interest.

References

1 Singh R, Cheng KM, Silversides FG. Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. Poult Sci 2009; 88:256–264.

2 Jones DR, Anderson KE, Musgrove MT. Comparison of environmental and egg microbiology associated with conventional and free-range laying hen management. Poult Sci 2011; 90:2063–2068.

3 Aygun A, Sert D, Copur G. Effects of propolis on eggshell microbial activity, hatchability, and chick performance in Japanese quail (Coturnix coturnix japonica) eggs. Poult Sci 2012; 91:1018–1025.

4 Ibrahim FA, Kout Elkloub MEM, El Sabry MI, Jihan MB, Hassan ASI. Effect of Egg Disinfection by Silver Nanoparticles on Eggshell Microbial Load, Hatchability and Post-hatch Performance of Quail Chicks. Int J Poult Sci 2018; 17:234–242.

5 North MO, Bell DD. Commercial Chicken Production Manual. 4th ed. New York, NY: Chapman and Hall 1990.

6 Adler HE, DaMassa AJ, Scott WF. Studies on egg disinfection. Poult Sci 1979; 58:799–806.

7 Arhienbuwa FE, Adler HE, Wiggins AD. A method of surveillance for bacteria on the shell of turkey eggs. Poult Sci 1980; 59:28–33.

8 Proudfoot FG, Nash DM, Hulan HW. Effects of glutaraldehyde surfactant solution on the hatchability of the hen's egg. Poult Sci 1985; 64:2400–2402.

9 Sacco RE, Renner PA, Nestor KE, Saif YM, Dearth RN. Effect of hatching egg sanitizers on embryonic survival and hatchability of turkey eggs from different lines on eggshell bacterial populations. Poult Sci 1989; 68:1179–1184.

10 Whistler PE, Sheldon BW. Biocidal activity of ozone versus formaldehyde against poultry pathogens inoculated in a prototype setter. Poult Sci 1989; 68:1068–1073.

11 Coufal CD, Chavez C, Knape KD, Carey JB. Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. Poult Sci 2003; 82:754–759.

12 Oliveira GDS, McManus C, Dos Santos VM. Garlic as active principle of sanitiser for hatching eggs. Worlds Poult Sci J 2022; 78:1037–1052.

13 Zhai H, Liu H, Wang S, Wu J, Kluenter A. Potential of essential oils for poultry and pigs. Animal Nutrition 2018; 4:179–186.

14 Alali WQ, Hofacre CL, Mathis GF, Faltys G. Effect of essential oil compound on shedding and colonization of Salmonella enterica serovar Heidelberg in broilers. Poult Sci 2013; 92:836–841.

15 Witkowska D, Sowinska J. The effectiveness of peppermint and thyme essential oil mist in reducing bacterial contamination in broiler houses. Poult Sci 2013; 92:2834–2843.

16 Oliveira GS, Santos VM, Nascimento ST, Rodrigues JC. Alternative sanitizers to paraformaldehyde for incubation of fertile eggs. Poult Sci 2020b; 99:2001–2006.

17 Oliveira GS, Nascimento ST, Santos VM, Silva MG. Clove essential oil in the sanitation of fertile eggs. Poult Sci 2020a; 99:5509–5516.

18 Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45:493–496.

19 Brenes A, Roura E. Essential oils in poultry nutrition: main effects and modes of action. Anim Feed Sci Technol 2010; 158:1–14.

20 Solorzano-Santos F, Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. Curr Opin Biotechnol 2012; 23:136–141.

21 Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. J Anim Sci 2008; 86:140–148.

22 O'Bryan CA, Pendleton SJ, Crandall PG, Ricke SC. Potential of plant essential oils and their components in animal agriculture-in vitro studies on antibacterial mode of action. Front Vet Sci 2015; 2:1–8.

23 Ascençao VL, Filho VEM. Extraçao, caracterizaçao química e atividade antifungica de oleo essencial *Syzygium aromaticum* (cravo da índia) [Extraction, chemical characterization and antifungal activity of *Syzygium aromaticum* (clove) essential oil]. Cad Pesqui 2013; 20 (especial):137–144.

24 Loongyai W, Wiriya B, Sangsawang N. Detection of *Salmonella* and *Escherichia coli* in egg shell and egg content from different housing systems for laying hens. Int J Poult Sci 2011; 10:93–97.

25 Gentry RF, Quarles CL. The measurement of bacterial contamination on eggshells. Poult Sci 1972; 51:930–933.

26 Jones DR, Anderson KE, Curtis PA, Jones FT. Microbial contamination in inoculated shell eggs: I. Effects of layer strain and hen age. Poult Sci 2002; 81:715–720.

27 Andrews WA, Hammack TS. Bacteriological Analytical Manual on line. U. S. Food and Drug Administration. Center for Food Safety and Applied Nutrition 2001; https://www.academia.edu/42740282/BACTERIOLOGICAL_ANALYTICAL_MANUAL.

28 Abdulwahab K, Ali A, Abdulatif A. In Vitro Antimicrobial Activity of Clove Oil against Gram Negative Bacteria Isolated from Chickens. Approaches Poult Dairy Vet Sci 2019; 6:542–546.

29 Upadhyay RK, Dwivedi P, Ahmad S. Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. Asian J Med Sci 2010; 2:152–158.

30 Tona K, Onagbesan O, De Ketelaere B, Decuypere E, Bruggeman V. Effect of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick post hatch growth to forty-two days. JAppl Poult Res 2004; 13:10–18.

31 SAS Institute. SAS/STAT® 9.1 User's Guide. Cary, NC. USA: SAS Institute Inc; 2004.

32 Duncan DB. Multiple Range and Multiple F-Tests. Int Biometric Soc 1955; 11:1–42.

33 Smith A, Rose SP, Wells RG, Pirgozliev V. The effect of changing the excreta moisture of caged laying hens on the excreta and the microbial contamination of their eggshells. Br Poult Sci 2000; 41:168–173.

34 Protais J, Queguiner S, Boscher E, Piquet JC, Nagard B, Salvat G. Effect of housing systems on the bacterial flora of eggshells. Br Poult Sci 2003; 44:788–790.

35 De Reu K, Grijspeerdt K, Heyndrickx M, Zoons J, De Baere K, Uyttendaele M, *et al.* Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. Br Poult Sci 2005; 46:149–155.

36 Kim JH, Kim KS. Hatchery hygiene evaluation by microbiological examination of hatchery samples. Poult Sci 2010; 89:1389–1398.

37 De Reu K, Grijspeerdt K, Messens W, Heyndrickx M, Uyttendaele M, Debevere J, Herman L. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria including *Salmonella enteritidis*. Int J Food Microbiol 2006; 112:253–260.

38 Copur G, Arslan M, Duru M, Baylan M, Canogullari S, Aksan E. Use of oregano (*Origanum onites* L.) essential oil as hatching egg disinfectant. Afr J Adv Biotechnol 2010; 9:2531–2538.

39 Ulucay IO, Yildirim I. Hatching traits of quail (*Coturnix coturnix japonica*) eggs disinfected with carvacrol, cinnamaldehyde or thymol. J Appl Anim Res 2010; 38:139–142.

40 Oliveira GDS, dos Santos VM, Nascimento ST. Essential oils as sanitisers for hatching eggs. Worlds Poult Sci J 2021a; 77:605–617.

41 Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med 2006; 39:1–8.

42 Chaieb K, Hajlaoui H, zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. Phytother Res 2007; 21: 501–506.

43 Copur G, Arslan M, Baylan M, Canogullari S. Use of allicin Allicin as an alternative hatching egg disinfectant versus formaldehyde fumigation in broiler hatching eggs. Biotechnol Biotechnol Equip 2011; 25:2494–2498.

44 Baylan M, Akpınar GC, Canogullari SD, Ayasan T. The effects of using garlic extract for quail hatching egg disinfection on hatching results and performance. Braz J Poult Sci 2018; 20:343–350.

45 Magwood SE. Studies in hatchery sanitation I. Fluctuations in microbial counts of air in poultry hatcheries. Poult Sci 1964; 43:441–449.

46 Barott HG. Effects of temperature, humidity and other factors on hatch of eggs and on energy metabolism of chick embryos. United States Department of Agriculture. Tech Bull 1937; 553:1–46.

47 Tullet SG, Burton FG. Factors affecting the weight and water status of the chick at hatch. Br Poult Sci 1982; 23:361–369.

48 Meijerhof R, van Beek G. Mathematical modelling of temperature and moisture loss of hatching eggs. J Theor Biol 1993; 165:27–41.

49 Brake J, Sheldon BW. Effect of a quaternary ammonium sanitizer for hatching eggs on their contamination, permeability, water loss and hatchability. Poult Sci 1990; 69:517–525.

50 Peebles ED, Pansky T, Doyle SM, CR Boyle CR, Smith TW, Latour MA, Gerard PD. Effects of dietary fat and eggshell cuticle removal on egg water loss and embryo growth in broiler hatching eggs. Poult Sci 1998; 77:1522–1530.

51 Tona K, Bamelis F, Coucke W, Bruggeman V, Decuypere E. Relationship between broiler breeder's age and egg weight loss and embryonic mortality during incubation in large-scale conditions. J Appl Poult Res 2001; 10:221–227.

52 Rosa PS, de Avila VS. Variaveis relacionadas ao rendimento da incubaçao de ovos em matrizes de frangos de corte [Variables related to egg incubation yield in broiler breeders]. Concordia: EMBRAPA Suínos e Aves; 2000; pp. 1–3.

53 Schmidt GS, de Figueiredo EAP, de Avila VS. Incubaçao: estocagem dos ovos ferteis [Incubation: storage of fertile eggs]. Concordia: EMBRAPA Suínos e Aves; 2002; pp. 1–5.

54 Molenaar R, Reijrink IAM, Meijerhof R, Van den Brand H. Meeting embryonic requirements of broilers throughout incubation: a review. Braz J Poult Sci 2010; 12:137–148.

55 Aviagen. How to measure chick yield. 2011; pp. 1–4. https://en.aviagen.com/assets/Tech_Center/BB_Resources_Tools/Hatchery_How_Tos/02HowTo2MeasureChickYield.pdf

56 Boleli IC, Morita VS, Matos JrJB, Thimotheo M, Almeida VR. Poultry egg incubation: integrating and optimizing production efficiency. Brazilian Journal of Poult Sci 2016; 18:1–16.

57 Willemsen H, Everaert N, Witters A, De Smit L, Debonne M, Verschuere F, *et al.* Critical Assessment of Chick Quality Measurements as an Indicator of Posthatch Performance. Poult Sci 2008; 87:2358–2366.

58 Oliveira GDS, Nascimento ST, Dos Santos VM, Lima Dallago BS. Spraying hatching eggs with clove essential oil does not compromise the quality of embryos and one-day-old chicks or broiler performance. Anim 2021b; 11:2045.