

Antimicrobial effect of essential oil of *Gallesia integrifolia* (Phytolaccaceae) against bacteria and fungi on hatching eggshells and its influence on the production parameters of day-old chicks

Efeito antimicrobiano do óleo essencial de *Gallesia integrifolia* (Phytolaccaceae) frente a bactérias e fungos da casca de ovos incubáveis e sua influência nos parâmetros de produção de pintainhos de um dia

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Highlights

Count of aerobic mesophiles on fertile eggs ranged between 2.92 and 3.42 Log₁₀ CFU.

The count of molds and yeasts on fertile eggs ranged between 1.74 and 3.78 Log₁₀ CFU.

The chicks exhibited optimal to good quality scores.

Abstract

Formaldehyde-based disinfectants, although widely used for hatching egg sanitation and pathogen control in poultry production, pose serious occupational health risks and face increasing consumer opposition, driving the search for eco-friendly alternatives. In this context, this study aimed to evaluate the potential of *Gallesia integrifolia* (Phytolaccaceae) fruit essential oil, known for its antibacterial and

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antifungal properties, as a natural biocide for eggshell disinfection. For the experiment, 192 eggs were selected from the nests of two breeder houses located in the northwestern region of the state of Paraná. The selected eggs were divided into control and treatment groups and identified using sequential numbers in groups of four eggs. Eggs in the control group were disinfected by spraying them with an aqueous solution containing 2% polysorbate at a rate of 2 mL per egg. Eggs in the treatment group were disinfected with an aqueous solution containing 2% polysorbate and *G. integrifolia* essential oil at a concentration of 0.44 mg mL⁻¹. The efficacy of *G. integrifolia* essential oil was assessed by counting aerobic mesophiles and fungi on the eggshell surface. The eggs were incubated, and the production and quality parameters of the hatched chicks were evaluated using scores. The results showed that *G. integrifolia* fruit essential oil did not alter the evaluated production parameters. Aerobic mesophile counts, chick quality scores, the egg weight-to-chick weight ratio, total hatchability, and hatchability of fertile eggs did not differ significantly ($p \geq 0.05$) between treatments in either breeder house. However, fungal counts in the first breeder house differed significantly ($p < 0.05$) between the treatment and control groups.

Key words: Aerobic mesophiles. Disinfection. Fungi. Organosulfur. Pau-d'alho.

Resumo

Desinfetantes à base de formaldeído, embora amplamente utilizados na higienização de ovos incubáveis e no controle de patógenos na avicultura, apresentam riscos graves à saúde ocupacional e enfrentam crescente rejeição por parte dos consumidores, impulsionando a busca por alternativas ecologicamente corretas. Nesse contexto, este estudo teve como objetivo avaliar o potencial do óleo essencial dos frutos de *Gallesia integrifolia* (Phytolaccaceae), conhecido por suas propriedades antibacterianas e antifúngicas, como um biocida natural para a desinfecção da casca de ovos. Para execução do experimento foram selecionados 192 ovos de ninho de dois matrizeiros localizados na região Noroeste do estado do Paraná. Os ovos selecionados foram separados em grupo controle e tratamento e identificados com números sequenciais em grupos de quatro ovos. Os ovos do grupo controle foram desinfetados por aspersão com uma solução aquosa contendo polisorbato a 2% na proporção de 2 mL por ovo. Já os ovos do grupo tratamento, foram desinfetados com solução aquosa contendo polisorbato a 2% e óleo essencial de *G. integrifolia* na concentração de 0,44 mg mL⁻¹. A eficácia do óleo essencial de *G. integrifolia* foi avaliada por meio da contagem de mesófilos aeróbios e fungos da superfície da casca dos ovos. Os ovos foram incubados e ao nascimento dos pintainhos foram avaliados os parâmetros produtivos e de qualidade dos pintainhos nascidos por meio de escores. Os resultados obtidos demonstraram que o óleo essencial do fruto da *G. integrifolia* não alterou os parâmetros produtivos avaliados, a contagem de mesófilos aeróbios e a avaliação de qualidade dos pintainhos, relação peso do ovo e peso do pintainho, assim como, a eclosão total e a eclosão sobre os férteis não diferiram ($p \geq 0,05$) entre os tratamentos em ambos matrizeiros. Já a contagem de fungos do primeiro matrizeiro demonstrou diferença ($p < 0,05$) quando comparado ao grupo teste com o controle.

Palavras-chave: Desinfecção. Fungos. Mesófilos aeróbios. Organossulfurados. Pau-d'alho.

Introduction

Brazil's prominence in food production is supported by its abundant natural resources, integrated production systems, and differentiated sanitary status, which have contributed to productivity gains and increased competitiveness in the sector over the past few decades. As a result, Brazilian chicken meat production has increased more than elevenfold over the past four decades, with per capita consumption reaching 45.5 kg in 2024 (Associação Brasileira de Proteína Animal [ABPA], 2025).

This growth reflects the increasing demand from both domestic and international markets, as chicken meat provides high-quality protein at a competitive cost. Consequently, the poultry sector requires strategies to further improve production efficiency and cost-effectiveness (Schmidt & Silva, 2018). In this regard, egg disinfection reduces contamination and, when effective, can improve hatchability and chick quality. Currently, fumigation with formaldehyde is the most widely used method for this purpose (Lauvers et al., 2011). However, despite being an effective antimicrobial agent, formaldehyde can cause severe damage to embryos if fumigation is performed incorrectly. Furthermore, its use is discouraged primarily because of the health risks associated with its application (Melo et al., 2019).

Given this scenario, natural products and alternative methods have been increasingly explored, and essential oils have

emerged as a promising option. These oils are complex natural mixtures that exhibit a broad range of biological activities, including antimicrobial activity (Abers et al., 2021).

The use of essential oils as an alternative for bacterial control in fertile eggs is promising, as bacterial contamination can compromise the entire production chain, resulting in reduced hatchability and substantial economic losses in hatcheries (Nogueira et al., 2019).

In this context, the species *Gallesia integrifolia* (Spreng.) Harms stands out. This arboreal species, commonly known as "pau-d'alho" (garlic tree), is named for its strong garlic-like odor and is widely used in folk medicine to prepare teas and treat influenza, coughs, pneumonia, worm infections, gonorrhea, prostate tumors, and rheumatism (Pinto et al., 2024). Aromatic plants such as *G. integrifolia* contain high concentrations of organosulfur compounds, which often exhibit various biological activities, including antimicrobial and antioxidant effects, as well as cytotoxicity against certain cancer cells (Anwar et al., 2008).

Given the diversity of its reported biological activities and evidence of its antimicrobial potential, this study aimed to evaluate the potential of *G. integrifolia* fruit essential oil as a natural biocide for hatching egg disinfection. Specifically, its effects on eggshell microbial loads (aerobic mesophiles and fungi), as well as on the production and quality parameters of hatched chicks, were assessed.

Material and Methods

Egg selection, identification, and disinfection

For the experiment, hatching eggs were collected from the nests of two broiler breeder houses located in the northwestern region of the state of Paraná and operated by a regional agribusiness company. A total of 192 eggs were collected from each breeder house, resulting in 384 eggs evaluated. The collected eggs were divided into two trays, each containing 96 eggs: one designated as the control group and the other as the treatment group. After selection and group allocation, the eggs were identified using sequential numbers in groups of four, resulting in 24 replicates.

Once identified, the eggs were weighed. Subsequently, eggs in the control group were disinfected with an aqueous solution containing polysorbate 80 (2 mL per egg). In contrast, eggs in the treatment group were disinfected with an aqueous solution containing polysorbate 80 and *G. integrifolia* essential oil at a concentration of 0.44 mg mL⁻¹, using the same application rate of 2 mL egg⁻¹ and the same application method.

The *G. integrifolia* essential oil used to disinfect the eggs in the treatment group was obtained from the plant's fruit, collected in June 2020, and extracted by hydrodistillation using a modified Clevenger apparatus for 3 h, as described by Silva et al. (2023).

To determine the concentration of essential oil used for egg application, the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) reported by Raimundo et al. (2018) for *G. integrifolia* fruit essential oil were used as references. In that study, the MIC

against the *Escherichia coli* ATCC strain was 0.15 mg mL⁻¹, while the MFC against fungi ranged from 0.02 to 0.18 mg mL⁻¹. For egg disinfection, the dose was set at 0.44 mg mL⁻¹, approximately three times higher than the MIC against *E. coli* and 2.4 times higher than the highest MFC reported for fungi. Thus, the selected concentration exceeded both the antibacterial and antifungal thresholds determined in the same study.

Sample collection and processing

After disinfection, the eggs were allowed to dry completely (approximately 20 min). Only after this period were swab samples collected from the eggshell surface, following the methodology described by Andrade (2018), which involved an exposure time of 10-20 min. For sample collection, swabs were moistened in a tube containing 0.1% peptone water and pressed against the tube walls to remove excess liquid. The swabs were then rubbed over the entire eggshell surface. Each sample consisted of a pool of four eggs, which were placed in sterile bags containing 12 mL of sterile 0.1% peptone water. The collected samples were kept refrigerated until processing at the Laboratory of Preventive Veterinary Medicine and Public Health, within the Graduate Program in Animal Science, with an emphasis on Bioactive Products, at Universidade Paranaense.

In the laboratory, the samples were processed to determine aerobic mesophile and fungal counts on the eggshell surface. Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) were used for the enumeration of aerobic mesophiles and fungi, respectively. The samples were homogenized for 60 s to

obtain the 10^{-1} dilution. Serial dilutions were then prepared in 0.1% peptone water to obtain 10^{-2} and 10^{-3} dilutions. For plating, 0.1 mL (100 μ L) of each dilution (10^{-1} , 10^{-2} , and 10^{-3}) was inoculated onto the respective culture media. The samples were incubated at 37 °C for 48 h for aerobic mesophile enumeration and at room temperature for 120 h for fungal enumeration. After incubation, colony-forming units (CFUs) were counted, and the results were expressed as Log_{10} values.

Chick quality evaluation

After sample collection, the eggs were sent for incubation. At 19 days of incubation, during transfer from the incubator to the hatcher, the eggs were placed in tulle ("filó") bags in groups of four

according to their identification numbers. This procedure prevented the chicks from mixing upon hatching and enabled individual group-based quality evaluation (Andrade, 2018). Upon hatching, the chicks were weighed according to their respective group identifications and evaluated based on the following quality parameters: activity, down, eyes, navel, residual membranes, abdomen, legs, and shanks. Each parameter was assigned a score, and the total score was used to classify the chicks into four quality categories: excellent (4; 100 points), good (3; 80-99 points), average (2; 50-79 points), and weak (1; ≤ 49 points), according to the methodology described by Tona et al. (2003) (Table 1). This study was submitted to and approved by the Ethics Committee on Research Involving Animal Experimentation (approval no. 40144/2023).

Table 1
Quality score classification of newborn chicks

Variable	Definition	Characteristic	Score
Activity	Evaluated by placing the chick on its back and observing its ability to return to a standing position. A rapid return to the standing position is considered indicative of good activity, whereas remaining in a supine position is classified as weak activity.	Good	16
		Average	8
		Weak	0
Down	Chicks with clean and dry down are considered to have good quality, whereas wet, dirty, or both wet and dirty down is classified as poor quality.	Clean and dry	12
		Clean and wet	6
		Dirty and wet	0
Eyes	Evaluated with the chick in a standing position by observing eye brightness and the extent of eyelid coverage over the eye.	Open and bright	10
		Open and dull	5
		Closed	0

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Navel	Evaluated by examining the navel and surrounding area for closure and coloration. A navel color that differs from the surrounding skin color is considered indicative of poor quality.	Closed and clean	12
		Not completely closed, typical coloration	6
		Not closed and typical coloration	0
Residual membrane	Evaluated at the navel by assessing the size of any remaining membrane. The membrane is classified as very large, large, or small.	No membrane	12
		Small	6
		Large	0
Abdomen	Evaluated by visually examining the chick's abdomen. A large or distended (bloated) abdomen is classified as poor quality.	Normal	12
		Average	6
		Distended	0
Legs	The chick is placed in a standing position to assess its ability to remain upright and stable. Toe conformation and the appearance of the knee joints are also examined.	Normal legs/toes, and joints	10
		One leg/toes and joints affected	5
		Two legs/toes and joints affected	0
Shanks	Evaluated by observing their color and brightness. Normal shanks are reddish and shiny.	Shiny, reddish	16
		Shiny, pale	8
		Opaque, pale	0

Source: Adapted from Tona et al. (2003)

Additionally, production parameters were evaluated by determining total hatchability, hatchability of fertile eggs, and the chick weight-to-egg weight ratio. Total hatchability was calculated as the ratio of hatched chicks to the total number of incubated eggs. Hatchability of fertile eggs was calculated as the ratio of hatched chicks to the number of fertile eggs incubated.

Statistical analysis

Descriptive analyses were performed by calculating the mean and standard error of the mean for aerobic mesophile and fungal

counts. The count data were transformed into Log_{10} values and tested for normality using the Lilliefors test and for homogeneity of variance using Levene's test. When these assumptions were met, differences between treatments were assessed using the independent-samples t-test. Descriptive statistics for the qualitative chick quality variables were expressed as absolute and relative frequencies (%). Inferential analyses of the quality scores were performed using the *Mann-Whitney U* test. A significance level of 5% was adopted for all statistical tests. All analyses were conducted using Bioestat 5.3 statistical software (Ayres et al., 2007).

Results and Discussion

The results obtained from eggs collected from broiler breeder nests showed no significant difference ($p \geq 0.05$) in aerobic mesophile counts between the control and

treatment groups in either breeder house. However, in breeder house 1, fungal counts were significantly higher ($p \geq 0.05$) in the treatment group than in the control group (Table 2).

Table 2

Mean $\pm$ standard error (Log_{10}) of aerobic mesophilic and fungal counts in eggs from nests disinfected with *G. integrifolia* essential oil or unsanitized

Breeder house	Count	Treatment		P-value
		Control	Test	
1	Aerobic mesophiles	2.95 $\pm$ 0.12	2.92 $\pm$ 0.10	0.8992
	Molds and yeasts	3.54 $\pm$ 0.05b	3.78 $\pm$ 0.07a	0.0111
2	Aerobic mesophiles	3.51 $\pm$ 0.07	3.42 $\pm$ 0.07	0.3810
	Molds and yeasts	1.46 $\pm$ 0.19	1.74 $\pm$ 0.15	0.2691

* T-test for two independent samples

High aerobic mesophile counts are considered indicators of poor hygienic conditions and potential contamination (Morton, 2001). Aerobic mesophiles comprise microorganisms capable of growing in the presence of oxygen at temperatures ranging from 15 °C to 45 °C (Silva et al., 2020).

Studies in the literature indicate that the microbial load on eggshells tends to reflect the production environment and the sanitary management practices adopted. Clímaco et al. (2018), when evaluating eggs collected from nests, reported aerobic mesophilic bacterial counts ranging from 3.17 to 3.57 log_{10} CFU mL^{-1} before disinfection. Similarly, Costa et al. (2022) reported a count of 3.93 log_{10} CFU mL^{-1} for nest eggs, whereas floor eggs exhibited a count of 6.51 log_{10} CFU mL^{-1} . A similar pattern was observed in the

present study, in which nest eggs exhibited comparable contamination levels.

A common practice for reducing microbial loads is the sanitization of hatching eggs before incubation using methods such as fumigation, spraying, or immersion with chemical agents (Cony et al., 2008). Among these methods, formaldehyde fumigation is widely used because of its excellent antimicrobial activity. However, formaldehyde poses serious health risks to handlers, as it is a toxic and carcinogenic gas that can damage mucous membranes, cause eye and skin irritation, and induce respiratory problems (Cadirci, 2009). A study conducted by Clímaco et al. (2018) found that fumigation with paraformaldehyde at a concentration of 13.33 g m^{-3} for 20 min significantly reduced the microbial load

on eggshells. The total aerobic mesophilic bacterial count decreased from $3.42 \log_{10}$ CFU mL⁻¹ before treatment to $1.10 \log_{10}$ CFU mL⁻¹ after treatment, demonstrating the effectiveness of the procedure in reducing eggshell contamination.

However, it is essential to note that, according to the results of Santos et al. (2025) study, the use of formaldehyde gas during the hatching process can cause ultra- and microstructural changes in the trachea and lungs of broiler chicks. Cadirci (2009) also reported this, noting that although formaldehyde does not have prolonged residual effects, it can cause embryo mortality if fumigation is performed incorrectly at high concentrations. These results are consistent with those obtained by Moustafa (2004), who found that fumigation at a high concentration resulted in a complete reduction in eggshell bacterial count 24 h after fumigation. However, a higher percentage of culled chicks was also observed in the first week of life, due to its toxic action. Nevertheless, it should be emphasized that in the present study, the evaluation of the essential oil's action was performed 20 min after product spraying, which may justify the difference in action observed by Moustafa (2004), who evaluated 24 h after fumigation. Another relevant consideration is that the complete elimination of bacteria observed following high-concentration formaldehyde fumigation proves its sterilizing action, which differs from the disinfectant potential found in essential oils, as they are distinct products. Furthermore, there is a difference between fumigation and spraying: formaldehyde is administered as a gas, which is more active and has greater power of action over the area where they are located.

The use of essential oils diluted in solvents has contributed to reducing the reliance on chemical agents in egg sanitization (Oliveira & Santos, 2018). Specifically, *G. integrifolia* (Sprengel) Harms essential oil contains compounds with antimicrobial potential, as reported by Bortolucci et al. (2022), highlighting the importance of investigating *Gallesia* species (pau-d'alho). However, in the present study, the application of *G. integrifolia* fruit essential oil did not result in a significant reduction in aerobic mesophile counts.

Although no significant reduction in aerobic mesophile or fungal counts was observed following essential oil spraying in the present study, it is worth noting that Arunachalam et al. (2016) reported a broad bacteriostatic effect of the hydroethanolic extract of the inner stem bark of *G. integrifolia*, particularly against Gram-negative bacteria. These findings suggest that antimicrobial activity may vary according to the plant part used and the extraction method employed.

The most relevant fungal pathogens in poultry production are species of the genus *Aspergillus*, particularly *Aspergillus flavus* and *A. fumigatus*. These fungi are commonly found in litter materials and poultry house environments and can also contaminate eggs. Their importance lies in the respiratory complications that may develop when birds inhale fungal spores, resulting in the respiratory form of aspergillosis. This condition can cause substantial economic losses due to increased mortality among newly hatched chicks exposed to contaminated eggs (Tessari et al., 2004).

In a study by Raimundo et al. (2021), the authors reported high antifungal activity

and the medicinal potential of *G. integrifolia* oil. Furthermore, Raimundo et al. (2018) evaluated the *in vitro* fungicidal activity of *G. integrifolia* fruit essential oil and reported effective concentrations ranging from 0.02 to 0.18 mg mL⁻¹ against fungi of the genera *Aspergillus* and *Penicillium*. In comparison, the fungicidal compounds ketoconazole and bifonazole exhibited concentrations ranging from 0.20 to 3.50 mg mL⁻¹. These findings suggest that the essential oil may exhibit antifungal activity at lower concentrations than those required for the comparative compounds under the conditions evaluated. However, when compared with the present study, in which hatching eggshells were disinfected by spraying with *G. integrifolia* essential oil at a higher concentration (0.44 mg mL⁻¹), no significant reduction in mold and yeast counts was observed in eggs collected from breeder house 2 (Table 2). This difference in results may be attributed to the experimental conditions: the present study was conducted under field conditions (*ex vitro*), whereas Raimundo et al. (2018) conducted their study in a controlled *in vitro* environment using an ATCC strain.

It should be emphasized that pretreatment microbiological counts were not performed on the same eggs because surfacesampling methods, such as swabbing, mechanically dislodge microorganisms and may compromise cuticle integrity, potentially introducing experimental bias. To address this issue, a parallel control group was used, receiving only the vehicle (2% aqueous polysorbate solution) without the essential

oil. Although this design precludes the calculation of individual log reductions from baseline measurements, it allows the effects of the essential oil to be assessed relative to those of the vehicle and spraying procedure.

As regards dose selection, one limitation of the present study is that the MIC and MFC values used as references were not determined against the fungal species naturally present on the eggshell surface. Instead, the selected concentration was based on previously published values for the same essential oil (Raimundo et al., 2018).

Regarding contact time, the 20-min interval between spraying and swab collection was selected to reflect the operational exposure period of hatching eggs to disinfectants during routine hatchery sanitation, thereby representing a practical field scenario. Unlike *in vitro* assays, which often employ contact times ranging from hours to days to establish concentration-dependent endpoints, the present study evaluated the antimicrobial effect under practical field conditions.

In the evaluation of chick quality parameters, classifications in both breeder houses indicated that most hatched chicks were classified as being of good quality in both the control and treatment groups. Accordingly, the mean quality scores did not differ significantly ($p > 0.05$) between treatments. Similarly, no significant differences were observed in egg weight or chick weight parameters (Table 3).

Table 3

Mean $\pm$ standard error of egg weight (g), chick weight (g), and quality score of chicks from eggs from nests disinfected with *G. integrifolia* essential oil or unsanitized

Breeder house	Count	Treatment		P-value
		Control	Test	
1	Egg weight (g)	62.7 $\pm$ 0.52	62.6 $\pm$ 0.40	0.9719*
	Chick weight (g)	45.1 $\pm$ 0.43	44.7 $\pm$ 0.31	0.5587*
	Chick quality score	3.0 $\pm$ 0.04	3.1 $\pm$ 0.06	0.6207**
2	Egg weight (g)	63.8 $\pm$ 0.31	64.3 $\pm$ 0.46	0.4013*
	Chick weight (g)	44.2 $\pm$ 0.32	44.3 $\pm$ 0.40	0.8701*
	Chick quality score	3.2 $\pm$ 0.10	3.3 $\pm$ 0.10	0.6207**

* T-test for two independent samples ** Mann-Whitney U test

Given these results, and considering that the contamination levels of incubated eggs may be associated with chick quality, the present study found that a greater

proportion of chicks in the treatment group were classified as being of excellent quality. This observation may suggest a potential positive effect of the essential oil (Figure 1).

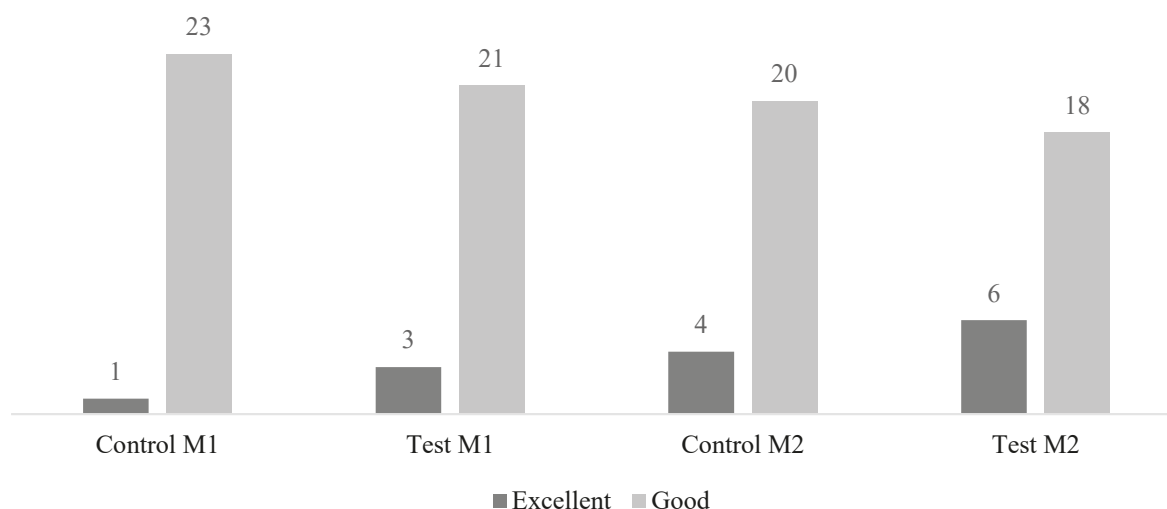


Figure 1. Quality of day-old chicks classified by scores according to treatments and origin (breeder houses). (M1 - Breeder House 1; M2 - Breeder House 2).

The cytotoxicity of *G. integrifolia* fruit essential oil against VERO cells (African green monkey kidney epithelial cells) was evaluated by Silva et al. (2023), who reported an IC₅₀ value (the concentration required to inhibit 50% of cell proliferation) of 226 µg mL⁻¹. To assess the significance of this value, it is necessary to compare it with reference values reported in the literature. According to Rogero et al. (2003), who evaluated biomaterials, IC₅₀ values greater than 100 µg mL⁻¹ were classified as non-toxic to VERO cells, indicating suitability for biological applications. The IC₅₀ value reported for *G. integrifolia* fruit essential oil exceeds this threshold, suggesting low cytotoxicity in the assay employed. This shows that it can be used in the disinfection of hatching eggs without affecting chick viability.

In a study by Andrade (2018) on egg disinfection using citronella essential oil (*Cymbopogon winterianus*), chick quality classification scores were generally similar to those observed in the present study. However, none of the chicks received a score of 100 points; instead, they were classified as average or good quality. Among the average chick quality scores reported by Andrade (2018), the treatment using *C. winterianus* essential oil at a concentration of 800 µL, despite significantly reducing eggshell fungal counts, yielded a lower mean score (75.71) than those observed in the present study. In the present study, the mean quality scores were higher, and no chicks were classified as being of average quality; all were classified as good or excellent (Table 4).

Table 4

Mean ± standard error of total hatchability, hatchability of fertile chicks, and chick weight/egg weight ratio

Breeder house	Count	Treatment		P-value
		Control	Test	
1	Total hatchability (%)	95.83±1.94	93.75±2.17	0.7728*
	Hatchability of fertile eggs (%)	98.96±1.04	97.92±1.44	0.8046*
	Chick weight/Egg weight ratio	71.91±0.29	71.43±0.16	0.1603**
2	Total hatchability (%)	87.5±3.40	87.5±3.00	0.9015*
	Hatchability of fertile eggs (%)	90.6±3.30	89.6±3.00	0.7029*
	Chick weight/Egg weight ratio	69.33±0.33	68.94±0.27	0.3732**

* Mann-Whitney U test ** T-test for two independent samples

Other production parameters, namely total hatchability, hatchability of fertile eggs, and the chick weight/egg weight ratio, also

showed no significant differences ($p > 0.05$) between treatments in either of the two breeder houses evaluated (Table 4).

One of the primary objectives of broiler production is to achieve high productivity and, consequently, profitability, which requires high hatchery yields and the production of high-quality chicks (Melo et al., 2019). However, microbial contamination of fertile eggs after laying can negatively affect the success of this process, as microorganisms can penetrate fertile egg and contaminate not only a single embryo but several others during the incubation process, thereby compromising hatchability (Wells et al., 2011).

One of the primary objectives of broiler production is to achieve (Wells et al., 2011).

In a study conducted by Oliveira et al. (2020), the total aerobic mesophilic bacterial count was significantly lower on eggshells sprayed with clove essential oil diluted in cereal alcohol at a concentration of 0.39% than on unsanitized eggshells. Significant differences were also observed in total hatchability and hatchability of fertile eggs between the treatment and unsanitized groups. The treatment groups exhibited mean rates of 70.81% and 84.69%, respectively, compared with 61.63% and 74.03% in the unsanitized groups, suggesting a potential bactericidal effect of clove essential oil.

Although *G. integrifolia* fruit essential oil did not reduce aerobic mesophile or fungal counts in the present study, no significant differences were observed between the treatment and control groups in terms of total hatchability, hatchability of fertile eggs, or the chick weight/egg weight ratio. It should also be noted that the fertile eggs collected from broiler breeders' nests were not subjected to an initial assessment of individual microbial contamination. Consequently, the number

of replicates was increased to facilitate comparisons of treatment efficacy.

Furthermore, the research objective of investigating antimicrobial biomolecules for egg disinfection aligns with Sustainable Development Goals (SDGs) 2 (Zero Hunger), 3 (Good Health and Well-being), and 12 (Responsible Consumption and Production), particularly through the development of biocides that are potentially less harmful to humans, birds, and the environment. This research opens new avenues for investigating other essential oils and extracts derived from plant species with potential commercial applications.

Conclusion

Spraying *G. integrifolia* fruit essential oil at a concentration of 0.44 mg mL⁻¹ did not affect chick production or quality parameters. However, it did not reduce bacterial or fungal contamination on the eggshells of hatching eggs collected from nests. Therefore, further studies are needed to investigate alternative application methods and experimental conditions to more effectively evaluate the efficacy, safety, and economic viability of this essential oil, as well as to establish protocols that produce more consistent results.

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