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Lavender Essential Oil in Sanitation on Fertile Egg

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ABSTRACT

This study aimed to assess the effectiveness of using lavender essential oil to sanitize fertile eggs instead of formaldehyde; on the eggshell bacteria count and quality of chicks. Under aseptic circumstances, 825 white eggs (67.93±0.63 g) were collected from 60 weeks old (Lohmann White breeder chickens) and randomly divided into five treatments (non-sanitized and sanitized with distilled water, lavender essential oil, Glutaraldehyde GPC-8, and formaldehyde fumigation) before incubation and at 10th day of incubation. The results showed that according to the fertile eggs, non-sanitized and formaldehyde groups was reduced hatchability compared with other treatments. All sanitized groups reduced the dead embryo compared with non- sanitized. Total aerobic bacterial counts on the hatching eggshell surface were significantly reduced due to using Lavender essential oil and GPC8 compared with formaldehyde, non-sanitized, and sanitized with distilled water before setting in an incubator. Also, at the 18th day of hatching eggs, spraying with lavender essential oil reduced significantly the number of total aerobic bacteria compared to all other treatments. Lavender essential oil treatment recorded the highest chick quality compared with all other treatments except Glutaraldehyde, and the lowest chick quality recorded for the formaldehyde group. Also, the abnormal chicks were significantly reduced with Lavender essential oil compared with formaldehyde. Eggs for incubation can be safely and effectively treated with lavender essential oil. It is strongly advised to use it in place of formaldehyde and other toxic compounds while sanitizing fertile eggs.

Keywords: Biological Sanitation, Fumigation, Chick Quality, Microbiology.

1. Introduction

The area of chicken hatching operations has grown over time with these modifications, there are the potential for increased hatchability, decreased mortality of chicks, and enhanced bird performance. Recently, incubation was not understood as other steps in the chicken production chain. The knowledge of incubation has not transferred from the information obtained compared to a few years ago in the poultry health, poultry nutrition, poultry management, and environmental conditions. Only lately have incubation parameters been acknowledged as having a considerable impact on chicken performance[1]. To maximize hatchability and produce chicks of the highest quality, a hatchery sanitation program must be successful[2].

One of the essential strategic areas where the poultry industry may enhance production efficiency is in the cleaning of fertile eggs. Eggshell microbial load must be decreased in order to improve hatchability and chick quality as well as to reduce an abundance of harmful microorganisms that gravely harm embryonic development[3]. The sanitizing agent paraformaldehyde, routinely used in farms and hatcheries, successfully maintains low levels of eggshell contamination[4, 5]. Paraformaldehyde, however, offers a significant risk to the environment, human health, and the growth of chick embryos[6,7,8]. For instance, paraformaldehyde has been linked to defects in developing chick embryos, according to the researchers[9].

According to researchers[10], lavender essential oil (*Lavandula angustifolia*) could be used as an alternative to sanitize fertile eggs, antibacterial, antifungal, antioxidant, and immune-stimulating properties of lavender essential oil. Linalool, linalool acetate, lavandula, and -terpinol are the significant components of lavender essential oils and exhibit a wide range of biological and pharmacological activity[9, 11, 12, 13]. Additional research confirming the usefulness of sanitation with essential oil of fertile eggs is required. Additionally, the success of the poultry industry depends on the creation of new active products instead those chemical compounds[10, 1].

Decontaminating eggshells is regarded as a successful method for getting rid of harmful bacteria and sanitizing eggs for human consumption. Chemical and radiation treatments have been recommended as some of the methods for sanitizing eggshells[14]. However, using these methods will have negatively affect on the

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environment, human health, and egg quality. Finding a secure substitute technique is desirable, thus, one possibility to clean eggshells is utilizing essential oils. So, essential oil products are necessary byproducts of secondary metabolism that are derived from a variety of plant organs, including leaves, stems, roots, and fruits[1].

The current study set out to determine the lavender essential oils as efficient at reducing microbial activity on eggshells, how they affect the ability of fertile eggs to hatch and the post-hatching improvement of quality in chicks.

2. Material and Methods

2.1 Ethics Approval

This study was approved by the Scientific Ethical Committee of the Animal Resources Department College of Agricultural Engineering Sciences, Salahaddin University (No.: 3/5/5756 at 14/5/2023).

2.2 Layout of the Experimental Design

This study was applied in Vano commercial hatchery in Erbil Governorate / Kurdistan Region, Iraq. A total of 825 white eggs with a weight value of 67.93±0.63 g from 60 weeks old Lohmann White breeders were collected in sterile conditions, then randomly dispersed among five groups before incubation, and sanitized at the 1st day and 10th days of incubation explained in (Table 1**)**. Lavender is used as commercial as a liquid that contains 60% Ethanol; the remaining, was Linalool, Butyl Phenyl Methyl Propionyl, Benzyl Salicylate, and Hexyl Cinnamic Aldehyde.

Table 1: The explanation of treatments, concentrations of the chemical, and technique of applying it to the eggs.

Treatment		Concentration	Application	Number of eggs
T1	Non-sanitized	--	--	165
T2	Sanitized with Distilled Water	--	Spraying	165
T3	Lavender essential oil	Commercial	Spraying	165
T4	Glutaraldehyde (GPC8™)	10 ml / L	Spraying	165
T5	Formaldehyde	6 g / m^3	Fumigation	165

2.3 Eggshell Microbial Count

The swab samples were collected on the surface area of about two cm^2 on the surface of each egg per treatment under aseptic conditions on the surface of eggshell on the first day before incubation and 18th days before setting in an incubator. They immediately transported to the laboratory, Salahaddin University, College of Education, Microbiology lab, according to the method of the researcher[15]. After serial dilution from an initial 10^{-1} dilution to 10^{-7}, 100 ul of each dilution was plated on MacConkey agar and Salmonella Shigella agar to the counting of total aerobic bacteria and *Salmonella*, respectively. Colonies were counted after the incubation time, and the findings were reported in $\log_{10}$ CFU/mL.

2.4 Incubation and Hatching

Before being put in an incubator with the temperature and relative humidity set to 37.5°C and 55%, respectively, the eggs were serially numbered and weighed. On the eighteenth day of incubation, the eggs were moved into hatchers to hatch them. Following that, they completed the final three days of incubation in a hatcher at 37.2°C and 65% relative humidity before hatching. The influences of disinfectant of hatching egg on fertile egg weight, egg weight loss%, and hatchability % were estimated.

2.5 Chick Quality

Thirty chicks per treatment were used to test the chick quality. Chick Quality is evaluated using the Pasgar© scoring method, which was developed by the researcher[1], and was used to visually evaluate the quality of the chicks after they had been removed from the incubators. Each bird typically began the test with ten points and reduced one point from each feature, the quality considered to be poor **(**Table 2**)**. To prevent interexaminer variation, only one individual was used to do this subjective evaluation.

Table 2: Evaluation of chicken quality using the Pasgar score.

Observed parameters	Assessment
Naval area	Healing state
Legs	Injury, red hooks, swollen hooks, or malformed are present.
Eyes	Brightness and the size of the eye gap
Beak	Injury, red dot, and deformed beak are all present.
Yolk	Absorption of the yolk sac degree
Reflex	Ability to react to stimuli, chicks need more than two seconds to turn from lying on its back to a normal position

Source: Adapted from (Gabriel *et al*., 2020).

2.6 Statistical Analysis

A one-way ANOVA table was used by SPSS version 27 software to evaluate the data[16]. Means and standard error were included in the results of summary statistics. The Duncan's test was use to determination of significant differences between the various parameters at 0.05 levels[17].

3. Results and Discussions

Table 3 showed that there were no significant (P>0.05) differences among treatments according to the initial egg weight and egg weight at the 18th and the 21st day of incubation. Moreover, in this investigation, there was no difference in the treatments for the percentage of egg weight loss throughout incubation except sanitized with distilled water at the end day of incubation (Table 4). Physical elements necessary for incubation, like temperature and relative humidity as two essential criteria, have a more substantial influence on this parameter[18, 19]. Since all the eggs in this experiment were incubated under identical conditions, no differences between the treatments were anticipated. Additionally, measuring the weight loss of eggs during incubation allowed us to infer the degree of cuticle destruction caused by sanitizers, which in turn affected embryonic development[20]. Our data imply that none of the sanitation procedures hurt the cuticle.

The results in (Table 5) show a significant difference in the percentage of fertility according to total and fertile eggs. The hatchability was 69.7% in non-sanitized with total eggs, but according to the hatch of fertile eggs, non-sanitized and formaldehyde groups was reduced hatchability compared with other treatments. This outcome seen as a result of the eggs coming from breeder hens at the poultry house that were 60 weeks old and under the same management. Also, Table 5 showed that dead embryos during the hatching period was significantly (P<0.05) reduces in Glutaraldehyde and lavender essential oil treatments compared with non-sanitized and formaldehyde.

The best of our knowledge, there is no study on using lavender essential oil to sanitize eggs instead of formaldehyde. The researcher did not find any statistically (P>0.05) significant differences between the treatments on fertile eggs with essential oil of cloves at 0.6 mg/mL concentration and paraformaldehyde[1]. The researchers examined the effects of oregano essential oil on the cleanliness of eggs for incubation at two concentrations (0.55 and 0.75 mL/cm^3) and two exposure times (3 and 6 h). They found that these egg's capacity to hatch was not significantly different from the ability of eggs that had been formaldehyde-treated[21]. Because the bacterial burden on the eggshell was reduced, sanitation with lavender essential oil boosted hatchability than to non-sanitized eggs.

In Table 6, data was acquired about the impact of hatching egg disinfection on the total aerobic bacterial count of the eggshell surface. This table finding showed that the GPC8 and lavender essential oil instead of formaldehyde reduced the total aerobic bacterial counts on hatching eggshell surface compared to non-sanitized and sanitized with distilled water. The counts of total aerobic bacteria were decreased from 5.72 log in non-sanitized to 4.30 and 4.53 in Lavender essential oil and GPC8, respectively. In contrast, there were no significant differences (P>0.05) observed between Lavender essential oil and GPC8 before setting in the hatchery machine. Also, on the 18th day of hatching eggs spraying lavender essential oil decreased significantly the overall aerobic bacterial population more than all other treatments. No salmonella was detected in all treatments at different stages of setting in the incubation. Linalool, linalool acetate, lavandula, and - terpinol are the significant components of lavender essential oils and exhibit a wide range of biological and pharmacological activity[11, 12, 13]. One property of lavender essential oil is inhibiting the growth of pathogenic bacterial microbiota, while on the other hand, it can promote the growth of good microbes[11,22, 23]. Due to the high concentration of aldehyde and linalool components in lavender essential oil, which have antibacterial activity, fewer total aerobic bacteria were detected as pathogens in this investigation.

Table 7 shows the effects of Lavender essential oil on the quality of chicks after hatching of chicks. The results showed significant (P<0.05) differences between all treatments. The lavender essential oil treatments recorded the highest chick quality (P<0.05) compared with all other treatments except Glutaraldehyde, and the lowest chick quality was recorded for the formaldehyde group. Also, the abnormal chicks were significantly (P<0.05) reduced with Lavender essential oil compared with formaldehyde.

Table 3: Effect of disinfection on fertile egg weight during incubation (mean±SE).

Treatment	Egg weight (g)		
	Zero day	18th day	21st day
Non-sanitized	69.66±0.80	62.16±0.49	49.20±0.98
Sanitized with Distilled Water	66.31±1.29	60.56±0.78	49.10±1.05
Lavender essential oil	69.74±1.38	61.43±0.52	47.86±1.23
Glutaraldehyde (GPC8TM)	67.53±1.39	61.20±0.56	46.76±1.36
Formaldehyde	66.43±1.45	61.36±0.94	45.63±
P. value	0.226	0.613	0.319

Table 4: Effect of disinfection on weight loss of egg weight during incubation (mean±SE).

Treatment	Egg weight loss (%)	
	0-18th day	0-21st day
Non-sanitized	7.50±0.40 ab	20.46±0.80 ab
Sanitized with Distilled Water	5.74±0.51 c	17.21±1.50 b
Lavender essential oil	8.31±0.46 a	21.87±0.63 a
Glutaraldehyde (GPC8TM)	6.33±0.49 bc	20.76±1.29 a
Formaldehyde	5.06±0.51 c	20.80±0.68 a
P. value	0.005	0.080

a,b,c Means within the same column with different superscripts differ (P<0.05).

Table 5: Effect of hatching disinfection on hatchability percentages and dead embryo (mean±SE).

Treatment	Hatchability (%)		Dead embryo
	Hatchability of total eggs (%)	Hatchability of fertile eggs (%)	
Non-sanitized	69.70±3.03 [ab]	88.42±0.46 [c]	13.10±0.60 [b]
Sanitized with Distilled Water	72.72±2.09 [a]	93.71±0.87 [b]	6.73±0.97 [c]
Lavender essential oil	61.82±3.78 [b]	95.41±0.68 [ab]	4.80±0.75 [cd]
Glutaraldehyde (GPC8^{TM})	66.66±2.18 [ab]	97.34±0.08 [a]	2.76±0.08 [d]
Formaldehyde	47.87±2.42 [c]	78.68±1.79 [d]	27.66±1.85 [a]
P. value	0.001	<0.001	<0.001

[a,b,c,d] Means within the same column with different superscripts differ (P<0.05).

Table 6: Effect of disinfection on total aerobic bacterial and Salmonella spp. count (Log_{10} CFU per egg) on eggshell at different stage of incubation (mean±SE).

Treatment	Total aerobic bacteria count		*Salmonella* spp.	
	Before setting in incubator	Before setting in Hatcher	Before setting in incubator	Before setting in Hatcher
Non-sanitized	5.72±0.04 [a]	8.04±0.005 [a]	ND	ND
Sanitized with Distilled Water	5.54±0.08 [a]	7.92±0.04 [ab]	ND	ND
Lavender essential oil	4.30±0.17 [b]	7.52±0.03 [d]	ND	ND
Glutaraldehyde (GPC8^{TM})	4.53±0.11 [b]	7.78±0.01 [c]	ND	ND
Formaldehyde	5.55±0.08 [a]	7.84±0.01 [bc]	ND	ND
P. value	<0.001	<0.001	ND	ND

[a,b,c,d] Means within the same column with different superscripts differ (P<0.05).
ND: Not detected.

Table 7: Effect of hatching disinfection on chick quality (mean±SE).

Treatment	Chick quality	
	Activity of chicks	Abnormal chicks
Non-sanitized	7.96±0.24 [b]	1.66±0.33 [b]
Sanitized with Distilled Water	8.20±0.32 [b]	1.66±0.88 [b]
Lavender essential oil	8.86±0.14 [a]	0.66±0.66 [b]
Glutaraldehyde (GPC8^{TM})	8.60±0.11[ab]	2.00±0.00 [ab]
Formaldehyde	7.30±0.05 [c]	3.66±0.33 [a]
P. value	0.002	0.032

[a,b,c,d] Means within the same column with different superscripts differ (P<0.05).

Conclusions

In conclusion, the lavender essential oil is successful and safe for eggs during incubation. Using lavender essential oil reduced the abnormal chicks significantly with Lavender essential oil compared with formaldehyde. Total aerobic bacteria on the eggshell surface of fertile eggs were reduced and subsequently improving the chick quality with lavender essential oil. So, its use as an alternative to formaldehyde and other chemical compound in the sanitation of fertile eggs.

Conflict of interests

There is no conflict of interest.

Authors contribution

The authors have written, drafted, analyzed data, and finalized the manuscript.

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