مجلة جامعة بني وليد للعلوم الإنسانية والتطبيقية
Bani Waleed University Journal of Humanities and Applied Sciences
تصدر عن– جامعة بني وليد – ليبيا
Website:https://jhas-bwu.com/index.php/bwjhas/index
المجلد التاسع ـ العدد الخامس ـ 2024 ـ الصفحات (295-309)
ISSN3005-3900

استخدام مستخلصات أوراق الريحان (*Ocimum Basilicum L.*) كمعقم غير تقليدي لبيض التفريخ بدلا من التعقيم بالفورمالديهايد في بيض البهيج

د. محمود أحمد عثمان ابومهارة[1] *
[1]. قسم الإنتاج الحيواني، كلية الزراعة، جامعة بني وليد، بني وليد، ليبيا
mahmoudabomahara@bwu.edu.ly

Usage of basil leaf extracts (*Ocimum Basilicum L.*) Herbs plant as Unconventional Hatching Egg Sanitizer Versus Formaldehyde Fumigation in Baheig Eggs

mahmoud ahmed othman abo mhara[1]*
Department of Animal Production, Faculty of Agriculture, Bani Waleed University, Bani Walid, Libya

تاريخ الاستلام: 28-10-2024 تاريخ القبول: 22-11-2024 تاريخ النشر: 05-12-2024

الملخص:
قارنت الدراسة فعالية مستخلصات أوراق الريحان (.Ocimum basilicum L) والتدخين بالفورمالديهايد في تطهير قشور بيض التفريخ في بهيج ومطروح لتحسين نتائج الفقس وأداء الكتاكيت. كانت نسبة الفقس متسقة عبر سلالات الطيور، لكن كتاكيت مطروح أظهرت جودة أفضل من حيث وزن الفقس عند الولادة ومعدل تحويل العلف والقدرة على البقاء مقارنة بدجاج بهيج في عمر 4 أو 8 أسابيع. والجدير بالذكر أن المعاملة بمستخلصات أوراق الريحان أدى إلى زيادة نسبة الفقس وانخفاض معدل وفيات الأجنة مقارنة بالتدخين بالفورمالديهايد. أظهرت الكتاكيت من مجموعة مستخلصات أوراق الريحان وزنًا أفضل عند الفقس وأداءً متفوقًا من حيث وزن الفقس ومعدل تحويل العلف في عمر 4 أو 8 أسابيع. أظهرت الدراسة أن مستخلص مستخلصات أوراق الريحان قلل بشكل فعال من إجمالي عدد البكتيريا على قشور بيض التفريخ، مما يسلط الضوء على إمكاناته كمطهر طبيعي.
الكلمات المفتاحية: مستخلصات أوراق الريحان، التلوث، الحضانة، قشر البيض، أداء الكتكوت.

.

Abstract:
The study compared the effectiveness of basil leaf extracts (*Ocimum basilicum L.*) and formaldehyde fumigation in disinfecting Baheig and Matrouh hatching eggshells to Optimize hatching outcomes and chick performance. Hatchability was consistent across bird breeds, but Matrouh chicks showed better quality in terms of LBW, FCR, and viability compared to Baheig pullets at 4 or 8 Wks of age. Notably, *ocimum basilicum* treatment resulted in higher hatchability and lower embryo mortality compared to formaldehyde fumigation. Chicks from the *ocimum basilicum* group exhibited better weight at hatch and superior performance in terms of LBW and FCR at 4 or 8 Wks of age. The study demonstrated that *ocimum basilicum* extract effectively reduced total bacterial count on hatching eggshells, highlighting its potential as a natural disinfectant.

Keywords: *basil leaf extracts, contamination, incubation, eggshells, chick performance.*

Introduction

Microbial pollution of eggshells can occur through the laying process and after the egg is laid, leading to bacterial penetration and potential infection of the embryo. This can result in decreased hatch rates, lower chick quality, and infections in growing pullets, (Al-Shammari *et al*., 2022).

Maintaining a clean hatchery is crucial for completing great hatchability and generating quality chicks (Adame, 2023). Formaldehyde is commonly used in hatcheries as a fumigant because of its effectiveness and ease of use, (Rebolledo *et al*., 2023). However, health and safety laws like the Control of Substances Hazardous to Health (COSHH) legislation are making it difficult to employ formaldehyde (Pees *et al*., 2023). Formaldehyde also has a strong odor, irritates the eyes and nose, and is difficult to remove from the environment (Whistler and Sheldon, 1989). Moreover, its potential carcinogenic properties have led to regulatory actions by the Environmental Protection Agency (Solt *et al*., 2019).

Therefore, there is a need for effective alternative disinfectants to replace formaldehyde in hatcheries in case its use is banned by regulatory authorities. Several research have looked into the antibacterial activities of several spices and compounds (Liu *et al*., 2017). Spices have antimicrobial effects due to their essential oil content, which can inhibit microbial growth through their chemical structures. The reactivity of the panic-OH group in essential oils allows them to form hydrogen bonds with enzyme active sites (de Sousa *et al*., 2023). Spices such as Ocimum basilicum have been used for their antimicrobial properties in meats like poultry, turkey, and beef (Krishnan *et al*., 2014). Researchers have documented the antimicrobial activities of volatile oils (Chouhan *et al*., 2017), but their effects in food systems have been less studied (Kadhim *et al*., 2016). Optimal water vapor loss during incubation is crucial for hatchability and chick quality. Eggshell porosity has theaters a key part in keeping water stability and given that gas exchange for the developing embryo. Insufficient gas exchange and water loss can lead to embryonic mortality in poultry (Bilalissi *et al*., 2022). Manipulating factors like eggshell permeability can impact hatchability, with the cuticle affecting permeability (Rodríguez-Navarro *et al*., 2013). Changes in conductance constant (k) can affect oxygen consumption and gas exchange during metabolic development (Chen *et al*., 2021). Sanitisers can influence eggshell permeability and embryonic development by influencing the cuticle (Oliveira *et al*., 2021b).

This revision intended to equate the properties of spraying basil leaf extracts and formaldehyde fumigation on disinfecting Baheig and Matrouh hatching eggshells to sustain hatching grades and bird enactment.

MATERIALS AND METHODS

Basil leaf preparation and phytochemical analysis

Collection and extraction of basil leaf extracts (Ocimum Basilicum) were conducted using leaves from O. basilicum gathered in North West Egypt (34_29052.3” N 4_47011.9”W). The plants were verified in the Botany Department of the Plant Research Institute in Cairo, Egypt. The oil was extracted from 100 g of dried aerial parts using hydro-distillation in a Clevenger-type apparatus (VWR, Radnor, PA, USA). The extraction process involved boiling the plant material in water for 3 hours. The extracted oil was stored at 4°C. The plant extract's phytochemical components were identified using the method described by Altemimi *et al* (2017) with a Thermo Scientific TRACE-1300 series gas chromatography/mass spectrometry (GC/MS) system. The system used a fused silica DB-5 capillary column (30 m x 0.32 mm, 0.25 µm film thickness) connected to a Triple Quadrupole Mass Spectrometer (TSQ 8000 Evo; Thermo Fisher Scientific Inc.). Mass spectra were scanned from 40 to 700 amu at a rate of 5 scans/s.

Laying hens housing and treated egg for hatch

The study involved 15 male and 150 laying hens of Bahieg and Matrouh types, aged 32 Wks. housed in birdcages and managed below consistent procedures throughout. Artificial insemination was performed twice a week, and the hens were fed a diet covering 17% crude protein and 2950 Kcal metabolizable energy per kilogram. Eggs were collected from the cages at nine am and four pm daily. Shells with visible cracks and facial contamination were observed. The eggs were kept for three days at 16-18 °C and 76% RH before the trial began. The revision used a whole of 4000 eggs, with 2000 eggs from each strain in a 2x4 factorial plan test. Each breed's eggs were separated into 4 groups of 500 eggs both, further subdivided into three replicates. The 1st group was a control with no treatment, the 2nd group was treated with propyl alcohol (125 ml per liter), the 3rd group with a solution of *Ocimum Basilicum* essential oil (125 ml alcohol + 0.2 m Oregano oil per liter), and the 4th group with formaldehyde gas (3X strength: 42.3 ml formalin + 21.15 g potassium permanganate per cubic meter). Tripartite strength formaldehyde fume was generated in the setter at apartment temperature (16°C) and 89% RH for 20 min. Disinfectants were applied to all eggs using a hand sprayer until completely wet, followed by air drying at room temperature (24°C) for 30 minutes. The treated eggs, known as hatching egg sanitizers (HES), were then randomly placed in the incubator. The eggs were incubated in hatchery at 99.7°F and 55% virtual moisture in the setter till the 18th d, then transferred to the hatchery and maintained at 98.7°F and 76% relative damp. Eggs were amount to and balanced to the adjacent 0.1 gram before being placed in the incubator. The platters were casually positioned inside the incubator. The eggs were reweighed on the 24th and 36th times, and on the 7th day of incubation to determine weight loss (%). Hatching (%) was calculated by way of the ratio of hatched chicks to over-all fertile eggs. Each hatched chick was numbered and raised until 8 weeks old. BW was measured at 1st d, 4 and 8 weeks of stage, along with feed intake and death data. For the bacterial inactivation examine, 18 eggs were sited on malleable egg rooms. Shell-associated microorganisms were recovered using a total egg laundry technique to estimate full bacteria amount, Coliforms, and fungi and mold totals from 3 eggs/ treatment (Baheig type only). Dilutions (10^{-3}) were inoculated into sterile petri dishes and cultured at 37°C for 48 hrs. Colony counts were examined by nutrient agar, violet red bile agar, and fungi and mold potato dextrose agar (oxoid), and expressed as cfu/mL (Damena *et al*., 2022).

Statistical examination

The experiment data were analyzed by SAS program (2000). % data were changed, and microbial amounts were converted to log10 before numerical study. Significant differences in means were determined using Duncan, test (1955) through a impact level of $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Phytochemicals of the Basil leaf extracts:

The methanol extract of *O. basilicum* was analyzed using GC-MS, revealing thirty-four main points. The constituents consistent to these peaks were identified, as shown in Table 1. The first highest was identified as β-Cyclocitral, and the second top as 2-Propenoic acid, 3-phenyl-, methyl ester, (E)-. Subsequent peaks were determined to be á-opaene, à-ylangene, alfa.-Copaene, Bicyclo[4.1.0]-3-heptene,2-isopropenyl-5-isopropyl-7,7-dimethyl-, Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, á-ylangene, á-copaene, and Dihydrofuranno(3,2-f) coumaran (Figures 3 to 10).

Table 1. Chemical constituents in Basil leaf extracts recognized by gas chromatography/ figure spectrometry investigation.

Peaks	Compounds	RT (min)	Peak Area (%)	Molecular Formula
1	β-Cyclocitral	5.6	6.64	C10H16O
2	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	9.26	2.22	C10H10O2
3	á-copaene	10.29	0.64	C15H24
4	à-ylangene	10.29	0.64	C15H24
5	.alfa.-Copaene	10.29	0.64	C15H24
6	Bicyclo[4.1.0]-3-heptene,2-isopropenyl-5-isopropyl-7,7-dimethyl-	10.29	0.64	C15H24
7	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	10.67	0.57	C15H24
8	á-ylangene	11.17	8.63	C15H24
9	á-copaene	11.17	8.63	C15H24
10	Dihydrofuranno(3,2-f)coumaran	11.17	8.63	C15H24
11	2-Propenoic acid, 3-phenyl-, methylester	11.58	1.07	C10H10O2
12	1,3-Bis(cinnamoyloxymethyl)adamantane	11.87	0.96	C30H32O4
13	Germacrene D	12.45	8.10	C15H24
14	Guaia-1(10),11-diene	12.93	1.25	C15H24
15	2-(3-Isopropyl-4-methyl-pent-3-en-1 -ynyl)-2-methyl-cyclobutanone	13.33	0.51	C14H20O
16	Neophytadiene	19.12	2.81	C20H38
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.12	2.81	C20H40O
18	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	19.54	0.52	C20H40O2
19	17-Octadecynoic acid	19.54	0.52	C18H32O2
20	13-Heptadecyn-1-ol	19.84	0.80	C17H32O
21	Z,Z-3,15-Octadecadien-1-ol acetate	19.84	0.80	C20H36O2
22	Hexadecanoic acid, methyl ester	20.65	2.42	C17H34O2
23	l-(+)-Ascorbic acid 2,6-dihexadecanoate	21.73	1.56	C38H68O8
24	7,10-Octadecadienoic acid, methylester	23.26	1.97	C19H34O2
25	Methyl 9-cis,11-trans-octadecadienoate	23.26	1.97	C19H34O2
26	Phytol	23.59	7.87	C20H40O
27	Heptadecanoic acid, 16-methyl-, methyl ester	23.78	0.69	C19H38O2
28	Heptadecanoic acid, 9-methyl-, methyl ester	23.78	0.69	C19H38O2
29	trans-13-Octadecenoic acid	24.35	4.97	C18H34O2
30	cis-Vaccenic acid	24.35	4.97	C18H34O2
31	Azuleno[4,5-b]furan-2(3H)-one, 9a-[(acetyloxy)methyl]decahydro-6a,9 -dihydroxy-6-methyl-3-methylene-, [3aS-(3aà,6á,6aà,9á,9aá,9bà)]-	26.65	1.33	C17H24O6
32	Benzo[e][1,3]-thiazine-2-one, 8a-cyano-4,4-pentamethylene-perhydro-	26.65	1.33	C14H20N2OS
33	Alanine, N-acetyl-3-phenyl-N-trifluoroacetyl) -, methyl ester, L-	26.78	1.07	C14H14F3NO4
34	Benzo[e][1,3]-thiazine-2-one,8a-cyano-4,4-pentamethylene-perhydro-	26.78	1.07	C14H20N2OS

RT = retention time.

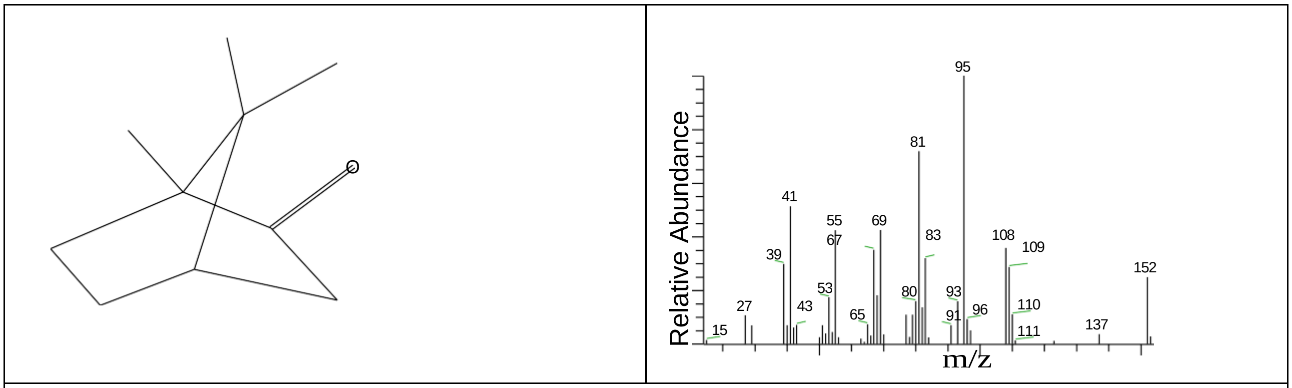


Figure 1. Construction of Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- with 5.6 (RT) present in *Ocimum basilicum*.

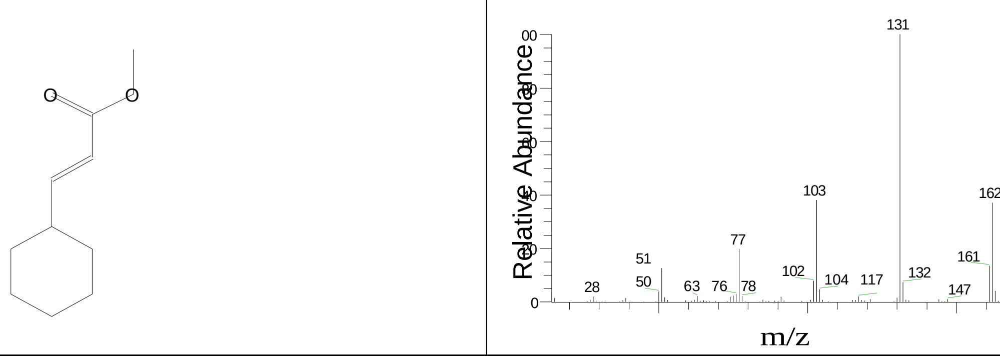


Figure 2. Structure of Cinnamic acid, methyl ester, (E))- with 9.26 (RT) present in *Ocimum basilicum*.

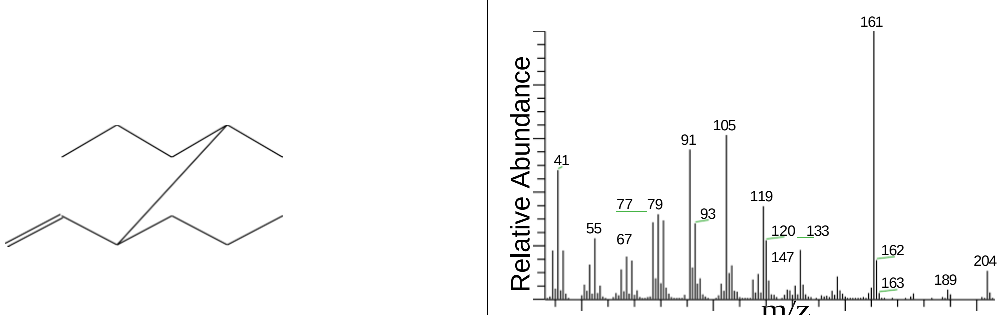


Figure 3. Assembly of á-copaene)- with 10.29 (RT) present in *Ocimum basilicum*.

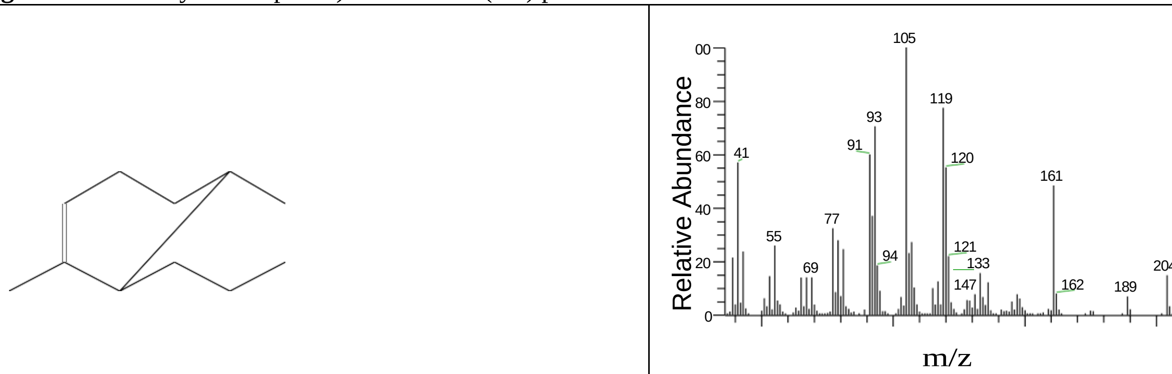
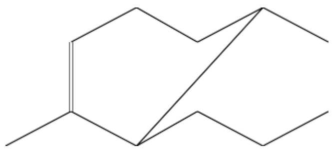


Figure 4. Construction of à-ylangene)- with 10.29 (RT) present in *Ocimum basilicum*.



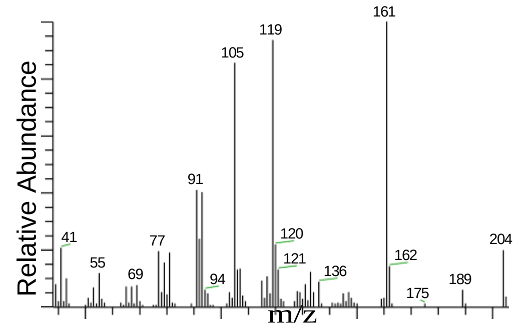
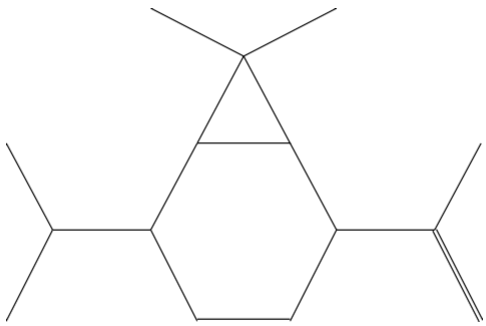


Figure 5. Structure of alfa.-Copaene)- with10.29 (RT) present in *Ocimum basilicum*.



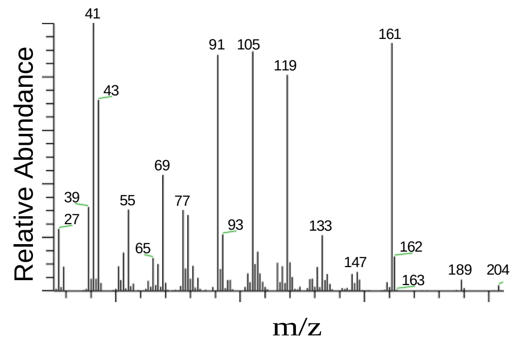
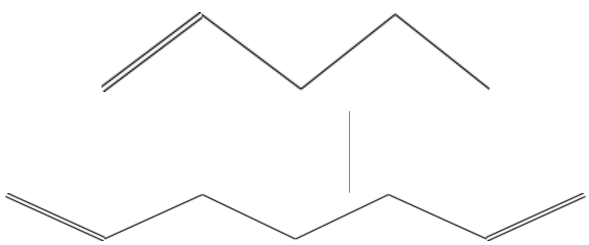


Figure 6. Structure of Bicyclo[4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-)- with 10.29 (RT) present in *Ocimum basilicum*.



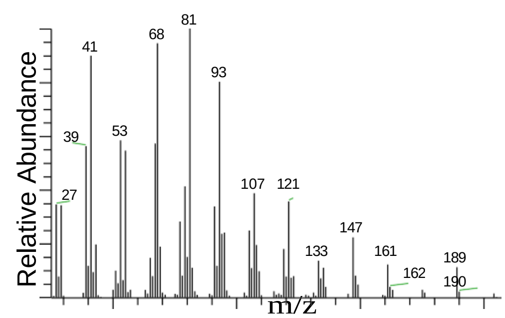


Figure 7. Structure of Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-)- with 10.67 (RT) present in *Ocimum basilicum*.

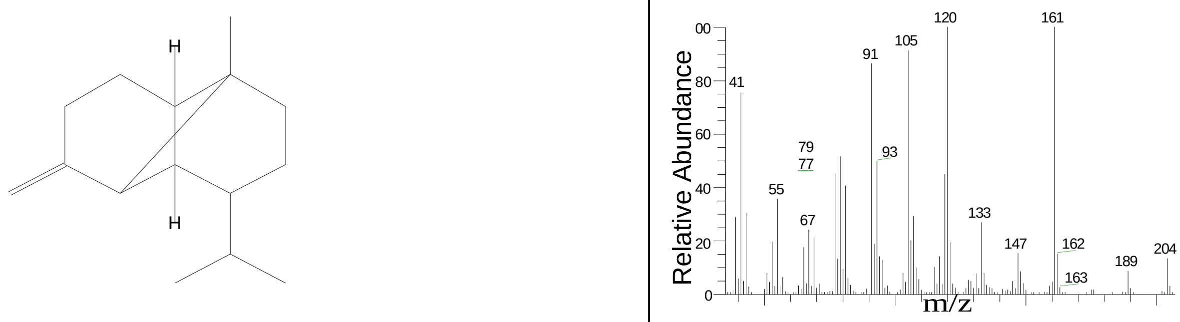


Figure 8. Structure of á-ylangene)- with 11.17 (RT) present in *Ocimum basilicum*.

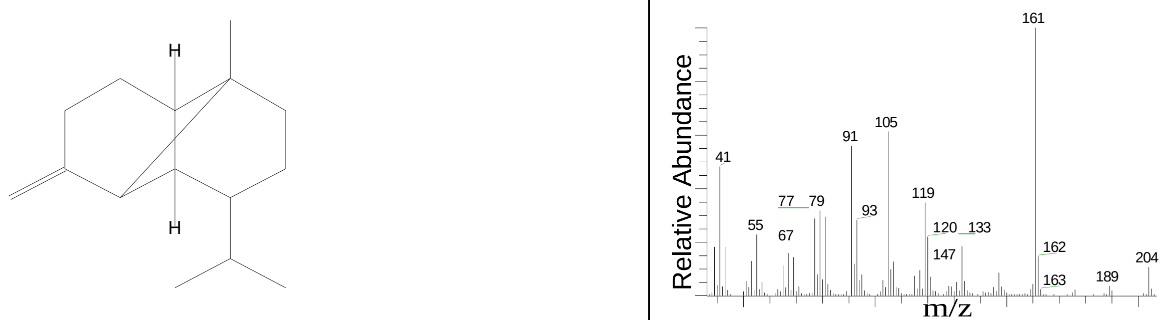


Figure 9. Structure of á-copaene)- with 11.17 (RT) present in *Ocimum basilicum*.

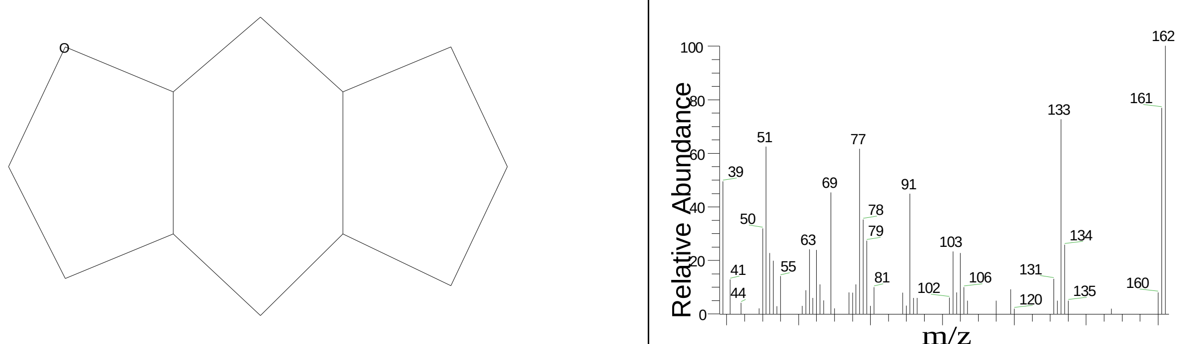


Figure 10. Structure of Dihydrofuranno(3,2-f)coumaran)- with 11.17 (RT) present in *Ocimum basilicum*.

Table 2 shows the impact of various sanitizers (Formalin, alcohol, Basil leaf extract) and their interactions on egg weight loss percentages at different time points (24 hours, 36 hours, and 7th day) during incubation for two types of laying hens (Baheig and Matrouh local strains). Regardless of the sanitizer used, the results indicate that egg weight loss was not influenced by the type of laying hens at the specified time intervals. Iqbal *et al*. (2014) found that eggs from larger hens had the lowest weight loss ($P<0.05$) during incubation, and weight loss decreased with increasing breeder hen age. Compared to other disinfectants and fumigation methods, spraying eggs with 14% propolis and 0.5-0.7% thyme oil significantly reduced egg weight loss during the setting phase (Shahein and Sedeek, 2014).

Table 2: Impact of strain and hatching egg sanitizers on egg weight loss (%) through incubation times.

Items	Egg loss (%)		
	24 hours	**36 hours**	**7 days**
Influence of type			
Baheig	0.722	0.917	3.314
Matrouh	0.936	1.130	3.123
SEM	0.081	0.10	0.049
P-value	0.057	0.091	0.061
Effect of treat			
Control	1.144	1.279	3.156
Formalin	0.777	0.993	3.093
Alcohol	1.059	1.249	3.464
Basil leaf	0.656	0.950	3.282
SEM	0.151	0.123	0.052
P-value	0.145	0.078	0.059
Influence of the interaction of type×Treat			
SEM	0.177	0.185	0.086
P- value	0.132	0.201	0.067

Significant at (P>0.05).

When the effect of types of laying hens was over looked, the results indicated that egg weight loss percentages at different time points (24 hours, 36 hours, and 7th day) during incubation were (P ≥0.05) affected by the type of sanitizers (Formalin, alcohol, Basil leaf extract). Oliveira *et al.* (2023) also found that essential oil sanitizers effectively condense microbial contamination on shells of egg. Li *et al*. (2018) initiate that treating eggs with (Inovapure TM) (LP) at 1.5% or 3.0% concentrations in distilled water, or a quaternary ammonium at 0.125% for 10 minutes, did not affect shell conductance or the eggshell conductance continuous. According to Sheldon and Brake (1991), the cuticle is an organic layer that is primarily made of protein but also contains some polysaccharides and lipids. Studies suggest that the cuticle helps protect eggs from microbial invasion and contamination, affecting shell permeability, (Bagley *et al*., 1988). Variations in conductance persistent and eggshell perviousness can affect the timing of the plateau stage in oxygen consumption during metabolic development, leading to changes in vital gas exchange. Using sanitisers can change how embryos grow and how easily eggshells can be broken. Weight loss of eggs at 24 hours, 36 hours, and 7 days was not impacted by treatment groups.

Table 2 displays the interaction between breed types and sanitizers (Formalin, alcohol, Basil leaf extract) on egg weight loss percentages at different time points (24 hours, 36 hours, and 7 days) during incubation. The results suggest that the interaction between breed types and sanitizers (Formalin, alcohol, Basil leaf extract) did not affect egg weight loss.

Table 3 shows the impact of breed, Basil leaf extract disinfectant, and formaldehyde fumigation on hatchability percentages of Baheig and Matrouh eggs. Breed did not significantly affect hatchability percentages of fertile eggs. Hatchability percentages varied significantly between eggs treated with basil leaves extract and those treated with fumigation with formaldehyde or propyl alcohol, regardless of breed. Basil leaf extract had a significant impact on various parameters including infertile eggs, primary and late embryonic mortality, un-hatched eggs, and hatchability percentages (P≤0.0001). The results align with Oliveira *et al*.'s studies (2023, 2022a), showing upper premature embryonic mortality in alcohol and formaldehyde groups compared to herb plant oil and control groups (P < 0.05). The Basil leaf extract group had lower total embryo mortality than the formaldehyde fumigation group (P< 0.05). Oliveira *et al*. (2021a) reported no differences in hatch rates or total embryo mortality between essential oil and formaldehyde sterilization related to the regulator group. In contrast to formaldehyde treat, Oliveira *et al*. (2022b) discovered that oregano oil disinfection enhanced first and late embryonic humanities while decreasing middle embryonic mortality and the rejected chick rate. Formaldehyde fumigation in the study was found to be toxic to the embryo, particularly in the early stages of incubation. This toxicity led to a higher rate of early embryonic mortality, ultimately increasing total embryo mortality. As a result, the hatchability of fertile eggs in the formaldehyde group was lower likened to the basil oil group. There was no substantial interaction effect between the two treatments on IE, EEM, LEM, Un-HE, and H (%). Enhanced hatchability might be attributed to reduced microbial infection of the eggs.

Hatching egg sterilization can help reduce contamination on eggshells, but it is not the only solution. It is important to focus on producing microbial-free eggs to avoid the need for disinfection. This can lead to cleaner and healthier chicks. The quality of the embryo and chick is unaffected by applying clove essential oil to hatching eggs, (Oliveira *et al* 2021b). Higher embryonic mortality was observed in eggs sanitary with ethyl and untreated groups, while lower rates were seen in eggs disinfected with 7% and 14% propolis concentrations and formaldehyde fumigation (Shahein and Sedeek 2014).

Table 3: Impact of breed and hatching egg sanitizers on embryonic mortality stages and hatchability percentages.

Items	IE %	EEM %	LEM %	Un-HE %	H %
Influence of type					
Baheig	5.249	2.888	1.905	3.417	91.8
Matrouh	4.885	3.075	2.186	3.945	90.8
SEM	0.026	0.030	0.025	0.56	0.206
P-value	0.145	0.066	0.071	0.059	0.063
Influence of treat					
Control	6.735^{a}	5.155^{a}	2.657^{ab}	5.719^{a}	86.5^{c}
Formalin	4.476^{b}	3.796^{b}	2.887^{a}	3.847^{b}	89.5^{b}
Alcohol	6.855^{a}	4.526^{ab}	2.038^{bc}	6.348^{a}	87.1^{bc}
Basil leaf	4.418^{b}	1.806^{c}	1.695^{c}	2.087^{c}	94.4^{a}
SEM	0.034	0.040	0.027	0.73	0.266
P-value	0.0001	0.0001	0.01	0.0001	0.0001
Effect of the interaction of type×Treat					
SEM	0.059	0.069	0.047	1.290	0.461
P-value	0.099	0.106	0.132	0.141	0.059

Significant differences (P<0.05) are denoted by different superscripts (a, b, c) within the same column. IE= in-fertility Egg; EEM= Early embryonic mortality; LEM= Late embryonic mortality; Un-HE= Un-hatched egg and H= Hatchability (%).

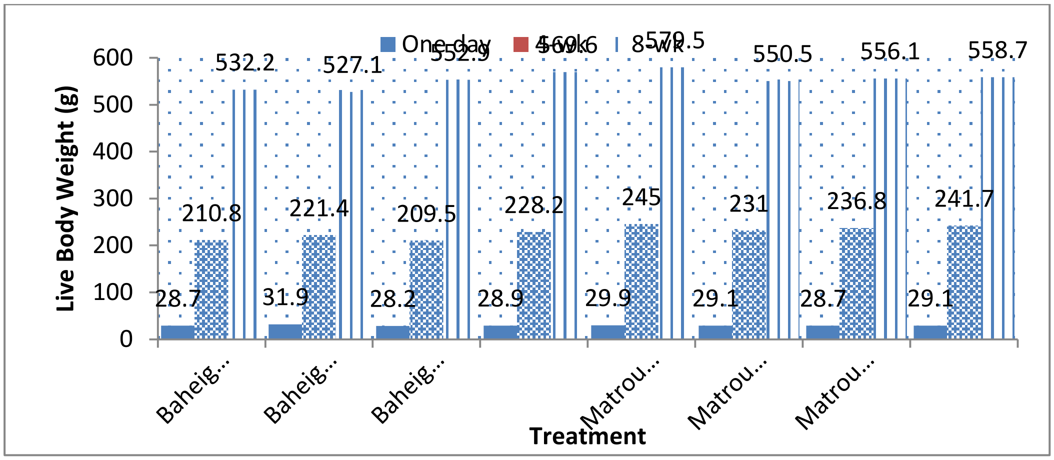
Table 4 offerings the consequences of the effects of changed breeds and types of Basil leaf extract disinfectant in comparison to Formaldehyde Fumigation on the performance of Baheig and Matrouh chicks, as well as their interactions. The performance of chicks posthatching indicated that the Basil leaf extract groups achieved the most favorable outcomes in terms of LBW and FCR at 8 weeks of age.

Table 4: Impact of strain and (HES) on chick performance at various ages

Item	LBW (g)			FCR		M %
	One day	4Wk	8Wk	0-4Wks	0-8Wk	
Effect of Type						
Baheig	30.21	228.3[b]	569.1[b]	2.267[a]	2.159[a]	7.88[a]
Matrouh	30.19	251.7[a]	583.8[a]	2.098[b]	2.177[b]	6.87[b]
SEM	0.049	3.21	5.399	0.036	0.662	0.029
P-value	0.082	0.011	0.031	0.044	0.043	0.049
Effect of treat						
Control	30.2[ab]	227.9[b]	559.9[b]	2.209[ab]	2.266[b]	8.56[b]
Formalin	32.5[a]	226.2[b]	568.8[b]	2.318[a]	2.337[a]	9.78[a]
Alcohol	30.5[b]	223.1[b]	569.5[b]	2.319[a]	2.228[b]	8.09[b]
Basil leaf	30.9[b]	234.9[a]	583.1[a]	2.017[b]	2.152[c]	6.27[c]
SEM	0.066	4.100	6.940	0.055	0.773	0.039
P-value	0.001	0.01	0.002	0.001	0.003	0.005
Effect of Type×Treatment						
SEM	0.104	7.166	12.03	0.09	1.29	0.07
P-value	0.05	0.001	0.0001	0.005	0.005	0.0001

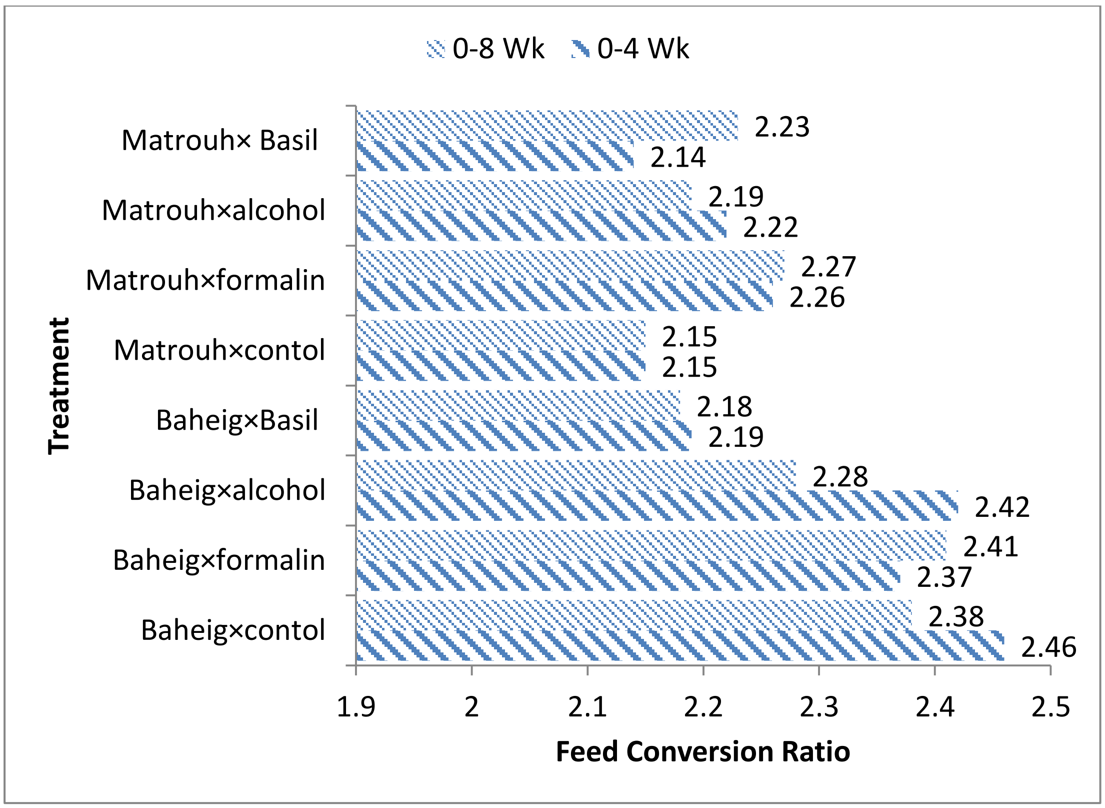
[a,b,c] Changed superscripts in the identical column direct substantial changes ($P < 0.05$) among mean values. M%=Mortality (%)

An interaction was observed among the studied traits for LBW and FCR, as shown in Figures 11 and 12. The groups treated with Basil leaf extract at 4 and 8 weeks exhibited the most favorable feed conversion ratio values for Baheig and Matrouh chicks.



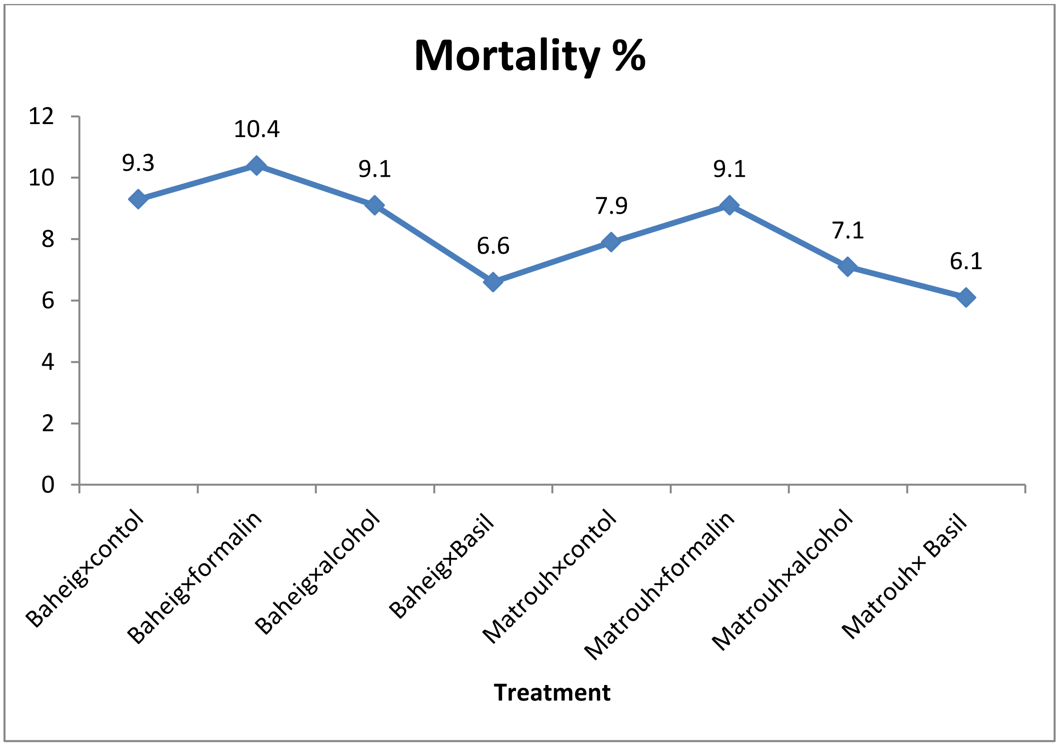
- **Figure 11: An interaction between the studied experiments was pragmatic for LBW**

Limited research has focused on the effect of disinfection processes on poultry performance. Li *et al*. (2018) and Fasenko *et al*. (2009) initiate that treating hatching eggs through electrolysed oxidising water did not affect BW after a 39-day growth phase. Ulucay and Yildirim (2010) concluded that thymol, carvacrol or cinnameldehyde were as effective disinfectants for hatching eggs as conventionally used quaternary ammonium. Chicks treated with 14% propolis had the highest body weight at hatch and pull-out associated to other egg treatments (Shahein and Sedeek 2014). In a study by Copur *et al*. (2010), the use of formaldehyde and oregano oil as hatching egg sanitizers did not have any significant impact on broiler BW or BWG throughout the production period. Using hydrogen peroxide as a hatchery sanitiser had no effect on broiler liveability, BW or FCR (Cox *et al*., 2000). Nevertheless, it had no effect on the incidence of residual yolk sac in chicks that were 42 days old. Çabuk *et al*. (2006) found that an herbal essential oil mixture had no substantial influence on body weight at 21 and 42 d in pullets from young (30 Wks or 80 weeks old) breeder groups, but did improve feed conversion ratio. Mortality was affected by basil leaf extract treatments, leading to decreased mortality over the entire experimental period Figure 13. The study indicates that Basil leaf extract can serve as a natural disinfectant for hatching eggs. Copur *et al*. (2010) tested oregano essential oil as a decontaminator on hatching eggs and observed significant things on BW, BWG and FI without notable differences between treatments.



- **Figure 12: An interaction between the treats was observed for Feed conversion ratio**

The data presented in Table 5 shows that the groups treated with basil leaf extracts (Ocimum basilicum L.) and formaldehyde fumigation exhibited a significant reduction in total bacterial population to 10.6×10^3 and in fungi amounts to 4.95×10^3, respectively, compared to the control group (52×10^3 for total bacteria and 14.3×10^3 for fungi counts). Coliform totals were only detected in the regulator treat (4.7×10^3).



\- **Figure 13: Mortality Percent**

Table 5: Antimicrobial commotion (cfu/egg)*10^3 HES on the micro flora of hatching eggs (geometric means log10, n = 3)

Items	Fungi	Coliforms	Total Bacteria
Control	14.3×10^3	4.67×10^3	52×10^3
Formalin	5×10^3	0	24×10^3
Alcohol	11×10^3	0	45×10^3
Basil leaf extracts	4.95×10^3	0	10.60×10^3

The high total bacteria population in the control and alcohol groups may have contributed to the low hatchability of eggs, while the formaldehyde fumigation group showed an increase in early and late embryonic mortality percentages, potentially affecting hatchability. *Ocimum basilicum* extracts exhibit antimicrobial effects by interacting with microbial cell membranes through their phytochemical assets and molecular forms, affecting enzymes, transporters, ion channels, and receptors. Previous research has shown that treating hatching eggshells with 70% ethyl alcohol significantly reduces the total bacterial amount over time. The total decreased from 24.16 to 8.30 X 10^3 cfu/egg afterward 1 and 4 d of packing, respectively (Shahein and Sedeek, 2014). Sun *et al*. (2021) evaluated the antibacterial properties of oregano essential oil on eggshell surfaces using an emulsion based on chitosan and including beeswax and basil essential oil. Arhienbuwa *et al*. (1980) found that quaternary ammonium-treated eggs had lower microorganism levels compared to formaldehyde-treated eggs, turkey eggs treated with formaldehyde or a quaternary ammonium solution still contained sizable bacterial populations, (Sacco *et al* 1989). Bekhet and Khalifa (2022) summarized that Oregano and Cumin oils act as effective disinfectant against microbes on the eggshell by significantly diminishing the bacterial count, increased egg weight, decreased weight loss percentage, increased hatchability.

CONCLUSION

These effects of this study advise that spraying basil leaf extracts and formaldehyde fumigation on disinfecting Baheig and Matrouh hatching eggshells able to maintain hatching results and laying hens performance.

REFERENCES

Adame, M.M. (2023): Review on Egg Handling and Management of Incubation and Hatchery Environment. Asian Journal of Biological Science. Vol 16(4): 474-484.

Al-Shammari, K.I.A. , Batkowska, J. , Gryzińska, M., Wlazło, Ł., Ossowski, M., Dębek, B.N., (2022): The use of selected herbal preparations for the disinfection of Japanese quail hatching eggs, Poultry Science, Volume 101, Issue 10,https://doi.org/10.1016/j.psj.2022.102066.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A. (2017): Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants (Basel). 2017 Sep 22;6(4):42. doi: 10.3390/plants6040042. PMID: 28937585; PMCID: PMC5750618.

Arhienbuwa, F.E., Adler, E. and Wiggins, A.D. (1980): A method of surveillance for bacteria on the shell of turkey eggs. Poult. Sci. 59:28-33.

Bagley, L.G.; Christensen, V.L.; and Bagley, R.A. (1988). Effect of altering eggshell permeability on the hatchability of turkey eggs at high altitude. Poultry Sci. 69: 451-456.

Bilalissi, A., Meteyake, H.T, Kouame, Y.A.E., Oke, O.E., Lin, H., Onagbesan, O., Decuypere, E. and Tona, K. (2022): Effects of pre-incubation storage duration and nonventilation incubation procedure on embryonic physiology and post-hatch chick performance. Poult Sci. 2022 May;101(5):101810. doi: 10.1016/j.psj.2022.101810.

Çabuk, M.; Bozkurt, M.; Alçiçek, A.; Akbaş, Y.; And Küçükyılmaz, K. (2006). Effect of an herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. South African Journal of Animal Science 36 (2): 135-141.

Chen L, Wang S, Feng Y, Zhang J, Du Y, Zhang J, Ongeval CV, Ni Y, Li Y. (2021): Utilisation of Chick Embryo Chorioallantoic Membrane as a Model Platform for Imaging-Navigated Biomedical Research. Cells. 2021 Feb 22;10(2):463. doi: 10.3390/cells10020463. PMID: 33671534; PMCID: PMC7926796.

Chouhan S, Sharma K, Guleria S. (2017): Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel). 2017 Aug 8;4(3):58. doi: 10.3390/medicines4030058.

Christensen, V. L. (1995). Factors affecting hatchability of turkey embryos. Poult. Avian Biol. Rev. 6 :71-82.

Copur, G.; Arslan, M .;Duru, M.; Baylan, M.; Canogullari, S. and Aksan, E. (2010). Use of oregano (Origanum onites L.) essential oil as hatching egg disinfectant. African Journal of Biotechnology Vol. 8 (17), pp. 2531-2538.

Cox,N. A.; Berrang, M. E.; Buhr, R.J. and Bailey, J. S. (2000). Bacterdical treatment of hatching egg IV. Hydrogen peroxide applied with vacuum and a surfactant to eliminate salmonella from hatching eggs.J. Appl. Poult. Res.9: 530-534.

Damena, A., Mikru, A., Adane, M. and Dobo, B. (2022): Microbial Profile and Safety of Chicken Eggs from a Poultry Farm and Small-Scale Vendors in Hawassa, Southern Ethiopia. Journal of Food Quality Volume 2022, Article ID 7483253, 16 pages https://doi.org/10.1155/2022/7483253 de Sousa, D.P.; Damasceno, R.O.S.; Amorati, R.; Elshabrawy, H.A.; de Castro, R.D.; Bezerra, D.P.; Nunes, V.R.V.; Gomes, R.C.; Lima, T.C. (2013): Essential Oils: Chemistry and Pharmacological Activities. Biomolecules 2023, 13,1144. https://doi.org/10.3390/biom13071144.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics 11 : 1-42.

Fasenko, G. M., Christopher, E. O. D. and M. McMullen. L. (2009): Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters. Poult. Sci. 88:1121–1127.

Bekheta, G. and Khalifaa, A.Y. Z. (2022): Essential oil sanitizers to sanitize hatching eggs. Journal Of Applied Animal Research, Vol. 50, No. 1, 695–701 https://doi.org/10.1080/09712119.2022.2138894.

Iqbal, J., Khan, S. H., Mukhtar, N., Ahmed, T. and Pasha, R. A. (2014): Effects of egg size (weight) and age on hatching performance and chick quality of broiler breeder. Journal of Applied Animal Research, 44(1), 54–64. https://doi.org/10.1080/09712119.2014.987294.

Kadhim, M.J., Sosa, A.A. and I.H. Hameed (2016): Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. Journal of Pharmacognosy and Phytotherapy. Vol. 8(6), pp. 127-146, June 2016

Krishnan, K. R., Babuskin, S. , P. Azhagu Saravana Babu, M. Sasikala, K. Sabina, G. Archana, M. Sivarajan, M. Sukumar, (2013): Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat, International Journal of Food Microbiology, Volume,171,Pages:32-40. https://doi.org/10.1016/j.ijfoodmicro.2013.11.011.

Li, X., Anderson, D., Rathgeber, B., McLean, N. and MacIsaac, J. (2018): Fumigating broiler hatching eggs with lysozyme product (Inovapure) to reduce eggshell microbial load, 2018 Poultry Science 97:4252–4261, http://dx.doi.org/10.3382/ps/pey288

Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB.(2017): Antibacterial and Antifungal Activities of Spices. Int J Mol Sci. 2017 Jun 16;18(6):1283. doi: 10.3390/ijms18061283. PMID: 28621716; PMCID: PMC5486105.

Oliveira G.d.S., dos Santos V.M., Nascimento S.T. (2021a) Essential Oils as Sanitisers for Hatching Eggs. Worlds Poult. Sci. J. 2021;77:605–617. doi: 10.1080/00439339.2021.1959276.

Oliveira G.d.S., McManus C., dos Santos V.M. (2022b): Garlic as Active Principle of Sanitiser for Hatching Eggs. Worlds Poult. Sci. J. 2022;78:1037–1052. doi: 10.1080/00439339.2022.2105275.

Oliveira G.d.S., McManus C., Salgado C.B., dos Santos V.M. (2022a): Effects of Sanitizers on Microbiological Control of Hatching Eggshells and Poultry Health during Embryogenesis and Early Stages after Hatching in the Last Decade. Animals. 2022;12:2826. doi: 10.3390/ani12202826.

Oliveira G.S., Nascimento S.T., Dos Santos V.M., Dallago B.S.L.(2021b) Spraying Hatching Eggs with Clove Essential Oil Does Not Compromise the Quality of Embryos and One-Day-Old Chicks or Broiler Performance. Animals. 2021; 11:2045. doi: 10.3390/ani11072045.

Oliveira, G.d.S.; McManus, C.; de Araújo, M.V.; de Sousa, D.E.R.; de Macêdo, I.L.; Castro, M.B.d.; Santos, V.M.d. (2023): Sanitizing Hatching Eggs with Essential Oils: Avian and Microbiological Safety. Microorganisms 2023, 11, 1890. https://doi.org/10.3390/microorganisms11081890.

Pees, M.; Motola, G.; Brüggemann-Schwarze, S.; Bachmeier, J.; Hafez, H.M.; Tebrün, W. (2023): Impact on Hatchability and Broiler Performance after Use of Hydrogen Peroxide Nebulization versus Formaldehyde Fumigation as Pre-Incubation Hatching Egg Disinfectants in Field Trial. Poultry 2023, 2, 1-11. https://doi.org/10.3390/poultry2010001

Rebolledo, F.P.O., Casillas, C.G.A., Isaías, T.G., Rivera, A.H.J. (2023): Natural Products as an Alternative to Formaldehyde for Disinfection of Fertile Eggs in Commercial Hatcheries [Internet]. Poultry Farming - New Perspectives and Applications. IntechOpen; 2023. Available from: http://dx.doi.org/10.5772/intechopen.112568.

Rodríguez-Navarro, A. B., Domínguez-Gasca, N., Muñoz, A. and Ortega-Huertas, M. (2013): Change in the chicken eggshell cuticle with hen age and egg freshness. Poultry Science, 92(11), 3026-3035. https://doi.org/10.3382/ps.2013-03230.

Sacco, R.E.; Prener, P.A.; Nestor, K.E.; Saif, Y.M.; and Dearth, R.N. (1989). Effect of hatching egg sanitizers on embryonic survival and hatchability of turkey eggs from different lines and on egg shell bacterial populations. Poultry Sci. 68: 1179- 1184.

SAS (2000). User's Guide: Statistics, Version 8.1. SAS Institute Inc. Cary, NC.

Shahein, E.H.A. and Sedeek, E.K. (2014): Role Of Spraying Hatching Eggs With Natural Disinfectants On Hatching Characteristics And Eggshell Bacterial Counts. Egyptian Poultry Science Journal, Vol (34) (I): (213-230).

Sheldon, B.W.; and Brake, J. (1991). Hydrogen peroxide as an alternative hatching egg disinfectant. Poultry Sci. 70 : 1092- 1098.

Solt, P. , Konnerth, J., Altmutter, W.G., Kantner, W., Moser, J. , Mitter, R. and van Herwijnen, H.W.G. (2019): Technological performance of formaldehyde-free adhesive alternatives for particleboard industry, International Journal of Adhesion and Adhesives, Volume 94, 2019, Pages 99-131.doi.org/10.1016/j.ijadhadh.2019.04.007.

Sun, R., Song, G., Zhang, H., Zhang, H., Chi, Y., Ma, Y., Li, H., Bai, S., & Zhang, X. (2021): Effect of basil essential oil and beeswax incorporation on the physical, structural, and antibacterial properties of chitosan emulsion based coating for eggs preservation. LWT, 150, 112020. https://doi.org/10.1016/j.lwt.2021.112020.

Ulucay, I.O. and Yildirim, I. (2010): Hatching Traits of Quail (Coturnix coturnix japonica) Eggs Disinfected with Carvacrol, Cinnamaldehyde or Thymol. J. Appl. him. Res. 38 (2010) : 139-142.

Whistler, P.E.; and Sheldon, B.W. (1989). Comparison of Ozone and Formaldehyde as poultry hatchery disinfectants as an alternative hatching egg disinfectant. Poultry Sci. 68 : 1345-1350.