Influence of Olive Leaves Extract as a Disinfectant for Hatching Eggs on Eggshell Contamination, Hatching Performance and Post-Hatch Productive and Immune Indices in Chickens

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***Abstract*–Several published reports have demonstrated the problems associated with the use of formaldehyde as a disinfectant for incubated eggs. Therefore, the aim of this experiment was to explore the efficient power of olive leaves (OL) as a safe alternative disinfectant to formaldehyde on the bacterial contamination of fertile eggs, hatching parameters, and productive and immune indices of hatchlings up to 35 days. A total of 288 eggs were used and distributed equally into four treatment groups, three replicates each, as follows: group 1 (T1) was a control, group 2 (T2) was eggs disinfected with methyl alcohol, while group 3 (T3) and group 4 (T4) were including eggs disinfected with aqueous and alcoholic extracts of OL, respectively. Compared with T1, the results exhibited that T4 reduced bacterial contamination of eggshells and shortened incubation and hatching times (P≤0.01). All groups did not differ significantly among them in terms of hatchability, chick physical characteristics, deformed chicks and total moisture loss from fertile eggs.T4 achieved superiority in dressing percentage and decreased mortality at post-hatch (P≤0.01) while T2 and T3 consumed less feed intake (P≤0.05). Moreover,T3 and T4 activated antibodies titers against Newcastle and Gumboro diseases, respectively in blood serum of chicks (P≤0.05) compared to T1. Therefore, the extract of OL, particularly the alcoholic extract, appears to be an effective natural disinfectant for hatching eggs, and** has the potential to be a safe alternative to formaldehyde disinfection

Keywords*– *olive leaves; hatching, contamination, disinfection; fertile eggs

INTRODUCTION

Bacterial contamination of hatching eggs is considered as one of the major challenges faced by poultry hatchery which negatively impacts hatchability rates and chick growth performance after hatching. Therefore, adhering to cleanliness and sterilization standards within the hatchery is crucial to maintain egg safety and reduce risk of egg shell contamination (1, 2). Contamination of hatching eggs is primarily caused by harmful microorganisms which could occur *via* two main routes, horizontal and vertical contaminations (3,4). Vertical contamination occurs when hen develops infections in her own reproductive tract, which leads to the transfer of pathogens to the eggs before ovulation or immediately after oviposition. Horizontal contamination occurs due to unfavorable conditions on farm or in hatchery, such as the use of contaminated water or exposure to polluted air, as well as unsanitary egg handling practices that allow contaminants to be transferred directly to the fertilized eggs (5).

Traditional methods for disinfecting incubated eggs involve using formaldehyde gas due to its effectiveness as a strong disinfectant, low cost, and ability to eliminate multiple pathogenic microorganisms. However, this chemical substance has toxic effects, stimulating the risks on both humans and chicks because of its considered a carcinogenic potential (4) .Nowadays, researchers are still trying to avoid the use of formaldehyde and replace it with safer and more effective alternatives for egg disinfection, such as various plant extracts due to their antimicrobial properties.

Olive leaves (OL) are an effective type of plant waste used as feed additives in poultry diet, distinguished by their phenolic compounds that help eliminate harmful bacteria, fungi and viruses in addition to their importance in alleviating inflammatory conditions (6,7,8). Previous studies have revealed that OL or their principle compound (oleuropein), played an essential role in enhancing antioxidant levels in chicks that were fed these leaves during the embryonic stage by *in ovo* injection (9 ,10). OL have high antioxidant capacity and antimicrobial activity, due to their richness in biologically active compounds, including secoiridoids, simple phenols, phenylethanoids, hydroxycinnamic acid derivatives, and flavonoids, making them a promising source of natural

antioxidants and antimicrobials (9). Additionally, the phenolic extract of OL exerted a pivotal role in increasing live body weight and improving feed conversion ratio of broilers by mechanisms of reducing malondialdehyde levels in tissues and promoting growth of beneficial Lactobacillus bacteria, which correlates with a decrease in harmful E. coli bacteria in the gastrointestinal tract (11). OL also participate actively in strengthening the immune system of broiler chickens through various biological mechanisms, including stimulation of both humoral and cellular immunity, as well as enhancing antibody production by immune cells (12). While the antimicrobial and immunomodulatory properties of OL extract have been extensively studied in poultry nutrition, its use as a direct egg disinfectant remains insufficiently explored .

Therefore, this study aims to evaluate the efficacy of aqueous and alcoholic olive leaf extracts as natural and safe disinfectants for hatching eggs, focusing on eggshell microbial load, hatchability, immune parameters, and post-hatch performance of chicks up to 35 days of age.

MATERIALS AND METHODS

Preparation of the OL extract

OLpowder was obtained from local markets in Babylon Governorate, Iraq and is characterized by its homogeneous texture and distinctive aroma. The extracts were prepared according to methods of (13).Briefly, 5 grams of OL powder was dissolved in graduated glass cylinders containing either 100 ml of distilled water for the aqueous extract, or in 100 ml of 76% methyl alcohol for the alcoholic extract. The solutions were left at room temperature for three hours and then filtered using Whatman filter paper. The resulting filtrates were collected in glass beakers, covered with aluminum foil, sealed, and stored in a refrigerator at 4 °C until use in experiment.

Screening of bioactivecompounds in OL

In brief, the active compounds OL powder (Table 1) were analyzed using high-performance liquid chromatography (HPLC). The analyses included the determination the total phenolics, total flavonoids, total alkaloids, total terpenoids, total tannins, and total saponins using the method described by (14, 15, 16, 17, 18, 19), respectively.The essential oil content of OP was also determined to measure the concentration of oleuropein based on essential oil using the method described by (20).

Table 1: Content of bioactive compounds present in olive leaves.

Bioactive compound	Unit	Amount
Total Phenolics*	g /mg gallic acid	112.36
Total Flavonoids*	g/mg rutin	31.56
Total Alkaloids*	%	14.58
Total Terpenoids*	%	2.58
Total Tannins*	%	12.48
Total Saponins*	%	1.87
Oleuropein**	%	3.65

*Based on dry powder
**Based on essential oil.

Treatments of experiment

A total of 288 clean and fertilized eggs of broiler chickens Ross 308 were used in this experiment, with an average weight of 55.60 ± 2 g. The eggs were equally distributed into four experimental treatments, each treatment contains three replicates (24 eggs per replicate). The first group (T1) was disinfected with formaldehyde as a control group, while the second group (T2) was disinfected with methyl alcohol. The third (T3) and fourth (T4) groups were disinfected with aqueous extract and alcoholic extract of OI, respectively.

Application of eggs disinfection

Formaldehyde gas was used to disinfect eggs inside the incubator for control group. Formaldehyde was generated by mixing 60 ml of 40% liquid formalin with 30 ml of water and 48 grams of potassium permanganate (KMnO4) per cubic meter of incubator volume for 20 minutes at 37°C and 80% relative humidity (21). In the other treatments, eggs were disinfected using methyl alcohol and aqueous and alcoholic extracts of (OI) before being introduced into the incubator. A manual spraying method was used to disinfect all sides of egg, using a hand sprayer. After disinfection, the egg was allowed to air dry for five minutes and then placed in an incubation tray to complete the hatching process. (22,23).

Eggs incubation

The eggs were incubated in a commercial incubator. The temperature was set at 37.7°C and the relative humidity was between 60 - 65%, with automatic egg turning from day 1 until the end of day 18 of incubation. After this period, the eggs were transferred to a hatching chamber for the last three days, where the temperature was set at 37.0°C and the relative humidity was between 80–85%.

Birds rearing

Totally, 204 healthy chicks were selected and distributed into four experimental treatments after hatching, with 51 chicks per treatment, each with three replicates. All birds were reared in a controlled environment under standard conditions, and were provided with balanced diets till termination of the experiment at 35 days.

Studied traits

Determination of bacterial contamination on eggshell

Three eggs were randomly selected from each treatment before and after disinfection to estimate the eggshell bacterial load on the surface. Samples were placed in plastic containers, 30 ml of physiological saline was added, and then stored at 4°C. To determine the total bacterial count on the shell surface, 30 µL of the solution was withdrawn using a micropipette, and the number of growing bacteria was counted using the pouring technique, as described in (24). Next, 15 ml of nutrient agar medium was added to each plate, which was immediately prepared and stored in a water bath at 46°C. The number of colony-forming units (CFU/ml) in the original culturewas calculated by multiplying the number of resulting colonies by the dilution factor.

Hatching traits

Hatching traits including the hatchability on fertile eggs and total eggs, were calculated based on the number of chicks hatched from fertilized eggs and from total number of eggs, respectively. The percentages of periodical (1–13 days, 14–21 days) and total (1–21 days) embryonic mortality were determined by counting the number of dead embryos based on fertilized eggs and total eggs. At the end of hatching, unhatched eggs were broken and individually examined to determine the percentage of internally pipped eggs which was calculated from the total number of fertilized eggs. The percentages of pipped-alive and pipped-dead chicks were registered based on fertile eggs. The percentage of deformed chicks relative to the number of healthy hatched chicks was also taken. Incubation time for each replicate in treatment was calculated as the number of hours from the start of embryonic development to the completion of hatching. Hatching time was calculated by counting the number of hours from the hatching of the first chick to last one in each replicate (25,26).

Moisture loss from eggshell

The percentage of moisture loss per fertile egg within each replicate was calculated periodically (1-13 days and 14 -21 days) and totally (1-21 days). This was done by calculating the difference between the initial weight of the egg and its final egg weight.

Chick characteristics

Hatched chicks were weighed individually in each replicated group using a sensitive digital balance, and the relative weight of the chicks was calculated based on the average initial egg weight. To determine the chick weight without yolk and the percentage of remaining yolk weight to body weight at hatching, 2 chicks were randomly selected from each replicate (a total of 6 chicks per treatment) and euthanized for yolk sac extraction. Nine chicks from each treatment were also selected for measuring several morphological parameters, including body length (10), wing length (27), and leg length (28) using a ruler graduated in millimeters. In addition, total chick quality scores were evaluated according to established criteria of (29).

Productive traits

Important productive traits, such as body weight (BW), feed intake (FI), and mortality rate were recorded daily and presented cumulatively throughout the experimental period. From BW and FI values, the feed conversion ratio (FCR) was calculated. The production efficiency factor (PEF) was also recorded (30). At 35 days of age, two birds were selected from each replicate (totaling 6 birds per treatment), with a ratio of 2 males and 4 females. Birds were subjected to 10 hours fasting to empty their digestive tracts. Each bird was then weighed individually, slaughtered, and feathered. The dressing percentageswithout or with edible organs (heart, liver, and gizzard) were calculated.

Immunological traits

Six birds per treatment were selected at 35 days of age,and were slaughtered to extract the immune organs (bursa of Fabricius, thymus, and spleen). The weights of these organs were recorded and their ratio to live body weight was calculated. The bursa weight index was recorded using formula of (31). Besides, 3 ml of blood was collected from each bird and preserved in serum- separating tubes. The blood was then immediately centrifuged at 3000 RPM for 15 minutes to obtain serum. Antibody titers against viral diseases (Newcastle, Gumboro, infectious bronchitis, and avian influenza)in serum were determined using commercial test kits provided by Innovative Diagnostics (France).

STATISTICAL ANALYSIS

Statistical analysis was performed using (32). To determine the influence of different treatments on the studied traits, analysis of variance (ANOVA) was conducted based on a completely randomized design (CRD). Treatment means were compared using Duncan's multiple range test (33) to identify significant differences.

RESULT & DISCUSSION

Bacterial contamination of eggshells

It is noted from Table (2) results that T2 and T4 did not differ significantly from control (T1) in contamination rate on eggshell , but T3 achieved significant value ($P \leq 0.05$) in this trait. After disinfection, a significantly low contamination rate ($P \leq 0.01$) was for T4 compared to T1 and other groups. No significant effect among groups regarding to absolute difference of contamination rate while T4 achieved similar significant value compared to T1 in relative difference of contamination rate. The egg may be exposed to various contaminations caused by microorganisms, including harmful bacteria. This contamination can threaten the safety of the embryo and negatively affect embryogenesis. Therefore, it is necessary to adopt effective hygiene practices, such as disinfecting hatching eggs, to reduce bacterial contamination within poultry farms and associated hatcheries. These measures contribute to ensuring a safer and healthier environment for the embryo, which positively impacts hatching rates and the quality of the chicks produced (34). In current data after disinfection of eggshell, alcoholic olive leaf extract played an important role in decreasing microbial contamination on eggshell surface which positively reflected on increasing relative difference in disinfection rate compared with control.

This may be attributed to the antibacterial, antifungal, and antiviral properties of olive leaf extracts which depends on active compounds detected in this extract (Table1). These compounds provide essential benefits by combating oxidation and inhibiting the growth of certain harmful microorganisms (35). Among the most important active compounds found in olive leaves are oleuropein and its derivatives, which include hydroxytyrosol, caffeic acid, vanillic acid, vanillin, and rutin. It has shown that both olive leaves and oils have health-promoting properties as probiotics and offer various medicinal benefits, without any noticeable toxic effects (36).

Oleuropein is considered one of the most prominent phenolic compound that contributes significantly to enhancing the immune, physiological, and therapeutic response (37). Oleuropein's antimicrobial activity is believed to result from its ability to disrupt cell membranes and cytoplasm in bacteria and fungi (38,39). Despite of formaldehyde effectiveness, it is a toxic

chemical, and improper use can harm the embryo by damaging the egg cuticle, increasing microbial penetration, and interfering with the hatching process (40). Similar results were obtained by Oliveira *et al* (41). who suggested that disinfection of hatching eggs with essential oil of clove (*Syzygium aromaticum*)proved to be highly effective in reducing the bacterial load on eggshells, with a significant decrease in bacterial counts after only one hour of disinfection.The current result partially contradicts what was indicated by Taşdemir *et al* (42). which indicated that thyme juice used as a disinfectant instead of formaldehyde had no significant effect on reducing the microbial load on eggshells of laying hens. Moreover, Al-Shammari *et al* (22). showed that using aqueous extracts of ginger, garlic, oregano, and cinnamon compared to formaldehyde exhibited strong antimicrobial activity on quail hatching eggs, depending on the extract used.

Table 2 : Effect of hatching eggs disinfection by aqueous and alcoholic extracts of olive leaves on bacterial contamination rate (CFU/mL eggshell liquid) (mean ± standard error).

Traits	Treatments				Significance
	T1	T2	T3	T4	
Contamination rate					
Before disinfection	50.25±1 0.98b	61.25±1. 65 ab	83.00± 9.53 a	36.00± 3.05 b	*
After disinfection	28.75±1 1.52b	40.25±9. 23 b	67.00± 1.52 a	18.67± 2.90 c	**
Difference in disinfection rate					
Absolute difference	21.50±7. 57	21.00±9. 26	16.00± 10.50	17.33± 2.40	NS
Relative difference	43.91±1 2.68 ab	34.25±1 4.82bc	17.09± 9.60 c	48.39± 5.79 a	*

T1: disinfection of hatching eggs by formaldehyde (control) ; T2: disinfection of hatching eggs by methyl alcohol; T3: disinfection of hatching eggs by aqueous extracts of olive; T4: disinfection of hatching eggs with alcoholic extracts of olive leaves.Different letters (a,b,c) between rows indicate to presence of significant differences at level of * (P≤0.05), ** (P≤0.01).

Hatching characteristics

Table (3) shows that there were no significant differences among T1 and other groups in hatchability and mortality of fertile and total eggs, internally pipped eggs, pipped-alive chicks, pipped-dead chicks and deformed chicks. Less hatching and incubation times (P≤0.01) were registered for T4 compared to T1and other groups.There was no changing in hatching results affected by disinfection treatments were obvious. However, only shortening of required time for hatching and total incubation was in T4.

The lack of significant differences between the experimental treatments and the control may be due to safety and efficacy using of our disinfectant, which did not causeany deleterious effects on embryo growthandhatchability (22). Similarly, Batkowska *et al* (23). conveyed that no significant differences between groups of eggs disinfected by 15% alcoholic propolis extract and control in embryonic mortality rate, total hatchability percentage, and chick weight of quails on the 1st, 7th, and 14th days of rearing. The results of our study are also consistent with what reported by Ahmed *et al* (43). that was a decreased time of incubation (502.7, 498 and 501 hours) for egg treated with 1.25%, 2.5% and 5% of a natural white vinegar solution as disinfectant for local Dandrawi chicken, respectively compared with untreated eggs (503.6 hours). Different results were achieved by Baylan *et al* (8). who noted that disinfection eggs with 5% garlic extract had a significant role in reducing embryonic mortality, increasing hatchability and improving hatched chick weight. Also, previously different data by Alhamed *et al*(44). who found that immersing quail eggs in a 5% vinegar solution for 2 minutes have decreased hatchability and increased embryonic mortality compared to control

Table 3: Effect of hatching eggs disinfection by aqueous and alcoholic extracts ofolive leaves on hatching results (mean ± standard error).

Traits		Treatments				Significance
		T1	T2	T3	T4	
hatchability of fertile eggs (%)		94.44 ±3. 67ab	92.69±1.38b	95.59±2.51a	95.65±4.34a	**
hatchability of total eggs (%)		90.28±2.77	87.50±0.00	90.28±2.77	90.28±2.08	NS
Mortality of fertile eggs (%)	1-13 day	2.78±1.38	2.89±1.45	1.45±1.44	2.89±2.17	NS
	14-21 day	2.78±2.77	4.41±0.07	2.96±1.48	1.45±0.15	NS
	1-21day	5.56±3.67 ab	7.31± 1.38 a	4.41±2.51 b	4.35±4.34 b	*
Mortality of total eggs (%)	1-13 day	2.78±1.38	2.78±1.38	1.39±1.38	2.78±2.08	NS
	14-21 day	2.77±2.15 ab	4.18 ±0.00 a	2.78±1.38 ab	1.39±2.08 b	*
	1-21day	5.56±3.67	6.93±1.39	4.17±2.40	4.17±4.16	NS
Internally pipped eggs (%)		2.78±2.77	1.45±1.44	2.96±1.48	1.45±0.17	NS
Pipped-alive chicks (%)		0.00±0.00	1.45±0.24	0.00±0.00	1.45±0.18	NS
Pipped-dead chicks (%)		0.00±0.00	0.08±0.07	0.00±0.00	0.00±0.00	NS
Deformed chicks(%)		1.45±0.94	1.59±0.53	0.00±0.00	0.00±0.00	NS
Hatching time (hour)		27.88±0.29a	28.23±0.4 5 a	28.32±0.30 a	22.24±0.30 b	**
Incubation time (hour)		503.36±0.52 a	502.02±0.84 a	502.28±1.08 a	498.09±1.68 b	**

T1: disinfection of hatching eggs by formaldehyde (control); T2: disinfection of hatching eggs by methyl alcohol; T3: disinfection of hatching eggs by aqueous extracts of olive leaves; T4: disinfection of hatching eggs with alcoholic extracts olive leaves.Different letters (a,b,c) between rows indicate to presence of significant differences at level of * (P≤0.05), ** (P≤0.01).

Amount of moisture lost from fertile egg shells

Table (4) exhibited that the lowest significant difference (P≤0.05) in percentage of moisture lost from fertile eggs during (1-13 days) was in favor of T2, T3 and T4 compared to T1. As for the period (14-21 days), there was no significant difference between T1 and T4 in this parameter; however, T2 and T3 recorded high percentage(P≤0.05). In the total period (1-21 days), there was a significant similarity between the control and the rest of the treatments in this trait. All disinfection treatments succeeded positively in reducing moisture loss from the egg during days 1 to 13, with similar effects detected between T4 and T1 from 14 to 21 days of incubation. However, no noticeable changes were observed in this trait over the entire incubation period (1–21 days). This is attributed to the fact that the OL extracts did not cause damage to the permeability of the shell or the internal water balance of the egg, especially with uniform incubation conditions (temperature and humidity), which may explain the absence of statistical differences. Egg weight loss is a negative factor that significantly impacts the incubation and hatching process. Moisture loss in the egg negatively affects embryo development and metabolic status, which is an unfavorable indicator of hatching process. Therefore, maintaining egg weight and moisture are vital to ensure successful hatching and proper embryo development (45). Furthermore, reduced shell thickness may affect the permeability of the shell to water vapor and biogases, which in turn can lead to a lower hatching rate and a higher embryo mortality rate (43). Similarly, Oliveira *et al* (46). suggested that spraying hatching eggs with clove essential oil as a safe disinfectant did not cause any crucial changes in egg weight loss. In previously different result, it was indicated that was a significant decrease in the percentage of egg weight lost during the period from 0 to 18 days of incubation in egg sprayed with propolis alcoholic extract (14%) and thyme oil (5 and 7%) in comparison to 70% alcohol or formaldehyde as controls (47).

Table 4: Effect of hatching eggs disinfection by aqueous and alcoholic extracts of olive leaves on moisture loss from fertile eggs (%) (mean ± standard error).

Traits	Treatments				significance
	T1	T2	T3	T4	
- 13 day 1	14.57 ±2.59 a	10.44 ±1.26 b	9.59 ± 0.58 b	10.36 ±0.54 b	*
-21 day 14-	14.16 ±3.51 b	21.48 ±1.55 a	20.86 ±0.29 a	18.11 ±2.07 ab	*
- 21 day 1	26.83 ±1.60 ab	29.72 ±0.43 a	28.45 ±0.56 a	26.59 ± 1.85 b	*

T1: disinfection of hatching eggs by formaldehyde (control); T2: disinfection of hatching eggs by methyl alcohol; T3: disinfection of hatching eggs by aqueous extracts of olive leaves; T4: disinfection of hatching eggs with alcoholic extracts of olive leaves.Different letters (a,b,c) between rows indicate to presence of significant differences at level of * (P≤0.05).

Morphological parameters of chicks

Table (5) indicates that were no significant differences were recorded among T1, T3 and T4 in chick weight, chick weight without yolk and total score of chick quality. Significant similar values of relative chick weight between T1 and T4. There were no significant differences among treatments regarding relative yolk weight and the length each of chick, wing and leg.

There is a close correlation between the quality of hatching eggs and characteristics of newly hatched chicks, as this directly affects the chicks' performance and post-hatch subsequent growth (48,49,50). Accordingly, chick quality assessment relies on the use of quantitative and qualitative methods that take into account a variety of quality criteria. It seems obviously in our data that using of OL extracts for egg disinfection had no side effects on physical characteristics of hatched chicks. Studies have shown that initial chick weight at one day has a significant impact on growth during the early stages of life, but this effect decreases over time. (51). Chick weight is an indicator of growth performance and is affected by the unabsorbed yolk in the abdomen; therefore, chick length becomes more important in predicting the growth performance of broiler chickens. Leg length has also been shown to be positively correlated with chick weight and length, significantly impacting their growth performance (51).Our results are consistent with those of Oliveira et al (52). who reported that spraying hatching eggs with clove essential oil as a natural disinfectant did not negatively affect the length and weight of developing embryos and one-day-old chicks compared to the control treatment. Also, Hassan *et al*(53). confirmed that the use of clove essential oil at different concentrations (0.5%, 1% and 2%) as disinfectant for hatching eggs did not lead to change the weight of hatched chicks. However, only the 1% treatment resulted in a significantly greater chick length compared to the control. Also, there were conflicting results that indicated a significant increase in body length, leg length, and body weight of one-day-old chick, accompanied by a lower percentage of yolk residue at hatching in quail chicks hatched from the group of eggs treated with a moringa oil solution(54). The current results also partially differed from those of Gatea *et al* (55). who reported an increase in the relative weight of chicks, an improvement in chick's phenotypic characteristics and a decrease in the percentage of deformed chicks in all treatments when hatching eggs were sprayed with pomegranate and anise extracts.

Table 5 : Effect of hatching eggs disinfection by aqueous and alcoholic extracts of olive leaves on physical characteristics of hatched chicks (mean ± standard error).

Traits	Treatments				significance
	T1	T2	T3	T4	
Chick weight (g)	40.00± 1.00 a	38.19± 0.09 b	39.04± 0.32 ab	40.13± 1.01 a	*
chick weight without yolk (g)	37.34 ±1.19 a	36.09± 0.18 b	36.67± 0.27 ab	37.98± 0.83 a	*
Chick weight(%)	73.17±1.60 a	70.28±0.43 b	71.55±0.56 b	73.40±1.85 a	*
Yolk weight (%)	6.69±0.81	5.49±0.25	6.08±0.16	5.32±0.42	NS
Chick length (cm)	15.160±.20 ab	14.90±0.29 b	15.03±0.16 b	15.77±0.12a	*
Wing length (cm)	4.89±0.12	4.66±0.16	4.80±0.05	4.67±0.00	NS
Leg length (cm)	3.88±0.05	3.87±0.25	3.87±0.06	3.83±0.11	NS
Total score of chick quality	96.80± 1.97 a	91.07 ±1.93 b	93.07± 0.81 ab	97.20 ±2.05 a	**

T1: disinfection of hatching eggs by formaldehyde (control); T2: disinfection of hatching eggs by methyl alcohol; T3: disinfection of hatching eggs by aqueous extracts of olive. leaves;T4: disinfection of hatching eggs with alcoholic extracts of olive leaves

Productive response

Table(6)shows that were no significant differences between T1 and other treatments with respect to BW, WG, FCR, dressing percentage without edible parts and PEF. Low FI (P≤0.05) was for T2 and T3 compared to T1. Decreased mortality and increased dressing with edible parts(P≤0.1) were all in T4 in comparison to T1.Most of the data on one-day-old chicks in Table T5 may have influenced the results observed in Table T6. Therefore, our results showed unchanged values in the important productive traits (BW, WG, FCR, dressing without edible parts and PER) which may attribute to safe using of OL as natural disinfectant for hatching eggs instead of formaldehyde. Increase dressing with edible parts obtained in T4 might depend on BW, although it was only numerical increase in BW for this treatment. The resulting reduction in FI for T3 is probably due to improved production performance, influenced by enhanced intestinal function and increased nutrient absorption efficiency (44). The positive decrease in mortality for T4 could be attributed to the effective disinfection of fertile eggs by alcoholic extract of OL. The findings are partially in agreement with those reported by Debes and Basyony (5). that disinfection of White Leghorn hatching eggs with thyme and ginger oils significantly reduced total mortality rates and improved BW and FCR at 4 and 8 weeks for hatched chicks compared with birds produced from formaldehyde-disinfected eggs. Indentical results by Taşdemir *et al* (42).whostated that using the thyme juice as an alternative to formaldehyde in disinfection of laying hens' eggs did not show differences in BW and WGof birds with lower amount of FI compared to chicks of formaldehyde-disinfected eggs in the first two weeks. Contrarily, it was concluded that spraying hatching eggs with garlic oil (1 and 2 ml/L) improved dressing with edible parts of hatched chicks (56). Also, Ahmed *et al* (43). confirmed that spraying of hatching eggs with 2.5% and 5% natural white vinegar solution resulted in increased BW, FI and relative weights of liver, gizzard, heart of hatched chicks compared to the control.

Table 6: Effect of hatching eggs disinfection by aqueous and alcoholic extracts of olive leaves on productive performance of hatched chicks (mean ± standard error).

Treatments	Traits							
	BW (g; 5 weeks)	WG (g; 1-5 weeks)	FI (g; 1-5 weeks)	FCR (1-5 weeks)	Mortality (%; 1-5 weeks)	dressing 1 (%)	dressing 2 (%)	PEF
T1	2450.33±116.22 ab	2410.33±15.24 ab	3267.65±39.45a	1.36±0.07	9.80 ±1.92 b	73.08 ±5.67 ab	76.85± 5.97 b	472.59±65.23
T2	2346.33±56.88 b	2308.15±56.97 b	3087.05±31.22b	1.34±0.06	13.73±2.19 a	71.58±2.40 b	75.01 ±2.29 b	437.02 ±48.68
T3	2431.67±37.02 ab	2392.63±37.24 ab	3061.07 ±32.65b	1.28±0.07	11.76 ± 4.79 ab	73.76±6.21 ab	77.42± 6.29 ab	481.12 ±43.81
T4	2485.33±32.05 a	2445.21±31.07a	3251.48±40.04a	1.33±0.03	5.88± 1.19 c	74.03±4.87 a	78.54± 5.31 a	504.39 ±36.21
Significance	*	*	*	NS	**	*	**	NS

T1: disinfection of hatching eggs by formaldehyde (control); T2: disinfection of hatching eggs by methyl alcohol; T3: disinfection of hatching eggs by aqueous extracts of olive leaves;T4: disinfection of hatching eggs with alcoholic extracts of olive leaves.Different letters (a,b) between columns indicate to presence of significant differences at level of * (P≤0.05), ** (P≤0.01), NS: non-significant. BW: body weight, FI: feed intake, FCR: feed conversion ratio, PEF:production efficiency factor. [1]: without edible parts, [2]: with edible parts**.**

Immunological indicators

Based on results presented in Table (7), there wasabsence of significant differences among treatments regarding the relative weight of bursa of Fabricius, thymus and spleen. Also, There were no significant differences among T1, T3 and T4 in Bursa weight index.

Table 7: Effect of hatching eggs disinfection by aqueous and alcoholic extracts of olive leaves on weights of lymphoid organs of hatched chicks at 35 days of age (mean ± standard error)

Traits	Treatments				Significance
	T1	T2	T3	T4	
Bursa of Fabricius	0.19±0.00	0.12±0.02	0.14±0.02	0.17±0.00	NS
Thymus	0.41±0.05	0.34±0.018	0.33±0.04	0.38±0.05	NS
Spleen	0.13±0.02	0.08±0.00	0.10±0.00	0.11±0.00	NS
Bursa weight index	1.00 ±0.00 a	0.62± 0.12 b	0.74 ±0.13 ab	0.92± 0.02 a	*

T1: disinfection of hatching eggs by formaldehyde (control); T2: disinfection of hatching eggs by methyl alcohol; T3: disinfection of hatching eggs by aqueous extracts of olive leaves;T4: disinfection of hatching eggs with alcoholic extracts of olive leaves.Different letters (a,b) between columns indicate to presence of significant difference at level of * (P≤0.05), NS: non-significant

Table (8) refers that there is a significant increase (P≤0.05) for T3 and T4 compared with the control and the rest of treatments in serum antibodytiters against Newcastle and Gumborodiseases, respectively. Also, T3 and T4 obtained high values in serum antibody titersagainst Newcastle (P≤0.01) and Gumboro(P≤0.05) diseases, respectively as a difference between these treated groups and T1. It was obvious that no significant difference among treatments was found in serum antibody titers against infectious bronchitis and avian Influenza diseases. High value (P≤0.05) as a difference between T2 and T1 regarding serum antibody titers against infectious bronchitis compared to T1.

Table 8: Effect of hatching eggs disinfection by aqueous and alcoholic extracts of olive leaves on antibody titers against viral diseases of hatched chicks at 5 weeks of age (mean ± standard error).

Traits	Treatments				
	T1	T2	T3	T4	Significance
Newcastle	624.33± 26.88 b	134.00± 78.58 b	1860.33 ± 93.81 a	368.33± 11.45 b	*
Gumboro	33.00± 7.65 b	46.00± 16.27 ab	41.00 ± 11.05 b	62.00 ± 15.32 a	*
Infectious bronchitis	9891.00± 172.19	10825.00±150.67	8400.00± 135.18	9402.33 ±100.28	NS
Avian influenza	25.67± 4.37	20.00 ± 9.64	33.00± 21.07	51.67± 40.46	NS
Newcastle [1]	0.00± 0.00 b	-490.33± 30.06 b	1236.00 ± 11.55 a	-256.33± 37.57 b	**
Gumboro [1]	0.00± 0.00 b	13.00 ± 7.50 ab	8.33 ± 9.46 ab	29.33± 6.12 a	*
Infectious bronchitis[1]	0.00± 0.00 b	934.00 ± 23.23 a	-1491.00± 70.44 b	-489.00 ± 99.32 b	*
Avian influenza[1]	0.00± 0.00	-5.67 ± 11.66	7.33 ± 19.05	26.00 ± 37.52	NS

T1: disinfection of hatching eggs by formaldehyde (control); T2: disinfection of hatching eggs by methyl alcohol; T3 : disinfection of hatching eggs by aqueous extracts of olive leaves; T4: disinfection of hatching eggs with alcoholic extracts of olive leaves.Different letters (a,b, c) between columns indicate to presence of significant difference at level of * (P≤0.05), ** (P≤0.01), NS: non-significant.
[1]Difference in serum antibody levels between the experimental treatments and control.

The increase of the antibody titers against viral diseases (Newcastle and Gumboro) after disinfection of embryonated eggs in T3 and T4 may indicate immune system stimulation, especially in the early stages of critically embryonic development. This positive effect is probably related to the phenolic compounds and antioxidant substances in olive leaf extract, such as oleuropein, which may enhance immune function without causing harmful effects on immunological tissues or leading to pathological hyperplasia. Also, OL containsvarious polyphenolic compounds (Table 1). Polyphenols are generally capable of modulating immune cell activity by binding to specific cellular receptors, thereby modifying cell signaling pathways and controlling host immune responses (57). These bioactive compounds in OL have demonstrated their immunostimulant, antimicrobialand anti-inflammatory properties. Perhaps, these combined properties contribute to enhancing bird immunity by stimulating innate immunity, through enhancing the birds' resistance to microbial infections and multiple inflammations (58). These results are previously similar to which reported by Fouad *et al* (56) . and Fouad *et al* (54) . that chicks produced from eggs sterilized with garlic oil and moringa oil, respectively had an improvement in immunity through increased concentration of immunoglobulin G and that this in turn was shown to be important in improving the general health status and many physiological aspects.

CONCLUSION

The current findings suggest that spraying hatching eggs with an alcoholic extract of OL as a pre-incubation disinfectant is more effective than using the aqueous extract of OL. While the aqueous extract showed effects comparable to formaldehyde, the alcoholic extract demonstrated superior efficacy in reducing microbial contamination on eggshells, shortening incubation duration, and enhancing immune responses—without causing negative impacts on hatchability or chick physical quality. Additionally, a modest improvement in post-hatch productive performance was observed in the group treated with the alcoholic extract of OL.

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