Journal of Sohag Agriscience (JSAS) 2024, 9(2): 65-71

ISSN 2357-0725
https://jsasj.journals.ekb.eg
JSAS 2024; 9(2): 65-71

Received: 11-08-2024
Accepted: 04-12-2024

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Effect of some natural products on microbial infection in Quail Eggs

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Abstract

This study investigates the potential of clove oil and anise oil as natural alternatives for controlling bacterial pathogens in poultry production, as an experiment was done on incubating quail eggs. Bacterial contamination, particularly from *Salmonella* spp and *Escherichia* coli (*E. coli*), poses significant risks to animal and human health, leading to economic losses and foodborne illnesses. The study aims to assess the antibacterial properties of clove oil and anise oil against these pathogens in incubation eggs and throughout poultry production. Experimental trials will assess the antibacterial activity of various essential oil concentrations using disc diffusion assays. The findings enhance our understanding of natural alternatives for reducing bacterial contamination in the poultry industry, guiding strategies for producing safe, high-quality poultry products. In addition to understanding the ability of Clove and Anise essential oils as disinfectants in incubation eggs and poultry farms and consequently the impact of these essential oils on the overall health of poultry.

Keywords: Bioactive compounds, Bacteria, *Salmonella*, *E. coli*, Poultry, Clove oil, Anise oil, natural products, antibacterial, pathogenic, incubation eggs, Infection, Microbial

INTRODUCTION

Poultry farming is an important business that provides poultry meat and eggs to the people all over the world. Nevertheless, bacterial infection and the existence of pathogenic bacteria, such as *Salmonella and Escherichia coli* (*E. coli*) are threats to both animal and human lives, leading to losses and possible foodborne diseases. Knowledge of the source and mode of bacterial contamination is very important in preventing and controlling the spread of bacteria and producing quality poultry products. The research in this paper is aimed at identifying the potential of using natural agents, such as clove oil and anise oil, in the management of bacterial pathogens in poultry farming. Some of these essential oils have shown good potential in combating different bacteria such as Salmonella and *E. coli*. Nevertheless, their ability to influence incubation eggs and manage bacterial infection in the poultry sector is still not well researched. Given that bacterial contamination is a common issue in various stages of poultry production and eggs are found to be contaminated with bacteria, there is increasing concern to look for natural ways of reducing the risks associated with bacterial contamination. The purpose of this study is to assess the impact of clove oil and anise oil on incubation eggs and their ability to reduce the presence of Salmonella and *E. coli*. The study will therefore include experimental trials involving the various forms of these oils and the antibacterial activity against the tested pathogens will be determined. The results of this study will help to enrich the existing body of literature on the application of natural remedies such as clove oil and anise oil to reduce bacterial presence in the poultry sector. The outcomes can be used to design proper measures that will help in producing quality poultry products, reduce losses and incidences of foodborne diseases.

MATERIALS AND METHODS

Eggs preparing

Fertile quail eggs were sourced from Sohag University's poultry farm, known for healthy breeding stock. Clove and Anise essential oils were obtained from a reputable local company," Al Hawaj". The collected eggs were stored at 10°C for 2 days. Eggs were divided into three groups, each with three replicates. Egg weights were recorded using a calibrated digital scale. Baseline data was obtained for comparison, with similar average weights across groups. Each egg was given a name following the format GxRy–X, where x was the group number, y was the replicate number, and X was the serial number of the egg in the replicate.

Eggs Treatment

The eggs were divided into three groups, with Group 1 and Group 2 receiving Clove oil and Anise oil treatments, respectively, at concentrations of 2.5%, 5%, and 10%. Group 3 served as the control, with three replicates treated with distilled water as a positive control and three replicates left untreated as a negative control. The oils were prepared by diluting them in a carrier solvent, Tween 80, following the method described by Singh et al. (2021). The eggs in Group 1 and Group 2 were evenly sprayed with the respective oil solutions using a fine mist sprayer to ensure complete coverage of the eggshells Fouad Abdelfattah (2019). The eggs were then left undisturbed for a few minutes to allow the oils to adhere. The control group was treated similarly, with three replicates sprayed with distilled water only, as described by Abuoghaba (2016). Then eggs were incubated in incubators with standard turning and incubation conditions as Nora A. (2023). Allowing for the assessment of the specific effects of the Clove oil and Anise oil treatments.

Bacteriological tests

For the total bacterial count on eggshells, sterile plastic cups were used to collect three random eggs from each replicate Fasenko et al., (2009) in Group 1, Group 2, and the control group, totalling nine eggs per group. Cross-contamination was avoided during sample collection. The eggs were prepared by adding 1 ml of sterile distilled water to each cup and gently stirring to mix with the eggshells. Serial dilutions were performed following the BAM manual (2005), and the diluted bacterial

suspensions were spread onto Mueller-Hinton agar plates using the spread plate technique, study used this medium because It is a non-selective, non-differential medium that allows the growth of a wide range of non-fastidious bacteria. After overnight incubation at 37°C, colonies were manually counted, and the total bacterial count per sample was calculated, considering the dilution factor. For the isolation of *Salmonella* and *E. coli*, selective media were prepared according to established protocols. Xylose Lysine Deoxycholate (XLD) agar and Salmonella-Shigella (S.S) agar were used for *Salmonella* isolation Giaouris et al., (2005), while MacConkey agar was used for *Escherichia coli* isolation. Aliquots from the original swab suspensions were streaked onto XLD agar plates and incubated overnight at 37°C Jotan Kar et al., (2017). Characteristic colonies were selected from the XLD agar plates and further confirmed. Swabs were taken from yellow spots in XLD agar and separated on MacConkey agar *for Escherichia coli* confirmation, while pink colonies were separated on S.S agar for *Salmonella* confirmation. The plates were incubated at 37°C for 24 hours, and selected colonies were subjected to PCR analysis for confirmation.

PCR confirmation

Genomic DNA extraction from selected *Salmonella* and *Escherichia coli* colonies was performed using a commercial DNA extraction kit following the manufacturer's instructions (QIAamp DNA mini kit). The protocol involved various steps, including the addition of QIAGEN protease, buffer AL, ethanol, and buffers AW1, AW2, and AE, as well as centrifugation at specific speeds and durations. For PCR analysis, specific primers targeting the genes of interest (inyA and PhoA) were designed and synthesized. A PCR master mix was prepared using Emerald Amp GT PCR mastermix (Takara) and aliquoted into separate PCR tubes or plates. The PCR cycling conditions were optimized based on the specific primers and DNA template, with denaturation, annealing, and extension steps at specific temperatures and durations. DNA molecular weight markers and agarose gel electrophoresis were used to analyze the PCR products Sambrook (1989), followed by gel documentation and data analysis

MIC tests

After confirmation from species *E. coli* and *Salmonella* MIC (Minimum Inhibitory Concentration) tests were conducted to assess the antimicrobial activity of clove oil and anise oil against *Salmonella* sp and *Escherichia coli* isolates. Stock solutions of clove oil and anise oil were prepared by diluting commercially available oils with Tween 80 as a solvent. Working solutions were then prepared by serial dilution of the stock solutions to achieve desired concentrations. Inoculum cultures of the bacterial isolates were prepared by incubating single colonies in autoclaved nutrient broth. The MIC test was performed by mixing the bacterial culture with Mueller-Hinton agar in petri dishes and applying different concentrations of clove oil and anise oil using disk-diffusion susceptibility testing Bauer (1966). The test was performed in four concentrations (0.08, 0.05, 1.0 and 0.5%) for Clove oil and (0.05, 0.1, 0.5 and 1.0%) for Anise oil in three replicates for every concentration. The plates were incubated, and after 24 hours, the inhibition zones around each disk were visually examined and measured using an inscribed ruler.

Record parameters for quail chicks and eggs

To assess the growth and development of quail chicks, various parameters were recorded. Considering that the experiment was approved by the Ethics Committee of the Department of Agricultural Microbiology, Sohag University. The embryonic mortality was monitored throughout the incubation process Mohamed Salah Eldein (2024), and the number of embryos that died at early (within the first 3 days), mid (between 4-10 days), and late (after 11 days) stages of development was documented. The date, time, and any noticeable abnormalities or reasons for mortality were recorded. The hatchability rate was determined for each group by counting the number of live chicks at hatch and calculating hatchability using the equation provided.

$$Hatchability\ (\%) = \frac{Number\ of\ chiks\ at\ hatch}{Total\ set\ number}\ x\ 100$$

Individual chick weights were measured at hatching and again at 20 days of age using a precise weighing scale. The average chick weight at hatching and at 20 days of age was calculated by dividing the total weight by the number of chicks.

RESULTS AND DISCUSSION

Bacteriological testing of eggs

The results revealed the effectiveness of Clove and Anise oil treatments in reducing the total bacterial count (TBC) on eggshells. The TBC on eggshells treated with a 10% concentration of Clove oil decreased from an average of 231x10³CFU in the control group to 38x10³CFU, while the Anise oil treatment in the same concentration resulted in a TBC of 87x10³CFU. In comparison, the TBC in the control group was 204x10³CFU it is high number in compare with Fouad (2017) that was 50.8. These findings demonstrate the potential antibacterial effects of Clove and Anise oil treatments on eggshell contamination (Table 1)

Table 1: Total bacterial count for every treatment

Treatment	Average TBC x103 CFU
CO1	204
CO2	61
CO3	38
AO1	268
AO2	144
AO3	87
Control	231

CO1, 2.5% Clove; CO2, 5% Clove; CO3, 10% Clove; AO1, 2.5% Anise; AO2, 5% Anise; AO3, 10% Anise

For confirmation of Bacterial species that study tests MIC on them the research team got PCR results showed that target gene was positive as equal to 284 bp in alignment with ladder bands for potential *Salmonella* isolation and that detecting invA gene in *Salmonella enterica* and in potential *E. coli* isolation equal to 720 bp for gene phoA as it detects *E. coli* (Figure 1)

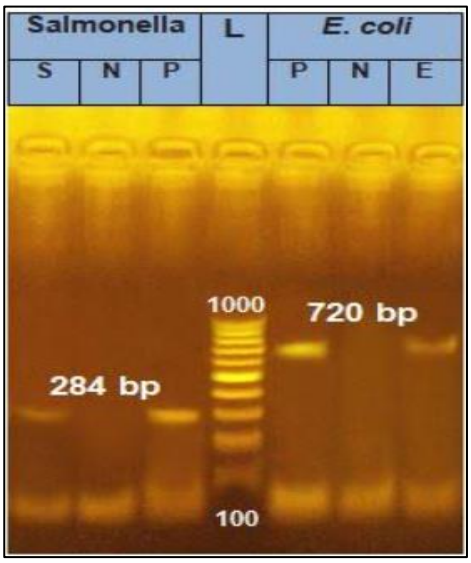

Figure 2: Gel electrophoresis for target genes in potential *Salmonella* sp and *E. coli*. S, potential *Salmonella* sp; E, potential *E. coli*; P, positive band; N, negative band; L, ladder bands

For MIC values (Table 2) for Clove oil ranged from 0.08% to 0.5% for *Salmonella enterica* and from 0.08% to 0.5% for *Escherichia coli*. For Anise oil, the MIC values ranged from 0.05% to 1.0% for *Salmonella enterica* and from 0.05% to 0.5% for *Escherichia coli*. These findings demonstrate the inhibitory effects of both Clove oil and Anise oil on the growth of the bacterial strains tested. The results are consistent with previous studies by Mukhtar (2011) and Radaelli et al. (2016).

Table 2: Minimum Inhibition Concentration test for Clove oil and Anise oil

Treatment	*Salmonella* enterica	*Escherichia coli*
	Inhibition zone (mm)	Inhibition zone (mm)
CO1	Non	Non
CO2	2	Non
CO3	5	4
CO4	8	7
AO1	Non	Non
AO2	2	Non
AO3	4	2
AO4	6	4

CO1, clove 0.08%; CO2, clove 0.05%; CO3, clove 0.1%; CO4, clove 0.5%. AO1, anise 0.05%; AO2, anise 0.1%; AO3, anise 0.5%; AO4, anise 1.0%.

Hatchability and Chick Quality

The results showed that the hatchability rates varied among the treatment groups, indicating the potential effects of the oil concentrations used. Group 1, treated with Clove oil, exhibited hatchability percentages ranging from 70% to 80% for concentrations of 2.5%, 5%, and 10%. Group 2, treated with Anise oil, showed hatchability percentages ranging from 70% to 77.5% for the same concentrations. The control group, consisting of Positive D.W (Distilled Water) and Negative treatments, displayed hatchability rates ranging from 60% to 67.5%. Average chick weight at hatching was found to be consistent across all treated groups and the control group, ranging from 6.5 grams to 6.9 grams. These findings suggest that both Clove oil and Anise oil treatments have the potential to positively influence hatchability rates in incubation eggs, as supported by previous studies Karrar (2015); Abuoghaba (2016). As results for the effects of different treatments of Clove, and Anise, on embryonic mortality in chicks. The embryonic mortalities ranged from 22.5% to 40% across the treatments and replicates. The control group showed mortalities of 32.5% to 40%. The Clove treatment exhibited mortality rates of 22.5% to 32.5%, and the Anise treatment showed mortalities ranging from 22.5% to 32.5%. These refer to that both clove oil and anise oil have potential benefits in reducing embryonic mortality at certain concentrations, complementing the study by Oliveira et al. (2021) that highlighted the positive effects of clove oil on late embryonic mortality. Further investigation is warranted to explore the impact of these treatments on overall chick quality and development (Table 3) on other hand, Oliveira (2021) reported that the effect of clove oil appears on late mortality only, as it reduces number of late embryonic mortality without early and mid-death.

Table 3: Records of parameters for quail chicks and eggs

Treatment	Control	CO1	CO2	CO3	AO1	AO2	AO3
TEM (%)	35.4	30.8	24.2	23.3	31.7	29.2	25
Hatchability (%)	64.6	69.2	75.8	76.7	68.3	70.8	75
Chick weight at hatch (gm)	6.8	6.6	6.5	6.9	6.5	6.8	6.7
RCWAH (%)	56.8	55	51.7	55.7	53.2	56	55.3
Chick weight at 20 days (gm)	117.5	115	119	118	115	115	118
TBWC (gm)	110.7	108.4	112.5	111.5	108.5	108.2	111.6

TEM, total embryonic mortality; RCWAH, relative chick weight at hatch; TBWC, total body weight change; CO1, 2.5% Clove; CO2, 5% Clove; CO3, 10% Clove; AO1, 2.5% Anise; AO2, 5% Anise; AO3, 10% Anise

CONCLUSION

As this study referred to the bacterial contamination in the poultry industry and explored the potential of natural alternatives, specifically clove oil and anise oil, for controlling bacterial pathogens. The study revealed significant differences in bacterial contamination levels at various sampling points in broiler chicken farms and identified key bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, and *Salmonella* species. The application of clove oil and anise oil demonstrated significant

antibacterial activity against *Salmonella* and *E. coli*, leading to reduced bacterial counts on eggshells, improved hatchability rates, and enhanced average chick weight at hatching. These findings highlight the potential of clove oil and anise oil as natural alternatives for mitigating bacterial infections in the poultry industry, thereby promoting safer and higher-quality poultry products.

REFERENCES

Bacteriological Analytical Manual (BAM). 2015 Prastuty Singh, Gurkirat Kaur and Arashdeep Singh. 2021. Physical, morphological and storage stability of Cloveoil nanoemulsion based delivery system: sagepub.com/journals-permissionsDOI:

Fouad W., Abdelfattah, M. G and Abdelnabi, M. A. 2019. EFFECT OF SPRAYING HATCHING EGGS BY DIFFERENT LEVELS OF VINEGAR ON EMBRYOLOGICAL DEVELOPMENT, HATCHABILITY AND PHYSIOLOGICAL PERFORMANCE OF DANDARWI CHICKS. Egyptian Poultry Science Journal, 2090-0570

Abuoghaba, A.A. (2017). Impact of spraying incubated eggs submitted to high temperature with ascorbic acid on embryonic development, hatchability, and some physiological responses of hatched chicks. Canadian Journal of Animal Science, 97, 172-182. DOI: 10.1139/cjas-2016-0025.

Nora A. Ismael, Usama M. Abdelmonem,y, Mohamed S. El-Kholy, A. G. El Nagar,zAtef F. Ahmed,xMohammed Almalki,xKhaled A. El-Tarabily , and Fayiz M. Reda. 2023. The relationship between eggshell color, hatching traits, fertility, mortality, andsome qualitative aspects of Japanese quail (Coturnix japonica) eggs. Elsevier Inc. on behalf of Poultry Science Association Inc

G.M. Fasenko, E.E. O'Dea Christopher, L.M. McMullen. 2009. Spraying hatching eggs with electrolyzed oxidizing water reduces eggshell microbial load without compromising broiler production parameters, Poultry Science: https://doi.org/10.3382/ps.2008-00359

Giaouris E., Chorianopoulos N., Nychas G.-J.E. Effect of temperature, pH, and water activity on biofilm formation by Salmonella enterica enteritidis PT4 on stainless steel surfaces as indicated by the bead vortexing method and conductance measurements. J. Food Prot. 2005; 68:2149–2154.

Jotan Kar , Tapas Roy Barman, Arup Sen, Sabuj Kanti Nath. (2017). Isolation and identification of Escherichia coli and Salmonella sp. from apparently healthy Turkey.

Int. J. Adv. Res. Biol. Sci. 4(6): 72- 78. DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.06.012

Sambrook, J.; Fritscgh, E.F.; andMentiates (1989): Molecular coloning. A laboratory manual.Vol !., Cold spring Harbor Laboratotry press, New York.

Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Microbiol. 40:2413–2415.

Fouad W. and Mohamed S. Abdel-Hafez. 2017. EFFECT OF SPRAYING HATCHING EGGS OF JAPANESE QUAILS BY LIVE YEAST ON PHYSIOLOGICAL CHANGES IN THE EMBRYONIC DEVELOPMENT, HATCHABILITY AND TOTAL BACTERIAL COUNT. Egypt.Poult. Sci.Vol(37)(IV): (1303-1321) (2017)

Mukhtar AM. 2011. The effect of dietary clove oil on broiler performance. Australian Journal of Basic and Applied Sciences, 5(7): 49-51.

Marcela Radaelli, Bárbara Parraga da Silva, Luciana Weidlich, Lucélia Hoehne, Adriana Flach, Luiz Antonio Mendonça Alves da Costa, Eduardo Miranda Ethur. 2016. Antimicrobial activities of six essential oils commonly used as condiments in Brazil against Clostridium perfringens. Brasileira de Microbiologia. Published by Elsevier Editora Ltda.

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. Sanitizing Hatching Eggs with Essential Oils: Avian and Microbiological Safety. Microorganisms 2023, 11, 1890. https://doi.org/10.3390/microorganisms11081890

Oliveira, G.d.S.; McManus, C.; Salgado, C.B.; dos Santos, V.M. 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. https://doi.org/ 10.3390/ani12202826

Oliveira, G.d.S.; Nascimento, S.T.; dos Santos, V.M.; Lima Dallago, B.S. 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. https://doi.org/10.3390/ ani11072045

Olivera, S.D.; Rodenbusch, C.R.; Ce, M.C.; Rocha, S.L.S. and Canal, C.W. (2003): Evaluation of selective and non-selective enrichment PCR procedures for Salmonella detection. Lett. Appl. Microbiol., 36: 217-221.

Karrar I.A. Al-Shammari, Justyna Batkowska, Magdalena M. Gryzi´nska. 2015. ASSESSMENT OF ULTRAVIOLET LIGHT EFFECTIN HATCHING EGGS DISINFECTIONON HATCHABILITY TRAITS OF TWO BREEDSOF QUAILS AND CHICKENS. Wydawnictwo Uczelniane Zachodniopomorskiego Uniwersytetu Technologiczne-go w Szczecinie