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Research Article

Effect of oregano essential oil against selective microorganisms in raw mutton

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Abstract

The current study was conducted to ascertain the impact of various concentrations of oregano essential oil on the microbial population in raw mutton. The experiment was conducted using a complete randomized design (CRD) with six (6) treatments as T_0, T_1, T_2, T_3, T_4, T_5 and three (3) replications. The drop plate method was utilized to detect the presence of certain microorganisms. The outcomes showed that adding oregano oil reduced the microbial population in samples. Colony-forming units gradually decreased where T_5 being the lowest number of colony-forming units. There was a highly significant difference ($p<0.0001$) in the colony-forming unit of *Staphylococcus epidermidis* and *Proteus vulgaris*. For *Klebsiella pneumonia*, there was a significant reduction ($p<0.01$) of the microbes among samples. On the other hand, *Staphylococcus epidermidis* significantly outperformed *Proteus vulgaris* in terms of growth inhibition. In light of the findings, it is possible to conclude that the addition of oregano essential oil at a 200 µl level reduces the microbial population of raw mutton.

Introduction

The meat industry has been looking for effective methods to preserve and guarantee the safety of meat products for a long time, (Islam et al., 2022; Oleynikov, 2020; Rahman et al., 2023) because different meats—for example, goat meat that is high in protein (Williams, 2007) and has a pH range that creates a favorable environment for microorganisms—are thought to be great growth mediums for germs (Das et al., 2022; Jay et al., 2005). Along with microbial invasion, meat can also lose its quality due to alterations in the protein and lipid fractions brought on by the autolytic activities, which can also induce contamination or spoilage (Akter et al., 2009; Akhter et al., 2009; Tauro et al., 1986). Consumers can be exposed to serious health issues upon their consumption, regardless of the environment in which the pollutants pave their way in them (Pokorný, 2007). In response to these consequences, the food industry on a commercial scale uses synthetic antioxidants that can stabilize free radicals by either giving them hydrogen (H) or taking their electrons to create a complex (Azad et al., 2021; Jahan et al., 2018; Maisuthisakul et al., 2007) or additives with bacteriostatic properties, e.g., acetic acid or lactic acid and their salts, which can preserve meat and extend their shelf life (Hashem et al., 2022; Oleynikov, 2020; Rahman et al., 2017; Sarker et al., 2021). However, some of them have been prohibited due to consumer worries about their harmful effects on human health. (Pokorný, 2007). Due to this trend, the food industry has been pressured to switch over to natural, plant-based ingredients, particularly those derived from vegetables, fruit, berries, herbs, and spices (Ali et al., 2022; Hossain et al., 2021; Rather et al., 2016). According to Holley and Patel (2005), there has been an amplified interest in using plant essential oils as safer meat additives. Derived from the oregano (Origanum vulgare L.) leaves and flowers, Oregano oil has a wide distribution throughout the Mediterranean area (Karousou et al., 2007; Kofidis et al., 2004). This long-used meat flavoring agent has also illustrated effectiveness against spoilage microflora in meat (Govaris et al., 2010). Carvacrol and thymol, the main contributors responsible for causing the bacterial cell membrane to become permeable (Lambert, 2001), and their precursors- c-terpinene and q-cymene are the prime substances of this essential oil and, in unison, offer strong antioxidant qualities (Milon et al., 2017; Cervato et al., 2000). To investigate how oregano essential oil affects the color and oxidative stability of raw and cooked chicken breast meat, Al-Hijazeen et al., (2016) conducted a study revealing the possibility of replacing synthetic antioxidants in meat, as at 100–400 ppm levels, oregano essential oil showcased its effectiveness as an effective preservative. Jayasena and Jo (2014) conducted a study to determine the potential of Essential oils as antimicrobial agents in meat and meat products and discovered that Plant-derived essential oils (EOs) exhibit remarkable antimicrobial effectiveness against spoilage and pathogenic microorganisms in meat and meat products. Muranyi and Fraunhofer (2013) investigated the effects of an edible coating made of alginate that included rosemary and oregano essential oils as natural antioxidants on lipid oxidation, color preservation, water loss, texture, pH, and customer acceptance of beef steaks throughout a 14-day exhibition (Mia et al., 2023; Rahman et al., 2023). The consequence was an increase in meat product stability with the possibility of prospective usage in the food sector and general public acceptance of beef. The shelf-life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging was the subject of another study. The fresh lamb meat was kept at 4° C and

research revealed that the shelf life of lamb meat was 7 days for samples that were air packaged, 9–10 days for samples that contained 0.1% oregano essential oils, and 21–22 days for samples that were packaged in modified atmospheres and contained 0.1% oregano essential oils (Disha et al., 2020; Karabagias et al., 2011). Numerous studies have been conducted to determine how oregano oil affects meat. However, it is quite recent in Bangladesh, and hasn't been any study done yet. So, the following goal guided the current study's development:

To investigate the effect of oregano essential oil in raw mutton.

To study the efficacy of various strengths of oregano essential oil on *Staphylococcus epidermidis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Shigella spp*.

Materials and Methods

Sample Collection:

Mutton weighing 100 gm after 1-2h post-mortem was purchased from Santidham More, Khulna. Oregano oil was purchased from Australia.

Bacteriological Media & Chemicals:

Bacteriological Media used in the study were procured from Hi-Media (India). Media used for bacteriological analysis, detection, count and identification of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Shigella spp.*, in mutton were Mannitol Salt Agar (MSA), Xylose Lysine Deoxycholate (XLD) Agar, HiCrome^{tm} bacillus Agar Base, Hektoen Enteric Agar. Here two chemicals were used for cleaning and sterilization following an aseptic procedure Alcohol (98%) and Normal saline (0.9%NaCl).

Experimental Design:

A completely randomized design (CRD) was used to carry out the investigation, having six treatments (T_0 = 0 µl of oregano oil; T_1 =10 µl of oregano oil + 1 gm meat; T_2 = 50 µl of oregano oil+ 1 gm meat; T_3 = 100 µl of oregano oil+ 1 gm meat; T_4 = 150 µl of oregano oil+ 1 gm meat; T_5 = 200 µl of oregano oil+ 1 gm meat) with 3 replications of raw mutton formulated with oregano oil in comparison with the control one. Six mutton mixes were prepared; the 1st meat mix (control) was prepared with 1 gm mutton and 0.9% sodium chloride. The second mix was prepared with 10 µl of oregano oil, the third mix was prepared with 50 µl of oregano oil, the fourth mix was prepared with 100 µl of oregano oil; the fifth mix was replaced 150 µl of oregano oil and the sixth mix was prepared with 200 µl of oregano oil. All measurements were made in duplicate.

Preparation of Sample for Bacteriological Studies:

The experiment was conducted with 6 treatments having 3 replications. The mutton was washed in normal saline (0.9% NaCl). 1gm. meat sample was added with 1ml (1000 µl) saline and ground it properly. After grinding 9ml saline was added. The sample was ready without oregano oil. On the other hand, 1gm meat sample was soaked in 10 µl oregano oil (1 hour). Then 1ml ($1000\mu l$) saline was added and grinded it properly. Following grinding, meat (mutton) was assigned into six treatments. Oregano oil of six different concentrations (as treatments) T_0 (0 µl of oregano oil), T_1 (10 µl of oregano oil), T_2 (50 µl of oregano oil), T_3 (100 µl of oregano oil), T_4 (150 µl of oregano oil), T_5 (200 µl of oregano oil) were added into mutton meat. After grinding 9ml saline was added. The sample was ready. Each sample was examined for the microbial count.

Monitoring parameter - viable count:

1 g of meat samples for six treatments were each weighed and put in T_0, T_1, T_2, T_3, T_4, T_5. T_0 was left as the fresh sample while T_1, T_2, T_3, T_4, T_5 were subjected to surface treatment using 10 µl, 50 µl, 100 µl, 150 µl, 200µl of the oregano oil respectively. All samples were stored at 25^0 (room temperature) and were analyzed immediately, 30 minutes after treatment.

Sample plating (by using drop plate method):

A series of dilutions of the sample was prepared. Then two sterilized Petri dishes were taken and agar medium was poured into them and allowed to dry. Four quadrants were created out of each agar plate by marking over the lid with a glass marking marker. Each quadrant is reserved for one dilution in the series, then the plates were arranged, a sample was drawn by the pipette from the highest dilution first, the lid was lifted on one side and a drop of the suspension was delivered from the pipette aseptically on the marked segment. The 10 µl volume settings of the electronic pipette were preprogrammed. The first dilution tube was vortexed for approximately 6 s and 10 µl was picked up using the electronic pipette. Eighty microliters were dispensed in 8 evenly spaced 10 µl drops onto the designated quadrant of the Petri plate. The leftover sample and the tip were thrown away. In that way, suspensions of bacteria of different dilutions were separated by plates on marked areas of the Petri dish. The Petri plates were inverted and incubated at 37°C for 18 to 24 hours after the drops on the agar dried.

Observation:

While the incubation period was over the microorganisms were grown in the petri plates and it was counted the CFU/ml via visual method.

Enumeration of Total Viable Count of Bacteria (TVC) :

In the samples, bacteria were enumerated following the methods of Total Viable Count (selected) of Bacteria by Drop Technique with suitable modifications whenever necessary. Total Viable bacteria might be counted by diluting samples, plating the dilutions on the solid medium, and counting the colonies that arise. CFU (colony-forming units) were used to express results. The number of CFU per gram of the original sample was calculated by using the formula:

Number of CFU ml^{-1} (or g^{-1}) = N x 10^n x 10

Where,

N = number of colonies on the plate at the selected dilution.

n = number of dilution.

Identification of Bacteria:

Identification of bacteria was obtained by their morphological characteristics.

Statistical Analysis:

Microsoft Excel was employed for data entry and the data were analyzed using the GLM procedure of SAS version 9.1, (SAS Institute Inc., 1996). Effects of oregano oil in mutton meat were tested by analysis of variance and when differences were detected. DMRT (Duncan's Multiple Range Test) was used to compare the treatment means, with significance considered at P<0.0001.

Results and Discussion

The bacterial count of raw mutton:

For *Staphylococcus epidermidis***,** Bacteria content gradually decreased from T_0 to T_5 (Figure-1) with a dilution factor of 10^{-1}, and the lowest bacteria content was found in T_5. There were highly significant differences (p<0.0001) in the bacteria content of the raw mutton among the treatments.

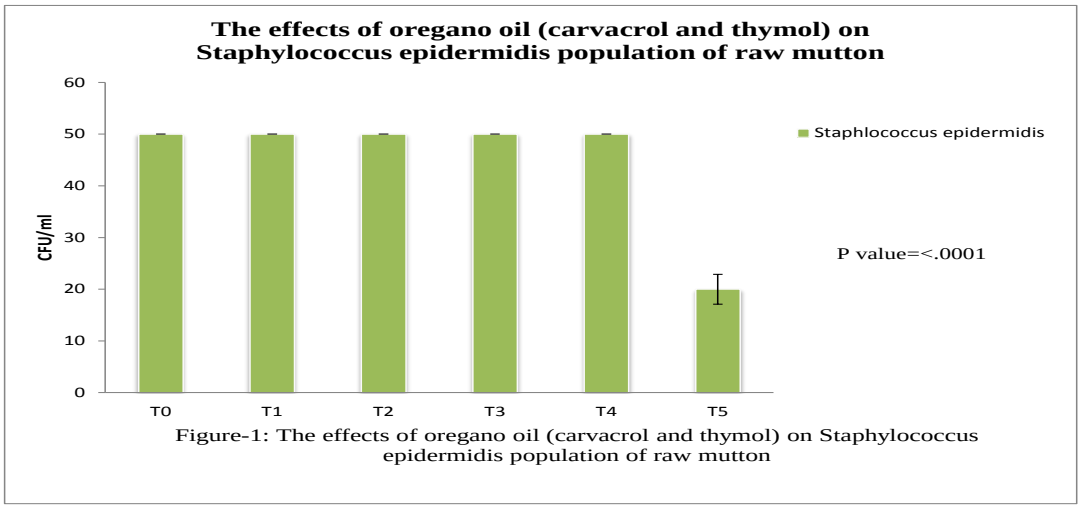

Figure-1: The effects of oregano oil (carvacrol and thymol) on Staphylococcus epidermidis population of raw mutton

T_0 (1gm meat: 0 µl of oregano oil), T_1 (1gm meat: 10µl of oregano oil), T_2(1gm meat: 50µl of oregano oil), T_3 (1gm meat: 100µl of oregano oil), T_4 (1gm meat: 150µl of oregano oil), T_5(1gm meat: 200µl of oregano oil);NS = Non significant; *= p<.05; **= p<0.01;***= p<0.0001.

For *Proteus vulgaris*, Bacteria content decreased from T_0 (Figure-2) with a dilution factor of 10^{-6} and there were highly significant differences (p<0.0001) in bacteria content of the raw mutton among the treatments. Özkalp et al, (2010) obtained data that showed that *Staphylococcus epidermidis* was more sensitive than *Proteus vulgaris* which can be concluded as the gram-positive bacteria were more sensitive to the oregano oil (antimicrobial agent) than did gram-negative ones.

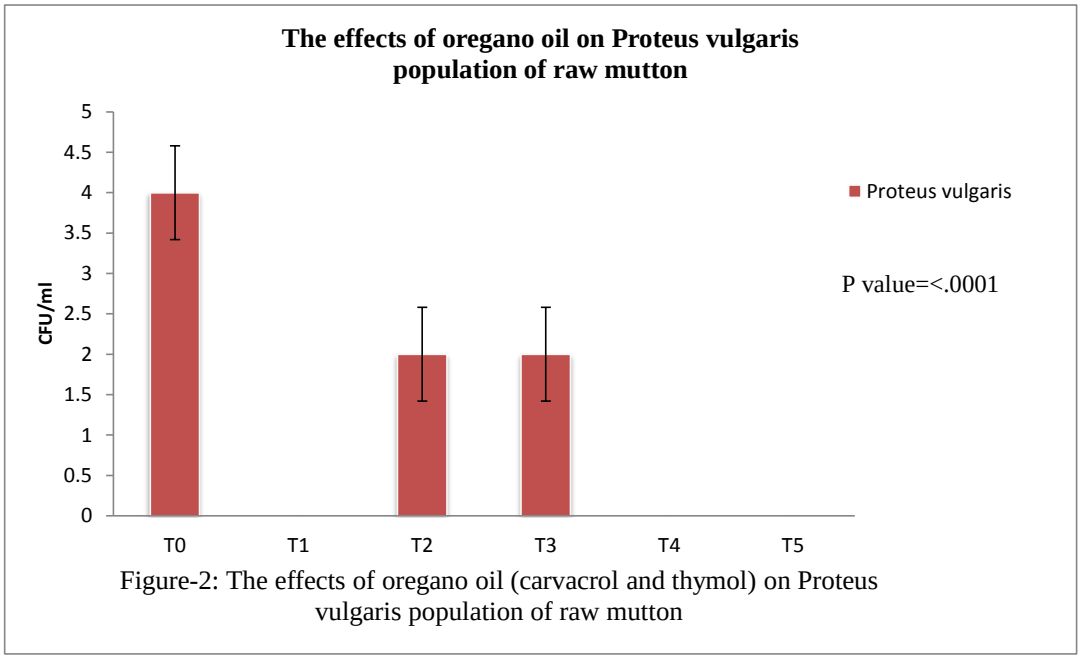

Figure-2: The effects of oregano oil (carvacrol and thymol) on Proteus vulgaris population of raw mutton

T_0 (1gm meat: 0 µl of oregano oil), T_1 (1gm meat: 10µl of oregano oil), T_2(1gm meat: 50µl of oregano oil), T_3(1gm meat: 100µl of oregano oil), T_4(1gm meat: 150µl of oregano oil), T_5(1gm meat: 200µl of oregano oil);NS = Non significant; *= p<.05; **= p<0.01;***= p<0.0001.

For *Staphylococcus aureus*, it had been shown that the highest value was 14.00 in T_0 while the lowest figure was 6.00in T_5 (Figure-3). There was a non-significance difference (p=0.2218) in the mutton among the treatments.

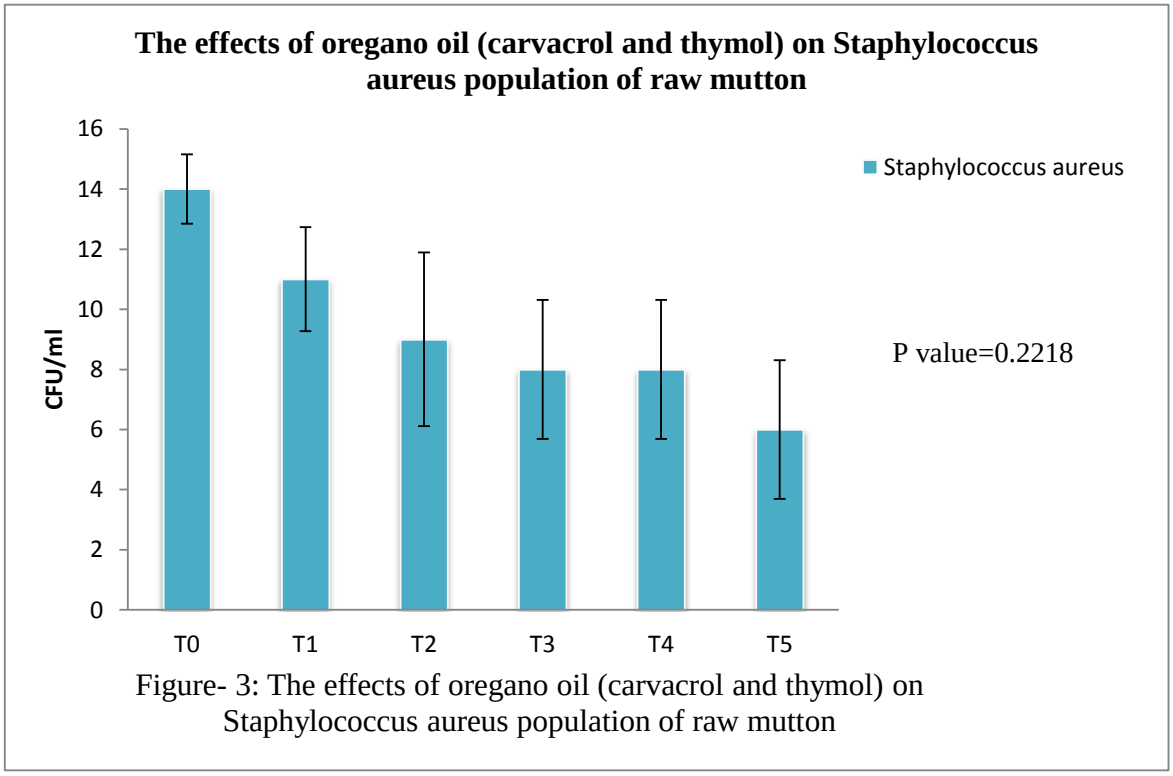

Figure- 3: The effects of oregano oil (carvacrol and thymol) on Staphylococcus aureus population of raw mutton

T_0 (1gm meat: 0 µl of oregano oil), T_1 (1gm meat: 10µl of oregano oil), T_2(1gm meat: 50µl of oregano oil), T_3(1gm meat: 100µl of oregano oil), T_4(1gm meat: 150µl of oregano oil), T_5(1gm meat: 200µl of oregano oil);NS = Non significant; *= p<.05; **= p<0.01;***= p<0.0001

For *Shigella spp.*, it had been shown that the highest value was 11.00 in T_0 while the lowest content was 2.00 in T_5 (Figure-4) There was a non-significance difference (p=0.1090) in the mutton among the treatments.

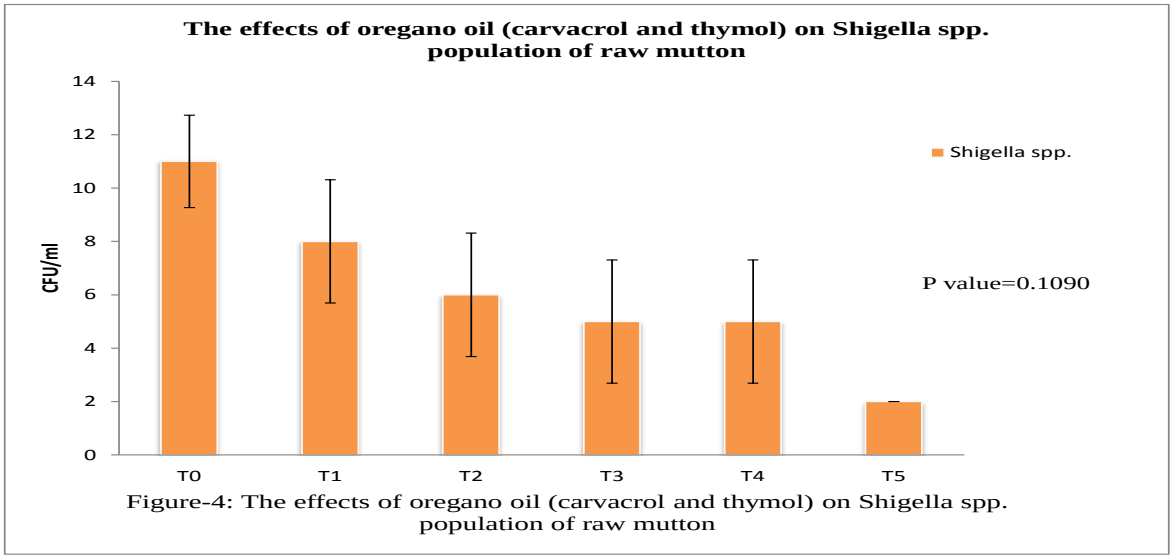

Figure-4: The effects of oregano oil (carvacrol and thymol) on Shigella spp. population of raw mutton

T_0 (1gm meat: 0 µl of oregano oil), T_1 (1gm meat: 10µl of oregano oil), T_2(1gm meat: 50µl of oregano oil), T_3(1gm meat: 100µl of oregano oil), T_4(1gm meat: 150µl of oregano oil), T_5(1gm meat: 200µl of oregano oil);NS = Non significant; *= p<.05; **= p<0.01;***= p<0.0001.

And for *Klebsiella pneumonia***,** it had been shown that the highest value was 35.00 in T_0 while the lowest figure was 5.00 in T_5 (Figure-5). There was a highly significance difference (p value= 0.0092) in the mutton among the treatments.

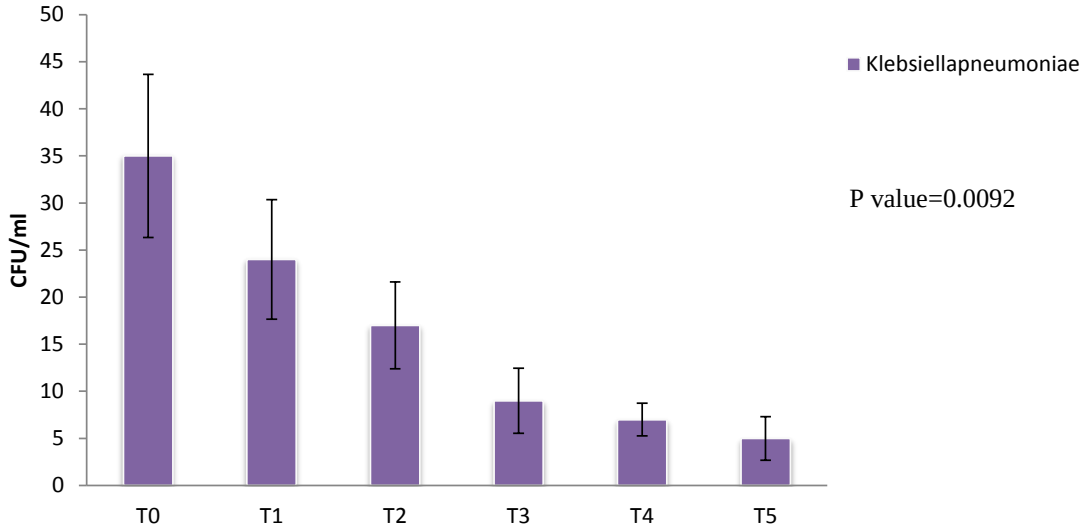

Figure-5: The effects of oregano oil (carvacrol and thymol) on Klebsiella pneumoniae population of raw mutton

T_0 (1gm meat: 0 µl of oregano oil), T_1 (1gm meat: 10µl of oregano oil), T_2(1gm meat: 50µl of oregano oil), T_3(1gm meat: 100µl of oregano oil), T_4 (1gm meat: 150µl of oregano oil), T_5(1gm meat: 200µl of oregano oil);NS = Non significant; *= p<.05; **= p<0.01;***= p<0.0001.

The main effects of these compounds are reducing microbial growth and lipid oxidation during storage. Here, the phenolic component of oregano oil is found to exert a profound inhibitory activity against spoilage organisms including certain pathogens in treated mutton meat. The main effects of these compounds are reducing microbial growth and lipid oxidation (Azad et al., 2022; Hashem et al., 2020 and 2021; Velasco and Williams, 2011). The final counts of the spoilage microorganisms were decreased and microbial development was slowed by oregano essential oil and supported by the result observed by the study of Skandamis and Nychas (2001).

Conclusion

This experiment was designed to see how the chosen bacterial population would respond to varying amounts of oregano oil added to raw mutton. Based on the data analysis, it can be concluded that mutton treated with 200 µl (T5) of oregano oil is extremely acceptable, and that adding additional oregano oil to raw mutton gradually reduces the amount of microorganisms present. There was a highly significant difference in colony-forming units among treatments. Future studies can be directed towards analyzing the sensory quality, proximate composition and consumer acceptance of highly concentrated oregano oil infused food in Bangladesh.

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Conflicts of Interest

The authors declare that there are no potential conflicts of interest.

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