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EFFECT OF EGYPTIAN PROPOLIS APPLIED IN DIFFERENT TECHNOLOGICAL FORMS TO CONTROL *S. TYPHIMURIUM* AND *STAPH. AUREUS* GROWTH IN CHILLED CHICKEN BREAST FILLETS

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ABSTRACT

The current study aimed to estimate the antibacterial inhibitory effect of various technological forms of the Egyptian propolis on Salmonella Typhimurium, as Gm-ve bacteria, and *Staphylococcus aureus*, as Gm +ve bacteria. In addition, to determine the most powerful concentration form of propolis, including ethanolic extract, capsulation and nanoparticles to inhibit the growth of the tested bacteria by the Minimum Inhibitory Concentration (MIC) test. Then, microbiological analysis and sensory evaluation (color, odour, texture and overall acceptance) were analyzed after inoculation of the strains in chilled chicken breast fillet. Additionally, the characterization of the Propolis capsule (PC) and Propolis nanoparticles (PN) was determined. Minimum Inhibitory Concentration test (MIC) result revealed that the propolis had an inhibitory effect on *Staph. aureus* more than *S.* typhimurium as *Staph. aureus* inhibitory concentrations were 10%, 3% and 1% for propolis ethanolic extract (PEE), Propolis capsule (PC) and propolis nanoparticles (PN), respectively. While *S. typhimurium* growth was inhibited by 10%, 5% and 2% for PEE, PC and PN, respectively. By applying the obtained results in chilled chicken breast fillet, which was inoculated with the two strains separately in concentrations of 10%, 5% and 2% for PEE, PC and PN, respectively. The propolis nanoparticles showed the highest reduction effect, reaching 100% on the 15th day and remained with acceptable sensory scores till the end of the experiment, while the salmonella was inhibited by 99.7% by PNs at the end of the experiment with low sensory scores. There were obviously significant variations ($P < 0.05$) between the control and the treated groups, especially at the end of the experiment.

Keywords*: Propolis; Capsulation; Nanotechnology; S.Typhimurium; Staph.aureus.*

INTRODUCTION

Foodborne illnesses cause substantial healthcare resource consumption and socio-economic losses, making them a serious global public health concern (He and Shi, 2021). According to the World Health Organization (WHO) (2022), food-borne illnesses affect about 600 million people each year and cause almost 420,000 fatalities globally (Havelaar *et al.,* 2015). Globally, there were 1729 foodborne illness outbreaks associated with eating livestock meat between 1991 and 2021, which led to 41,438 infections and 10,063 fatalities

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(Warmate and Onarinde, 2023). The main causes of these infections and outbreaks are mixed meats, beef, chicken and pork (Jeffer *et al.,* 2021). Salmonella and Staphylococcus are the most frequent pathogens that cause foodborne illnesses linked to livestock meat (EFSA and ECDC, 2018).

Meat and meat products are known to be a major source of *Staph. aureus* and *S.* typhimurium, which have been implicated in a number of outbreaks (Wu *et al.,* 2018). One of the most commonly consumed meats in Egypt is chicken, and during the stages of processing and preparation, such as slaughtering, evisceration, cutting, distribution, and storage, chicken carcasses tend to be contaminated with those pathogens (Sallam *et al.,* 2015).

Salmonella is a Gram-negative, rod-shaped bacterium that is considered one of the Enterobacteriaceae family that causes a variety of infections in humans and animals, usually through water or food that has been contaminated (Talukder *et al.,* 2023). Salmonella is a major zoonotic pathogen that typically lives in the digestive tracts of warm-blooded animals as poultry. It can persist in food and water due to fecal contamination (*Galán-Relaño et al.,* 2023). To cause an infection, salmonella must be consumed, and it can be destroyed by cooking it properly. However, if raw meat or its drips come into contact with cooked food or raw foods like salads, cross-contamination is still a serious risk (*Galán-Relaño et al.,* 2023).

Salmonellosis affects approximately 1.4 million people in the United States each year, with the majority of cases being foodborne. *Salmonella Enterica* is divided into two serotypes, *S. enteritidis* and *S. typhimurium*, which account for roughly half of these infections (Nazari Moghadam *et al.,* 2023). Regardless of the fact that climate change creates favorable conditions for Salmonella growth, *Salmonella enterica* is expected to become more common and persistent, antibiotic-resistant strains emerge, and the diseases spread worldwide as a result of intensive farming and global trade (Morgado *et al.,* 2021).

Staphylococcus aureus (*Staph.aureus*) is a widespread Gram-positive bacterium and a significant cause of food poisoning outbreaks. It can colonize and infect a wide range of hosts, including both humans and animals (Wang *et al.,* 2017). Animal-derived foods, such as red meat and chicken, have been linked to numerous Staphylococcal food poisoning outbreaks. *Staphylococcus aureus* replicates rapidly at room temperature, resulting in toxin production that causes food poisoning (Wu *et al.,* 2018).

Recent and innovative technology can be used to apply natural products to control foodborne pathogens, paving the way for a different approach to treatment (Čengić *et al.,* 2025).

Apitherapy is the term for the use of bee products for medical purposes, which have been used for centuries in traditional ancient medical practices to treat and prevent diseases. Wax, venom, and royal jelly are examples of bee secretions that are used in apitherapy, while honey, pollen, propolis, and bee Perga are bee products made by modifying plant-derived materials (Al Naggar *et al.,* 2021).

Propolis is a resinous product of bees that is created from the mixing of wax, bee secretions, and plant exudates. The antibacterial properties of propolis were confirmed by a systematic review that included research data on 600 various bacterial strains. The antimicrobial qualities of propolis are due to its chemical composition. Resin, which is composed of phenolic and flavonoid compounds, is a significant component of propolis, in addition to specific polar and phenolic lipophilic molecules, especially flavonoid compounds. These molecules harm the

membranes and cell walls of bacteria when they contact them, ultimately leading to cell lysis and death. (Przybyłek and Karpiński, 2019). In the food industry, Propolis can be used in a variety of cutting-edge technological forms, such as capsulation and nanotechnology.

A significant breakthrough in food safety and quality preservation is propolis nanoparticles. These nanoparticles improve the antimicrobial activity and bioavailability of propolis. This novel method not only increases propolis's solubility but also makes it possible to incorporate and deliver it into a variety of food products to improve nutritional value and prevent foodborne illnesses (Bezerra *et al.,* 2023). Propolis capsulation is the process of coating propolis in a protective layer to improve its bioavailability, stability, and solubility. This process enhances the value of the encapsulated propolis in food and pharmaceutical applications by preventing its degradation, control the released bioactive compounds, and improving its solubility in water or any other fluids (Jansen-Alves *et al.,* 2023).

So, the aim of this study is to investigate the inhibitory effect of propolis in different forms, including nanoparticles, capsulated and ethanolic extract, on *S.*typhimurium and *Staph.aureus* in Vitro, to determine if the most active form that can be used later in food industry

MATERIALS AND METHODS

1-Propolis samples

In the spring and summer of 2024, propolis samples were gathered from honeybee hives in Benha, Al-Qaluobia, Egypt. Samples of propolis were hygienically cleaned, cleared of wood, paint, and wax, chopped into tiny pieces, and put in a sterile container.

2-Preparation of propolis ethanolic extract (PEE)

A volumetric flask containing 10 grams (gm) of propolis and 100 milliliters (ml) of 70% ethanol had been placed for five hours at room temperature in a shaker incubator with vigorously shaking (Ghavidel *et al.,* 2021). After cooling to room temperature and centrifuging the final extract for five minutes at 1500 rpm, the supernatant was moved to a new container for another use. The excerpt, which was kept at -20ºC until the experiments began, was filtered using the Whatman paper filter No. 1. A 0.45-m filter was used to filter and sterilize the samples prior to adding the extracts (Said *et al.,* 2006).

3-Preparation of propolis nanoparticles (PN)

In the Nanomaterials Research and Synthesis Unit, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Egypt, propolis nanoparticles (PNs) were made. A 1500-watt homogeneous blender was used to homogenize 10 mL of Propolis Extract (PE), 10 mL of between 80 and 80 mL of distilled deionized water for 30 minutes. Distilled water was then progressively added to the mixed oil phase (Rao., 2011).

4-Preparation of algeniate-propolis capsulated (PC)

After dissolving 1% (w/v) sodium alginate in 30% (v/v) ethanol solution, 20 mL of propolis extract was added to the 80 ml alginate solution and mixed thoroughly with homogenizer (Rajaonarivony *et al.,* 1993).

5- Determination of propolis nanoparticles characterization

High-resolution transmission electron microscopy (HRTEM) monitoring at Cairo University's Faculty of Agriculture was used to measure the electrical conductivity and characterization of the nanoemulsion using a JEM 1400F HRTEM adapted with a 300 keV beam energy (Cui *et al.,* 2022).

6-Determination of propolis capsule characterization

The PC's electrical conductivity, surface charge (zeta potential), droplet size, and size distribution (polydispersity indexes (PDI)) were all measured using a dynamic light scattering (DLS) instrument (MICROTRAC, USA). Before the zeta-potential measurements, the samples were diluted with distilled water (pH 3.2 and 7.5) at 26.7 ^{O}C and the viscosity equal to 0.857 (Saloka *et al.,* 2013).

7-Minimum inhibitory concentration test (MIC)

The lowest concentration of propolis extract that inhibited the examined microorganisms growth, was defined as the minimum inhibitory concentration. With a few modifications, the MIC-based macro-dilution method was determined according to the National Committee for Clinical Laboratory Standards (NCCLS) (2000).

7-1-Bacterial strain

Salmonella enterica serovar *typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 6538) were obtained from Animal Health Research Institute, Shibin Elkom branch, Food Hygiene Department, and used to investigate the antibacterial activity of PEE, PN and PC samples

7-2-Antibacterial activity test

Prior to testing, all strains were cultured for one night at 37 °C on Tryptic Soy Agar (TSA) (OXOID, Basingstoke, UK). Two bacterial suspensions with corresponding concentrations of 1 × 106 CFU/mL were made starting with each strain. In accordance with the Clinical and Laboratory Standards Institute (CLSI., 2018), bacterial suspensions with a 0.5 McFarland standard, or 1 × 10^8 CFU/mL, were made to achieve the predetermined concentrations. Plate bacterial counts and serial dilutions were used to verify the concentrations. After gradually diluting in sterile saline solution (0.9%), 10 microliters of the suspensions with a concentration of 10^8 CFU/mL were diluted to 10^6 CFU/mL in 10 µL of the diluent was then inoculated in every spot.

7-3-Preliminary test

The first preliminary efficacy test on both strains was conducted using a 10% concentration of each treated sample. Using standard inoculation techniques, as a bacteriological loop, a sterile swab, and a spot, 10^8 CFU/mL suspensions of the bacteria were obtained and inoculated in a plate containing XLD agar media for *S.* typhimurium and Baired Parker agar media mixed with egg yolk for *Staph. aureus* to which examined treated materials (PEE, PC, and PN) were added. Regardless of the inoculation technique, the growth of *S. typhimuruim* and *Staph. aureus* was completely inhibited when each antibacterial sample was present in the additional medium.

7-4-Preparation of efficiency test

Agar plates with XLD media and Baired Parker with egg yolk media were prepared in two groups. The bacterial concentration (10^6 CFU) for each strain was tested using concentrations of PEE, PN, and PC ranging from 10% to 1% (10%; 9%; 8%; 7%; 6%; 5%; 4%; 3%; 2%; 1%). The plates were incubated at 37 °C for 24 hours, and the presence of growth or not in the spot was used to determine the effectiveness of the antibacterial agents. Every experiment was conducted triple on two separate days.

8-Experimental design

8-1-Purchasing chicken samples

Chilled chicken breast fillets (CBF) were purchased from a freshly slaughtered chicken carcass local market in Benha, Al-Qaluobia, Egypt. Weighing nearly 6 kg and transferred into the laboratory in an ice box without any delay, then cut into pieces aseptically in the laboratory.

8-2-Inoculation of samples

The chilled chicken breast pieces were exposed to UV light for 30 minutes in a

safety cabinet (15 minutes on each side) to lower the number of native bacteria before inoculation. In order to allow for bacterial attachment to the samples, each chilled chicken fillet sample was inoculated with 1 mL of inoculum (10^6 CFU/g) on the meat sample, 500 µL on each side.

8-3-Preparation of the samples

The samples were separated into two major groups, each group weighing 3 kg. The first group was inoculated with *S.*typhimurium (1×10^6 CFU/ml), while the second major group was inoculated with *Staph.aureus* pathogen (1×10^6 CFU/ml). Then, each major group was divided into four sub-groups:

- The first group is control one (C),
- The second group was treated with propolis ethanolic extract (PEE10%),
- The third group was treated with propolis capsulated (PC5%)
- The fourth group was treated with propolis nanoparticles (PN2%).

Each group's samples were put separately into a sterile plastic bag, sealed hermetically, and chilled in the refrigerator at 4±1 °C for up to 18 days (until organoleptically spoiled). The difference in the concentration of each treated antibacterial sample was calculated according to the results of the MIC test applied previously in the same study. Each experiment was conducted in triplicate.

9-Bacteriological analysis

On days zero, three, six, nine, twelve, fifteen, and eighteen of storage, the total numbers of *S.* typhimurium and *Staph. aureus* in the chilled chicken breast fillet groups was examined. To prepare the initial dilution in a sterile stomacher plastic bag, 10 grams of each sample were aseptically weighted into 90 milliliters of 0.1% peptone water (NEOGEN NCM0015A) in a Stomacher 400 Lab Blender (Seward Medical, London, UK) for 60 seconds. Ten-fold serial dilutions were thus made for bacteriological analysis.

To count *S.* typhimurium, one milliliter (1ml) of the original dilution was spread-plated on XLD agar (Biolife 4022062) using the procedure outlined by the International Organization for Standardization (ISO, 2017). Salmonella colonies on XLD agar typically have a black center and a light reddish zone. For twenty-four hours, the inoculated plates were incubated at 37 °C. Using the spread method, *S. aureus* was spread on the surfaces of plates of Baird-Parker selective agar (NEOGEN NCM0024A) suppl-emented with 20% egg-yolk tellurite emulsion and then incubated for 48 hours at 37 °C (ISO, 2021). Colonies of *S. aureus* are typically round, smooth, convex, shiny, black, and 2–3 mm in diameter. They have an opaque halo encircled by a clearing zone that is 2–5 mm in diameter. As an initial count, plates with typical colonies were counted.

10-Sensory evaluations

Five experts' panelists assessed each sample. Ten grams of chicken breast fillet were given to each group, and they were asked to rate the texture, color, and odor. Random numbers were used to code the samples; panelists were unaware of the method of the experiment. They were requested to give a score indicating (color, odor and texture) of each sample. According to Pimentel (2016), a descriptive scale with nine points was employed, with nine being the highest and one being the lowest.

11-Statistical Analyses

Graph Pad Prism 8.0.2 and two-way analysis of variance (ANOVA) with $P<0.01$ (Geisser-Greenhouse's Epsilon) were used to statistically analyze the data. Statistical analysis was used to examine how propolis in different forms and concentrations can affect the growth of Gram-positive and Gram-negative bacteria in cold-stored chicken breast fillets. Tukey's HSD test was employed in post hoc analysis to determine which treatments significantly differed after

the two-way ANOVA yielded significant results. All results were reported by Greenhouse and Geisser (1959) as the mean±SD of three triplicates. A $P<0.05$ was used to define significant differences, and trends were reported when $0.05>P<0.10$.

RESULTS

Fig (1) shows the particle size, morphology, and size distribution, which are some variables that influence the size and shape of the particles during the nanoemulsion production process. Usually, measurements of the particles' size and shape are taken with HRTEM, the PN particles showed high homogeneity with an average length of 19.783 nm and a narrow size distribution (polydispersity index: 0.253). Subsequent investigation showed that there was no particle aggregation and that the morphology was uniformly spherical.

The propolis capsulated extract demonstrates moderate zeta potential stability (15.5 mV) with positive polarity, reducing aggregation risks (Fig. 2a). Narrow particle size distribution around 35-40 nm (Fig.2b), and PDI around 0.1305 indicated a narrow size distribution, low particles size variation, in addition to the suitability for applications requiring uniformity, such as nanomedicine. These properties highlight the extract's potential for stable dispersion and efficient performance in various formulations.

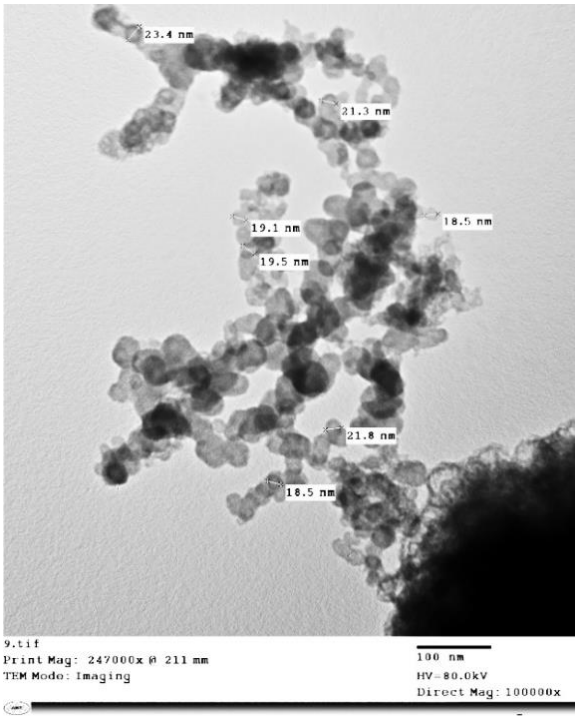

Figure 1: The droplet size of and propolis nanoparticles (PN) was measured using high-resolution transmission electron microscopy (HRTEM).

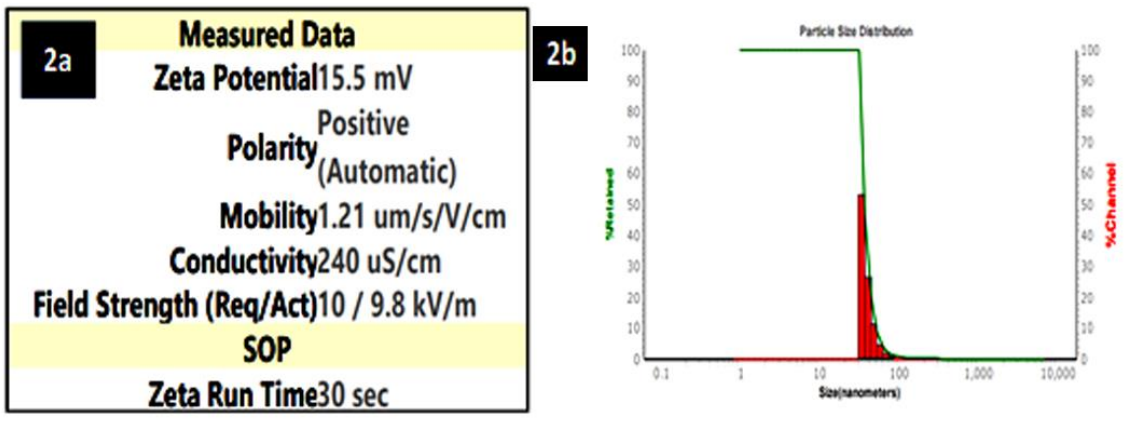

Figure 2: The characterization of Propolis capsule (PC).

The present study tested on the antibacterial efficacy of propolis against *S.*typhimurium and *Staph.aureus* using different forms of the extracts (PEE, PC and PN) (Fig. 3). It was observed that all three forms of extract showed antimicrobial activity against S. Typhimurium at different concentrations ranging from 10%, 5% and 2% of PEE, PC

and PN, respectively. While *Staph. aureus* was also affected by the different extracts, but at different concentrations that recorded 10%, 3% and 1% of PEE, PC and PN, respectively. Based on the calculated MIC of the three Propolis-treated forms, it was concluded that the best results were shown by the PN form rather than the other forms.

Table (1) describes the effect of treatments on the count of *S.typhymiurium* pathogen. On zero day, there is no significant difference between the control and PEE group, while a significant difference is recorded between them and PC as well as PN groups, with a value ($P<0.05$). On the 3rd day, the Salmonella levels increased in all groups, with a significant difference compared to Day zero. The rate of increase varied, as the control, PEE10%, PC5%, and PN2.5% values equal 6.97, 5.87, 5.34 and 5.05 log10cfu/g, respectively. On the 6th day, the control group showed a sharp increase, while the treated groups demonstrated significantly lower counts, especially PN 2%. In addition to the significant difference between each group, which is relatively consistent, reflecting reliable data collection. On the 9th day, the control group spoiled, and the remaining treatments continuously inhibited the bacterial count, with remarkable inhibition for the PN2% group till the 12th day. On day 15, the PEE10% group also spoiled, and the PC5% group showed a surprisingly increased count (4.15 ± 0.25 $\log_{10}$cfu/g) from the previous records, as well as a significant difference from the PN group ($P<0.05$). At the end of the experiment, the PN2% group continually steady, with a reduction equal to 2 Log from the beginning.

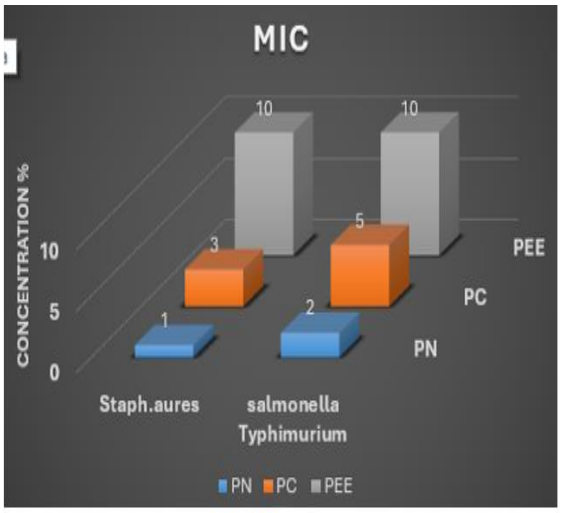

Figure 3: The result of MIC based on the microdilution method for *S. Typhimurium and Staph. Aureus.*

Table 1: The different effects of PEE, PC and PN on the viability of S. Typhimurium during the chilled storage period.

Groups / Storage period	Control	PEE (10%)	PC (5%)	PN (2%)
Zero day	$6.46 \pm 0.20^{A,a}$	$6.26 \pm 0.18^{A,a}$	$6.01 \pm 0.11^{A,b}$	$5.78 \pm 0.20^{A,c}$
3rd day	$6.97 \pm 0.15^{B,a}$	$5.87 \pm 0.25^{B,b}$	$5.54 \pm 0.20^{B,c}$	$5.25 \pm 0.12^{B,d}$
6th day	$7.35 \pm 0.25^{C,a}$	$5.56 \pm 0.14^{C,b}$	$5.11 \pm 0.17^{C,c}$	$4.61 \pm 0.19^{C,d}$
9th day	S	$4.95 \pm 0.24^{D,a}$	$4.45 \pm 0.15^{D,b}$	$4.15 \pm 0.20^{D,c}$
12th day	S	$5.68 \pm 0.35^{E,a}$	$3.95 \pm 0.20^{E,b}$	$3.24 \pm 0.15^{E,c}$
15th day	S	S	$4.15 \pm 0.25^{F,a}$	$2.11 \pm 0.11^{F,b}$
18th day	S	S	S	3.22 ± 0.15^{G}

*PEE: Propolis Ethanolic Extract *PC: Propolis Capsule
*PN: propolis Nanoparticles *S: Spoilage

The results are significantly different when P value<0.05. The different superscribed small letters represented the significance between different groups, while the different superscribed capital letters represented the significance between storage periods. The results are expressed as mean ± SD.

The *Staph. aureus* counts of the control and treated groups were shown in Table (2). According to the data, at zero day of chilled storage, the mean staph count ranged from 5.71 to 5.19 (log cfu/g) with no discernible variations. On the sixth day of cold storage, the control group's count rose to 7.05 log cfu/g. However, the count in the treated group dropped from 5.55, 5.26, and 5.19 to 4.77, 4.4, and 3.99 (log cfu/g) in PEE 10%, PC 2%, and PN 1%, respectively, at a slower rate than the control group. Day 12 showed a sharp rise in the PEE 10% group's count, whereas PC 2% and PN 1% showed total suppression of *S. aureus* growth by this time, indicating superior efficacy compared to PEE 10%.

The previously obtained results that *Staph.aureus*, which represents Gram+ve bacteria, is more affected by the propolis antibacterial effect than *S.typhimurium* confirmed via the data in Table (3), representing Gram-ve bacteria. The results also confirmed the fact that PN is a powerful form of propolis, when compared to other treated forms.

Table 2: The different effects of PEE, PC and PN on the growth of *Staph.aureus* during the chilled storage period

Groups / Storage period	Control	PEE (10%)	PC (5%)	PN (2%)
Zero day	$5.71 \pm 0.11^{A,a}$	$5.55 \pm 0.05^{A,a}$	$5.26 \pm 0.14^{A,b}$	$5.19 \pm 0.13^{A,b}$
3rd day	$6.61 \pm 0.03^{B,a}$	$5.15 \pm 0.07^{B,b}$	$4.77 \pm 0.09^{B,c}$	$4.41 \pm 0.05^{B,c}$
6th day	$7.05 \pm 0.19^{C,a}$	$4.77 \pm 0.01^{C,b}$	$4.40 \pm 0.06^{C,c}$	$3.99 \pm 0.02^{C,d}$
9th day	S	$4.20 \pm 0.07^{D,a}$	$3.79 \pm 0.12^{D,b}$	$3.67 \pm 0.04^{D,c}$
12th day	S	5.01 ± 0.13^{E}	ND*	ND*
15th day	S	S	ND*	ND*
18th day	S	S	S	ND*

*PEE: Propolis Ethanolic Extract *PC: Propolis Capsule
*PN: propolis Nanoparticles *S: Spoilage

The results are significantly different when P value<0.05. The different superscribed small letters represented the significance between different groups, while the different superscribed capital letters represented the significance between storage periods. The results are expressed as mean ± SD.

Table 3: The reduction % in *S. Typhimurium* and *Staph.aureus* of the examined chilled chicken fillet during storage.

Inoculated bacteria	Treated groups	Storage period (days)						
		0	3	6	9	12	15	18
S.Typhimurium	Control	-	-	-	S	S	S	S
	PEE10%	-	47.44	69.45	90.44	62.17	S	S
	PC5%	-	55.71	80.25	95.05	98.49	97.53	S
	PN2%	-	61.77	89.58	96.35	99.69	99.99	99.71
Staph.aureus	Control	-	-	-	S	S	S	S
	PEE10%	-	52.66	78.01	93.84	64.07	S	S
	PC5%	-	62.38	83.22	96.22	100	100	S
	PN2%	-	80.38	92.79	96.87	100	100	100

*PEE: Propolis Ethanolic Extract *PC: Propolis Capsule
*PN: propolis Nanoparticles *S: Spoilage

Figure (4) illustrates the sensory evaluation of all samples inoculated with the two different strains separately and treated with propolis. At zero day, all samples showed high scores with no significant differences (P≥0.5), while at the 3rd day, samples in all treated groups began to show differences, especially control group declined to 6.5±0.5 in salmonella group and treated groups (PN, PC) retained higher scores, especially PN (8.5±0.25). Samples inoculated with *Staph.aureus* bacteria also showed less decline in treated groups, with PN also performing better (8.5±0.2). Decline rates in sensory scores were generally steeper for Salmonella compared to Staph. aureus, particularly in the early storage period (3rd to 6th day). The sensory scores of control samples in the two major groups declined gradually, till reaching spoilage on the 9th day, while the PN groups remained high. On the 12th day, the Salmonella group showed an acceptable score (6 ± 0.5) for PN, and other subgroups showed significant declines. Propolis nanoparticles (PN) sub-group of *Staph.aureus* group on the same day showed better performance (6.5±0.25), but declines were less pronounced compared to Salmonella. The results continued in the same way to the end of the experiment, as Staphylococcus showed slightly better maintenance of sensory attributes in treated groups, especially beyond the 12th day

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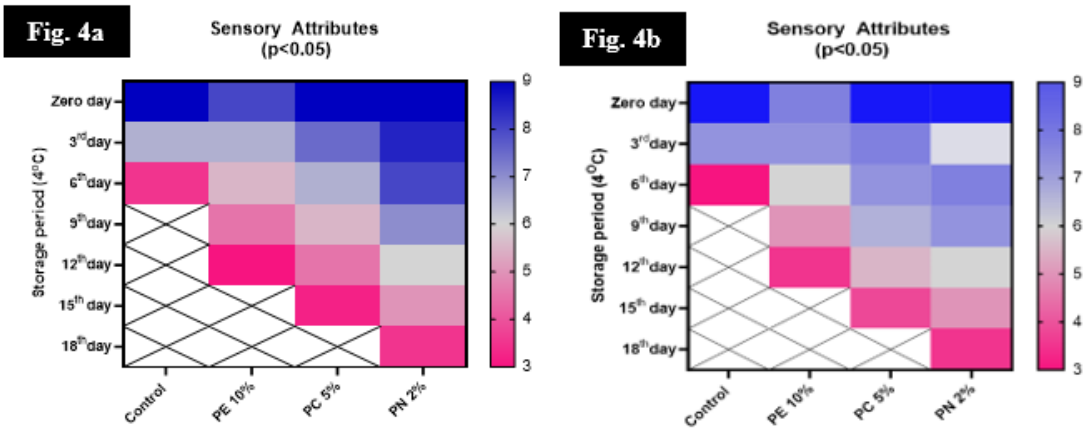

Figure 4: The sensory evaluation in the samples inoculated with *S. Typhimurium* (Fig. 4a) and the other inoculated with *Staph. aureus* (Fig. 4b)

DISCUSSION

Foodborne illnesses are a major problem in the Eastern Mediterranean Region. Foodborne illnesses affect 100 million people in the region annually, with 32 million cases involving children under the age of five, as recorded in World Health Organization estimates from 2015 (WHO, 2015).

About 70% of the foodborne illness burden in the region is attributed to pathogen-caused foodborne diarrheal illnesses. In the region, eating contaminated food kills about 37,000 people annually, mostly from brucellosis, typhoid fever, hepatitis A, and foodborne diarrheal illnesses. In order to reduce the negative effects of these illnesses on health and wellbeing both locally and internationally, food safety measures are essential (Abd El-Ghany, 2020). Egypt still needs additional efforts to overcome the problems of foodborne illness by creating innovative solutions with recent technology.

With the aid of modern technology, man has frequently looked to plants as a source of food, medications, and antioxidants to enhance his quality of life. According to AL-Ani *et al.* (2018), propolis is one of the greatest promising sources of bioactive

ingredients with antimicrobial activity. Propolis and its derivatives have demonstrated strong antibacterial activity against both Gram-positive and Gram-negative bacteria in numerous scientific investigations (Anjum et al., 2019, Przybyłek and Karpiński, 2019, Rufatto et al., 2017).

The current findings revealed that the propolis with different forms showed an inhibitory effect on both food-borne pathogens, *Staph.aureus* (Gram-positive bacterium) more than *S.typhimurium* (Gram-negative bacterium), depending on the propolis forms and concentration. The obtained findings are consistent with several earlier investigations that found that Gram-positive bacteria only needed a small concentration of propolis, whereas Gram-negative bacteria were significantly inhibited by it at higher concentrations. These results were consistent with those of Mahdavi-Roshan *et al.* (2022), who used propolis extract at varying concentrations (4, 8, and 12%) on marinated chicken fillet to record their effect on *Staph.aureus*, and *E.coli*. They found that the extract was more effective in inhibiting the growth of *Staphylococcus aureus*, exhibiting an inhibitory effect of 2 log/cfu at the lowest concentrated treated group, while *E.coli* did not even reach an inhibitory effect of 1 log/cfu at the highest concentrated treated group. The results of the study by Przybyłek and Karpiński., (2019) match up with these findings.

A study investigated the antibacterial effects of propolis (50 mg/mL to 0.78 mg/mL) and honey on 12 distinct bacterial species, including *S.typhimurium* and *Staph.aureus*, Vică *et al.* (2024) found that Gram-positive bacteria are more susceptible to propolis activity than Gram-negative bacteria. Also, Bryan *et al.* (2015) used the MTT assay to report on the antibiofilm activity of Russian propolis ethanol extracts (RPEE) on mature biofilm. In agreement with our findings, their study demonstrated a 50% reduction in *S.aureus* viability at a high concentration (5% w/v) of RPEE. Confocal and scanning electron microscopy images, however, showed complete cell lysis of bacterial biofilms following 18 hours of treatment at relatively high RPEE concentrations (20% w/v) (Bryan *et al.,* 2015). These findings can be compared with the achieved results in this study, which showed complete inhibition of *Staph.aureus* at the same concentration. This can be explained by the geographical origin of the propolis, which affects the bioactive chemical constituents on which the validity of the antibacterial extract.

Propolis' chemical components are responsible for its antimicrobial properties. One important component of propolis is resin, which is made up of phenolic and flavonoid compounds. There are also oils and candles with oleic and palmitic acids, as well as fiber from essential oils and aromatics. Low in quantity, pollen contains vitamins, minerals, free amino acids, and protein. Propolis also contains other substances like sugars, steroids, lactones, and ketones (Değirmencioğlu, 2018). Certain polar and phenolic lipophilic molecules, particularly flavonoid compounds, are responsible for propolis' antibacterial properties. When these molecules come into contact with bacterial cells, they damage their membranes and cell walls, which eventually results in cell lysis and death. According to Echeverría *et al.* (2017), propolis has antibacterial qualities because of its many highly polar and lipophilic groups, including carbonyl, electronegative, amine, imine, sulfide, thiol, methoxy, and hydroxyl groups. In the context of recent studies, phenolic compounds such as quercetin, naringenin, and caffeic acid are thought to be the most active and effective substances against the microorganisms under study (Ristivojevic *et al.,* 2016).

It's worth noting that the outer membrane of Gram-negative bacteria either prevents or delays propolis's penetration, which is

the most likely explanation for their low sensitivity to propolis extract. According to Tegos *et al.* (2002), they may also have multidrug resistance (MDR) pumps, which extrude amphipathic toxins across the outer membrane.

The current study's second purpose was to determine the most powerful and effective form of propolis as an antibacterial natural extract. So, based on the obtained results, it was referred to that the antibacterial effect of propolis nanoparticles was higher than that of propolis-capsule; however, propolis-capsule was more effective than the propolis ethanolic extract. This can be explained by the propolis nanoparticles' greater surface area-to-volume ratio and smaller size. Because of these characteristics, they are extremely reactive and can circumvent some of the drawbacks of raw propolis. Propolis nanoparticles are typically used to improve pharmaceutical substances' delivery to their intended targets (Afrasiabi *et al.,* 2020).

Regarding the greatest antibacterial effective results of propolis-capsule, most people concur that propolis extract has greater antibacterial activity than propolis-capsule (Jansen-Alves *et al.,* 2019), some have found that propolis-capsule had more inhibitory effects than their extracts (Hegazi *et al.,* 2019). The best assessment was likely provided by Jansen-Alves *et al.* (2019), who discovered that the mass ratio of propolis to wall material determined the antibacterial activity of encapsulated propolis, which in turn depended on the percentage of wall material used for encapsulation.

According to the study's findings, propolis's antimicrobial properties in various forms have the potential to be utilized as alternative medications because of their therapeutic potential. They may also be a useful source for creating functional foods.

CONCLUSIONS

The current study provides evidence that Egyptian propolis, especially propolis nanoparticles, had a powerful effect on limiting and inhibiting the growth of *Staphylococcus aureus*, representing Gram-positive bacteria, more than S*almonella* serovar *typhimurium*, representing Gram-negative bacteria in chilled storage chicken breast fillet.

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تأثير البروبوليس المصري المُطبق بأشكال تكنولوجية مختلفة في التحكم في نمو بكتيريا السالمونيلا تايفيموريوم (*Salmonella typhimurium*) والعنقودية الذهبية (*Staphylococcus aureus*) في شرائح صدور الدجاج المُبردة أثناء التخزين

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تهدف هذه الدراسة الى قياس مدى كفاءه مادة البروبليس بانماطها التكنولوجيه المختلفة متضمنة المستخلص الايثيلى والكبسلة وكذلك المواد النانومترية، وذلك لمعرفة قدرتها فى التحكم فى نمو بكتريا سالمونيلا تيفيميوريم (ممثلة للبكتريا السالبة غرام) وبكتريا المكورات العنقودية الذهبية (ممثة للبكتريا الموجبة غرام). كما اشتملت الدراسة على معرفة افضل تركيز ونمط تكنولوجى يمكن استخدامه من تلك المواد التجريبية لتحقيق هدف التحكم فى نمو كل بكتريا على حدى، بالاضافة الى بعض القياسات الميكروبيولوجية و الحسية التى تم تقديرها فى صدور الفراخ المخلية المبردة بعد حقنها بالبكتريا المذكورة وتطبيق عليها المواد محل الدراسة، والتى خضعت ايضا لبعض الاختبارات لمعرفة قياساتها وخواصها. فكانت النتيجة ان مادة البروبليس اظهرت كفاءه وقدرة تاثيرية على بكتريا المكورات العنقودية الذهبية اكثر من تاثيرها على بكتريا السالمونيلا تيفيميوريم، حيث تم تقدير التركيزات المؤثرة على نمو بكتريا الكرات العنقودية الذهبية كالاتى: 10% لمستخلص البروبليس الايثيلى، 3% للبروبليس الذى تم كبسلتة، و1% لجزيئات البروبليس النانومترية. وبالنظر الى التركيزات المؤثرة على نمو بكتريا السالمونيلا تيفيميوريم، فقد تم تقدير الاتى: 10% لمستخلص البروبليس الايثيلى، 5% للبروبليس الذى تم كبسلتة، و2% لجزيئات البروبليس النانومترية. اشتملت ايضا الدراسة فى جزئها الاخير على تطبيق تلك المواد (بتركيزات 10% لمستخلص البروبليس الايثيلى، 5% للبروبليس الذى تم كبسلتة، و2% لجزيئات البروبليس النانومترية) فى صدور الفراخ المخلية المبردة والتى تم حقنها مسبقا بالبكتريا المذكورة بتركيز معلوم والذى اوضح مدى قدرة جزيئات البروبليس النانومترية على القضاء على بكتريا المكورات العنقودية الذهبية والتى وصلت الى 100% بحلول اليوم الخامس عشر مع الحفاظ على الخواص الحسية، بينما وصلت قدرة نفس المادة الى 99.7% فى القضاء على بكتريا السالمونيلا تيفيميوريم بحلول نهاية التجربة ولكن الخواص الحسية لم تكن فى افضل احوالها. لذا، فدراستنا اكدت على حقيقتين علميتين وهما ان مادة البروبليس ذات قدرة تثبيطية هائلة لنمو بكتريا المكورات العنقدية الذهبية (ممثة للبكتريا الموجبة غرام) اكثر من بكتريا السالمونيلا تيفيميوريم (ممثة للبكتريا السالبة غرام)، وكذلك فان افضل نمط تكنولوجى لتطبيق مادة البروبليس هو جزيئاتها النانومترية والتى اظهرت قوة تحكمية هائلة فى نمو كلا من البكتريا محل الدراسة مع الحفاظ على الخواص الحسية لصدور الفراخ المخلية المبردة.