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Research article

Bee venom: A potential natural alternative to conventional preservatives for prolonging the shelf-life of soft cheese 'Talaga'

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ABSTRACT

The study aims to explore bee venom (honey-BV) as a potential natural preservative for "Tallaga" soft cheese. Characterization of the active compounds in honey-BV was conducted via chromatographic analyses. Antimicrobial efficacy against pathogenic bacteria and fungi was evaluated, and minimum inhibitory concentration (MIC) was determined. Subsequently, honey-BV was applied to Tallaga cheese at 15 mg/g concentrations. The main active ingredients identified in bee venom were apamin (2%) and melittin (48.7%). Both concentrations of bee venom (100 and 200 mg/mL) exhibited significant antifungal and antibacterial properties against tested organisms, with MIC values varied from 0.2 to 0.5 mg/mL for bacteria to 3–13 mg/mL for fungi. Application of honey-BV in Tallaga cheese resulted in complete elimination of Staphylococcal populations after 2 weeks of cold storage, with no detectable growth of molds or yeasts throughout the storage period. Additionally, a steady decrease in aerobic plate count was observed over time. In summary, honey-BV holds promise as a natural preservative for soft cheese, however, more investigation is required to optimize the concentration for economic viability, taking into account health benefits and safety considerations.

1. Introduction

Honey bee venom (honey-BV), apitoxin, is a crucial weapon that honey bees (*Apis mellifera*) use for self-defense, and is produced in poison glands within the abdomen. According to Park et al. [1] and Lee [2], honey-BV is a clear liquid with an acidic response, a fragrant smell, a bitter taste, and it dissolves in water. It has long been utilized in traditional medicine to treat inflammatory conditions including rheumatism and arthritis and to reduce pain [3–5]. It has been demonstrated that honey-BV contains a number of biochemically or pharmacologically active substances, such as enzymes (phospholipase, histidine decarboxylase, hyaluronidase), amines (histamine, dopamine, serotonin, and norepinephrine), and polypeptides (apamin, melittin, and mast cell degranulating peptide) [6–9]. Nowadays, interest in honey-BV as a complementary and preventative medicine has increased [10]. In addition, the inherent antibacterial properties of honey-BV have long been recognized [11–14]. As well, honey-BV has been likened to natural penicillin and

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has been reported to exhibit antibacterial activity against both gram-positive and gram-negative bacteria [15–18]. Moreover, the antifungal activity for honey-BV was reported by Ref. [19]. AS well, Elfiky et al. [20] studied the safety of bee venom preparation for marketing strategy. They assessed the safety of bee venom aqueous preparation by hematological, biochemical and histopathological examinations using experimental rabbits and concluded that bee venom was considered as a safe medication.

Currently, antimicrobial resistance is one of the most significant global challenges. It threatens the efficient protection against infections caused by viruses, bacteria, fungi, and parasites [21]. The bioactive natural products serve as a vital source of novel antimicrobial agents with broad effectiveness and a reduced likelihood of inducing antimicrobial resistance. Given the rising demand for natural products and the escalating threat posed by multidrug-resistant microorganisms, it is crucial to explore biologically active substances from diverse taxonomic groups and ecological niches.

Preservatives are compounds added, whether natural or synthetic, to edible products, medications, and beauty products. Their purpose is to prolong freshness, maintain overall characteristics, ensure harmlessness, and prevent the presence of microorganisms, as well as subsequent chemical breakdown and deterioration [22]. Milk and dairy products like cheese are full of vitamins, minerals, and other nutrients like calcium, magnesium, phosphorus, zinc and protein. Due to the high nutritional value of these products, which make up a large portion of human food, they provide an excellent medium for the growth and propagation of many microbes [23,24]. Moreover, these microbes are xerotolerant, acid tolerant, and to a certain extent can tolerate chemical (synthetic) preservatives which are occasionally applied to products to lengthen their shelf lives. However, some of these synthetic preservatives may have long-term adverse effects on humans as well as damaging consequences on the environment [25]. Also, synthetic preservatives have been linked to major health risks such cancer, allergies, hyperactivity, asthma, and nervous system damage, according to research [22,26]. Sodium benzoate has been associated with skin allergies [27], an increased risk of chronic disease due to oxidative stress in human erythrocytes in vitro [28], and activation of inflammatory pathways contributing to cancer development [29]. The carcinogenic risk of sodium benzoate in beverages is also a concern, as it has the ability to convert to benzene [30]. Additionally, high consumption of sodium benzoate in beverages has been associated with symptoms of attention deficit hyperactivity disorder (ADHD) symptoms in both kids and college students [31,32]. On the other hand, both in vivo and in vitro, potassium sorbate has been shown to be mutagenic, genotoxic, and to damage DNA when applied at high quantities or in conjunction with nitrites against human peripheral blood cells [33–35]. Consequently, researchers are currently investigating natural antimicrobial compounds with substantial effectiveness for prolonging the shelf life of cheese, aiming to find safer alternatives.

Natural antimicrobial agents are primarily derived from secondary metabolites found in plants, animals, and microorganisms, contributing to healthier food options [36–38]. In various studies, honey-BV has been identified for its antimicrobial properties against both bacteria and fungi [21,39,40]. Furthermore, recent research suggests that honey-BV possesses antimicrobial properties that could potentially be utilized in food preservation [41]. Therefore, using honeybee venom as a food preservative is a relatively novel concept that has gained some attention in recent years. To date, as far as we are aware, there hasn't been any published research on the application of honey-BV in food preservation.

In this regards, the primary objective of the present investigation was to explore the antimicrobial properties of honey-BV as a potential food preservative, specifically targeting various food spoilage microbes in a soft cheese variety known as 'Tallaga'. Additionally, this research serves as a crucial step in assessing the feasibility of honey-BV as a natural and effective cheese preservative.

2. Materials and methods

2.1. Harvesting of honey-BV

Honey-BV was collected from the F1 Carniolan hybrid honey-bee (*Apis mellifera carnica*) by the electric shock device at Department of Beekeeping, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt [42,43]. The honey-BV collector device is powered by continuous electric current of 18 V/3 Am./hr). The collection of honey-BV was scheduled every 15 days/for 30 min/3 times. The apparatus was positioned at the hive's entrance, creating a landing platform for bees that were returning. This device uses electrical impulses to encourage bee workers to sting through rubber sheets placed on glass plates, to guarantee the prevention of honey-BV's oxidation and the elimination of contaminants, to obtain pure, white, dry honey-BV that is free of contamination. When bees come into contact with the wires, they received a little electrical shock and sting on a sheet of glass. After the collection is complete, the honey-BV collector device is turned off, and the bees are shook off the collector frames. The venom deposited on the plate upon drying was taken immediately and scrapped off the plate using a sharp scraper and the dry venom was kept at 10 °C in a dark glass containers and stored cooled in a dry place [44,45].

2.2. LC-MS/MS analysis for active compounds in honey-BV

LC-MS/MS analysis for the bioactive polypeptides (apamin and melittin) in honey-BV was conducted according to the following method. Liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) with an ExionLC AC system was used for the separation of polypeptides in honey-BV sample. While the detector SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionization (ESI) for the detection of the target polypeptides.

An Ascentis® Express 90 Å C18 Column (2.1 × 150 mm, 2.7 μm) was used to perform the separation. Two eluents made up the mobile phase were A: 5 mM ammonium formate pH 3 and B: acetonitrile (LC grade). Following a set of programming, the mobile phase gradient was set to 5% B (0–1 min), 5–100% B (1–20 min), 100% B (20–25 min), 5% B at 25.01, and sustained 5% B (25.01–30 min).

The injection volume was 5 μL, and the flow rate was 0.3 mL/min. For MS/MS analysis, negative ionization mode was used with a

scan (EMS-IDA-EPI) from 100 to 1000 Da for MS1 using the following parameters: curtain gas: 25 psi; source temperature: 500 °C; IonSpray voltage: 5500; ion source gas 1 & 2 were 45 psi and from 50 to 1000 Da for MS2 with a declustering potential: 80; collision energy: 35; collision energy spread: 15.

2.3. GC-MS profiling of honey-BV

The dried honey-BV sample was re-suspended in 50 μL of Bis (trimethylsilyl) trifluoroacetamide (BSTFA) then, incubated in a dry block heater at 70 °C for 30 min. The used GC-MS system (Agilent Technologies) consisted of a gas chromatograph (model: 7890B) and a mass spectrometer detector (model: 5977A). The used GC column was HP-5MS (30 m × 0.25 mm I.D. and 0.25 μm film thickness). Sample analysis was performed using a hydrogen gas with a flow rate of 1.0 mL/min at a splitless, injection volume of 1 μL. The following temperature program was maintained: 50 °C for 1 min; rising at 10 °C/min to 300 °C and held for 20 min. The temperature of the injector and detector was maintained at 250 °C. Electron ionization (EI) at 70 eV was used to obtain mass spectra, with a solvent delay of 9 min and a spectral range of *m/z* 30–700. It was quadruple 150 °C and mass temperature 230 °C. The different constituents were identified by comparing the pattern of spectrum fragmentation with those stored in Wiley and NIST Mass Spectral Library data.

2.4. Antifungal assay

The antifungal activity of honey-BV was tested against eight fungal species; *A. niger* ATCC- 16888, *A. parasiticus* SSWT 2999, *Aspergillus flavus* NRRL 3357, *A. carbonarius* ITAL 204, *A. westerdijikia* CCT 6795, *A. ochraceus* ITAL 14, *Penicillium verrucosum* BFE 500 and *F. proliferatum* MPVP 328.

The antifungal activity of honey-BV was tested using the disc diffusion method on potato dextrose agar. A spore suspension of the tested fungi, a 100 μL was spread onto the surface of solidified potato dextrose agar (PDA) plates then applying the discs saturated with tested concentrations of honey-BV (100 and 200 mg/mL). For 24–48 h, the plates were incubated at 28 °C. A control positive, consisting of 1.0 mg/mL of miconazole (Sigma-Aldrich), was employed. The disc diameter was measured along with the inhibitory zones (in millimeters) [46].

2.5. Antibacterial assay

The obtained honey-BV was assayed for its antibacterial activity against five species of pathogenic bacteria, two Gram-positive bacteria: *Staphylococcus aureus* ATCC 13565 and *Bacillus cereus* EMCC 1080, and three Gram-negative bacteria: *Pseudomonas aeruginosa* NRRL B-272, *Escherichia coli* 0157H7 ATCC 51659, and *Salmonella typhi* ATCC 25566.

Two concentrations of honey-BV (100 and 200 mg/mL) were prepared in a sterile distilled water. Disc diffusion technique on nutrient agar was used to evaluate the antibacterial efficacy of the prepared concentrations. The tested pathogenic bacteria were inoculated to Tryptic soya broth tubes and incubated at 37^{o}C for 4 h. On the surface of solid nutrient agar plates a uniform bacterial lawn was developed using sterile cotton swabs. The nutrient agar plates were left for half an hour to dry, then the discs impregnated in the tested concentration and loaded of the surface of dried plates. Ceftriaxone sodium salt (1.0 mg/mL) was used as a positive control. The plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the diameter of inhibition zone (mm) [47].

2.6. Minimum inhibitory concentration (MIC)

Determination of MIC against fungi was performed by using the technique of Sokmen et al. [48]. Different concentrations of honey-BV were separately dissolved in 0.5 mL of 0.1% Tween 80 (Merck, Darmstadt, Germany). This mixture was then combined with 9.5 mL of melting PDA at 45 °C and transferred into a 6-cm-diameter Petri dish. A 3 μL of the fungal suspension (10^8 CFU/mL; 0.5 McFarland standards) was centrally injected into the prepared plates. The plates were incubated for 48 h at 25 °C. The mycelial growth was tracked and the MIC value was determined at the end of the incubation period.

The determination of MIC against bacteria was conducted using the tube dilution method [49]. A 24 h culture of the tested bacterial species was diluted in 10 mL of tryptic soy broth (TSB) with reference to the 0.5 McFarland standards to achieve inoculum of 10^8 CFU/mL. In culture tubes containing 15 concentrations of honey-BV (50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.125, 0.062 and 0.01 mg/mL in sterile distilled water) were prepared. A 100 μL bacterial cell suspension was added to each tube, and it was then incubated for 24 h at 37 °C. The turbidity of the broth indicates the inoculum's growth, and the minimum inhibitory concentration (MIC) was determined by taking the lowest concentration that prevented the test organism's growth.

2.7. Preservation of soft low salt white cheese (Talaga)

A batch of Talaga cheese was manufactured by the conventional method as described by Mehaia [50] with slight modifications. In details, 2 kg of whole buffalo milk were pasteurized at 65 °C for 30 min, then cooled to 42 °C. After the addition of table salt at 4% of ratio, yoghurt starter (*Lactobacillus delbruckii* subsp. *bulgaricus* 1:1 and *Streptococcus salivarius* subsp. *thermophilus*) was added as 1% (w/w) and let incubated for 10 min. Rennet (CHY-MAX Powder Extra NB, Chr. Hansen; Denmark) solution was added as 45 IMCU/Liter and let incubated for 3 h till full coagulation. To the manufactured cheese (treated batch), honey-BV was added by 15 mg/g, then all batches were stored in refrigerator for further investigations.

2.8. Microbiological analysis of Talaga cheese sample

Microbiological analysis was performed during storage periods (28 days) at 2 : 6 °C to evaluate the effect of honey-BV addition on the incidence and survival of pathogenic and spoilage microorganisms. Investigation of total viable count was performed on plate count agar. Enumeration of *Enterobactereaceae, Staphylococci*, and detection of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* were also accomplished as recommended by A.P.H.A [51]. and FDA [52] and described by El-Hadedy and Abu El-Nour [53].

2.9. Statistical analysis

The SAS statistical tool was utilized to perform a one-way analysis of variance (ANOVA) of the general linear model (GLM) on the obtained results. The average of the three experiments yielded the results ($p \leq 0.05$).

3. Results and discussion

3.1. LC-MS/MS analysis of bioactive polypeptides in honey-BV

The LC-MS/MS spectral chromatogram for apamin and melittin in honey bee venom (BV) is depicted in Fig. 1. Additionally, the mass spectrum of apamin and melittin can be found in Figs. S1 and S2, respectively. The results indicated that the active ingredients in the honey-BV sample were apamin and melittin, constituting 2% and 48.7%, respectively. These findings align with the study by Kokot and Matysiak [54], where they analyzed 38 honey-BV samples. The average levels of apamin and melittin were 0.93–4.34 and 25.40–60.27 (%), respectively. Rybak-Chmielewska and Szczêsna [55] found apamin levels comparable to those in our study. However, they reported a higher melittin content ratio (64.40%) than what we observed in the current study. In a recent study conducted by Sedat et al. [56], the apamin and melittin contents in honey-BV were found to be 3.85% and 70.49%, respectively. Variations in the existing ratios of active compounds in honey-BV can be due to a number of factors, including the strain and age of honeybees [57–59], nutritional supply (such as pollen replacements) [60], and climatic and seasonal influences [61–63].

3.2. GC/MS profiling of honey-BV

To examine the volatile profile of honey-BV, we conducted GC-MS/MS analysis, resulting in a diverse array of honey-BV components. The GC-MS/MS Spectral Chromatogram illustrating the active volatile compounds in honey-BV is depicted in Fig. 2. Among this diverse array of honey-BV components, several promising compounds stand out. These include: 9-Octadecenoic acid (Z)-, 9,12-Octadecadienoyl chloride, (Z,Z)- and 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z) which are shown in Table 1 and Figs. S3, S4 and S5. These bioactive fatty acids, which exist in some medicinal plants, have been reported as antifungal compounds according to several studies [64–68]. Additionally, other studies have confirmed the antibacterial activity of these fatty acids against bacterial strains such as *E. coli*, *S. aureus*, and *C. albicans* [69,70].

3.3. Antifungal activity of honey-BV

The antifungal activity of honey-BV against various mycotoxigenic fungi is summarized in Table 2. The results indicate significant antifungal effects across all the tested fungi at two different concentrations of honey-BV (100 and 200 mg/mL). The most pronounced

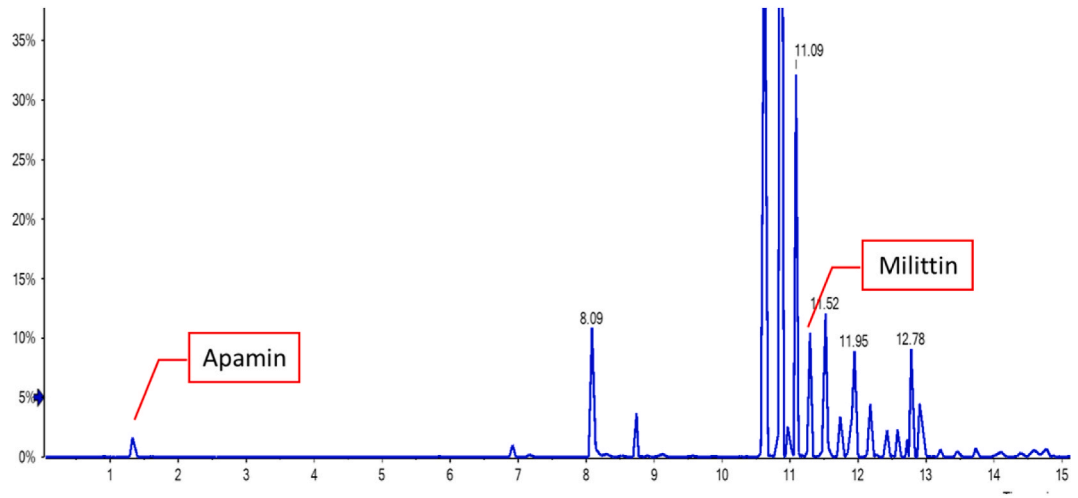

Fig. 1. LC-MS/MS spectral chromatogram for honey-BV (Apamin: 1.36 min; Milittin: 11.12 min).

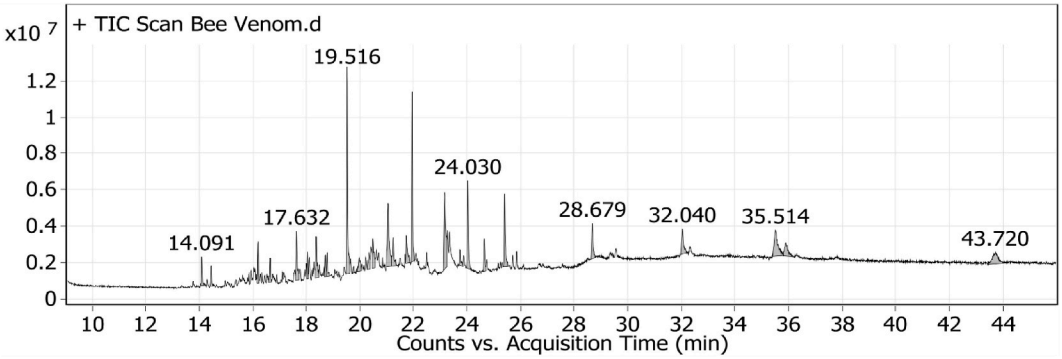

Fig. 2. GC-MS/MS spectral chromatogram for volatile compounds in honey-BV.

Table 1
Volatile compounds profile of honey-BV.

Name	Formula	RT (min)	Height	Width	Area
9-Octadecenoic acid (Z)-	C18H34O2	21.046	3454426	0.189	15897078
9,12-Octadecadienoyl chloride, (Z,Z)-	C18H31ClO	35.514	1429541	0.403	13963778
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4	35.906	727704	0.339	6660523

antifungal effect was observed against *F. proliferatum*, with diameters of 11.25 mm at 100 mg/mL and 14.0 mm at 200 mg/mL. Following closely were *A. ochraceus* and *P. verrucosum*, exhibiting diameters of 11.0 mm and 13.25 mm at 100 mg/mL, and 11.0 mm and 13.75 mm at 200 mg/mL, respectively. In contrast, the lowest activity was observed against *A. niger*, with average diameters of 8.75 mm at 100 mg/mL and 12.25 mm at 200 mg/mL. Notably, significant variations were noted between the inhibition zones obtained by the two honey-BV concentrations and miconazole (1 mg/mL), which served as a positive control against fungi.

The minimum inhibitory concentration (MIC) of honey-BV against the tested mycotoxigenic fungi is illustrated in Fig. 3. Notably, the honey-BV with the highest antifungal activity exhibited an MIC of 3 mg/mL against *A. westerdijikia*. Following closely were *A. flavus* and *A. ochraceus*, with inhibitory concentrations ranging from 4.0 to 4.5 mg/mL. In contrast, the lowest antifungal activity was observed at a dose of 13.0 mg/mL of honey-BV against each of *A. parasiticus*, *F. proliferatum*, and *P. verrucosum*. In the context of comparing the antifungal effects of honey-BV with other commercially used agents, natamycin demonstrated superior efficacy. Specifically, natamycin effectively inhibited fungal growth at concentrations ranging from 0.005 to 0.01 mg/mL [71]. Furthermore, according to Stanojevic et al. [72], the MIC values of sodium nitrite and sodium benzoate against fungi were located between 0.5 and 2.5 mg/mL. Meanwhile, higher MIC values of potassium sorbate and sodium nitrite against food-spoiling fungi were observed, ranging between 3.25 and 7.5 mg/mL [72].

Honey-BV has been reported in several studies as an active antifungal agent against various fungal-related diseases [2,73]. Notably, Al-Ani, Zimmermann, Reichling and Wink [74] found that melittin, a component separated from honey-BV, exhibits antimicrobial effects against different strains of fungi. The minimum inhibitory concentration (MIC) values for melittin ranged from 30 to 300 μg/mL. This antimicrobial effect is attributed to melittin's ability to generate oxygen free radicals (OH), leading to fungal cell death through disruption of the mitochondrial membrane via Ca2+ release [75].

3.4. Antibacterial activity of honey-BV

The data in Table 3 illustrates the antibacterial activity of two concentrations of honey-BV against five tested pathogenic bacterial

Table 2
Antifungal activities of honey-BV against tested mycotoxigenic fungi spp.

Inhibition zones in mm (Mean ± SD)								
Sample	*Aspergillus flavus*	*A. parasiticus*	*A. niger*	*A. carbonarius*	*A. ochraceus*	*A. westerdijikia*	*F. proliferatum*	*P. verrucosum*
1 (100 mg/mL)	10.75 ± 0.96^{c}	10.50 ± 1.00^{b}	8.75 ± 0.96^{c}	9.25 ± 0.96^{c}	11.0 ± 1.15^{c}	10.25 ± 0.50^{c}	11.25 ± 0.96^{c}	11.0 ± 1.15^{c}
2 (200 mg/mL)	13.25 ± 0.96^{b}	12.00 ± 1.15^{b}	12.25 ± 0.5^{b}	13.25 ± 0.50^{b}	13.25 ± 0.5^{b}	13.25 ± 0.50^{b}	14.0 ± 0.96^{b}	13.75 ± 0.96^{b}
Miconazole (1 mg/mL)	21.30 ± 1.2^{a}	25.00 ± 1.00^{a}	22.67 ± 0.6^{a}	24.00 ± 1.00^{a}	21.67 ± 0.6^{a}	22.67 ± 0.60^{a}	23.67 ± 0.6^{a}	22.30 ± 0.60^{a}

Mean with different letters in the same column were significantly different ($P < 0.05$).

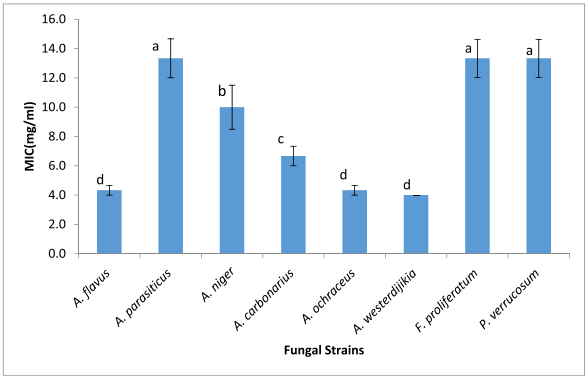

Fig. 3. Minimum inhibitory concentration of honey-BV against fungi.

strains. Notably, an increase in the honey-BV concentration correlates with a corresponding increase in the resulting inhibition zones against the tested bacteria. The tested Gram-positive bacteria exhibited high sensitivity towards honey-BV. Specifically, the highest inhibition zone (13.0 mm at 100 mg/mL and 16.25 mm at 200 mg/mL) was observed against *Staphylococcus aureus*. *Bacillus cereus* showed inhibition zones of 11.75 mm at 100 mg/mL and 13.25 mm at 200 mg/mL. In contrast, honey-BV recorded less inhibition zones against the tested Gram-negative bacteria. For *Pseudomonas aeruginosa* and *E. coli*, the inhibition zones at the concentrations of 100 mg/mL were 10.25 mm and 12.25 mm, while at 200 mg/mL, they increased to be 11.75 mm and 13.5 mm, respectively. On the other hand, honey-BV exhibited fewer inhibition zones against the tested Gram-negative bacteria which ranged from 10.25 to 11.75 mm at 100 mg/mL and they increased to be from 12.25 to 13.5 mm at 200 mg/mL for *Pseudomonas aeruginosa* and *E. coli* respectively. Significant differences were found between the obtained inhibition zones at both concentrations (100 mg/mL and 200 mg/mL) and the zones developed by the positive control, ceftriaxone (1 mg/mL).

The results mentioned above align with those of Hegazi et al. [39] who reported that honey-BV exhibited antibacterial properties and antimicrobial activity against both Gram-negative and Gram-positive bacteria. Specifically, it has demonstrated efficacy against strains such as *S. aureus*, *S. pyogenes*, *Klebsiella pneumoniae*, *E. coli*, and *P. aeruginosa*. Additionally, Farag and Swaby [21] found that honey-BV exhibited higher antimicrobial activity against gram-positive bacteria as compared to its sensitivity against gram-negative bacteria and fungal strains. Notably, the largest inhibition zones observed were 29.3 ± 1.5, 24.3 ± 1.9, 17.3 ± 1.8, 15.7 ± 1.5, and 14.0 ± 1.7 (mm) for honey-BV against *B. cereus*, *S. aureus*, *S. typhimurium*, *C. albicans*, and *E. coli*, respectively.

In our present study, the MIC values of honey-BV were investigated. Fig. 4 revealed that honey-BV exhibited potent antibacterial activity against *S. aureus* and *E. coli*, with a MIC of 0.2 mg/mL. Following closely were *B. cereus* and *S. typhi* with a MIC of 0.25 mg/mL. However, the antibacterial efficacy of honey-BV was lowest against *P. aeruginosa*, with a MIC of 0.5 mg/mL. These findings align with research of Leandro [76] reported similar MIC values for commercially available apitoxin (honey-BV) against the bacterial strains such as *Streptococcus sobrinus, S. salivarius, S. mitis, S. mutans, S. sanguinis, Enterococcus faecalis*, and *Lactobacillus casei,* falling within the range of 0.02–0.04 mg/mL. Furthermore, Farag and Swaby [21] reported comparable MIC values for honey-BV. Specifically, the corresponding MIC values were 0.32, 0.16, 0.625, 1.25, and 0.625 (mg/mL) for *S. aureus*, *B. cereus*, *S. typhimurium*, *E. coli*, and *C. albicans*, respectively. In the context of frequently used commercial preservatives, nisin demonstrated MIC values against Gram-positive bacteria, spanning from 0.6 to 0.8 mg/mL. However, it had no effect on Gram-negative bacteria [77]. Moreover, the MIC of potassium sorbate against *Escherichia coli* was 5 mg/mL, while that of sodium benzoate was also 5 mg/mL against both *Pseudomonas aeruginosa* and *E. coli* [72]. In contrast, honey-BV exhibited higher antibacterial activity and could serve as a promising alternative.

The antibacterial properties of honey-BV against both gram-positive and gram-negative bacteria are attributed to the potential action of melittin. Melittin, as reported by Oren and Shai [78], shows very little selectivity towards certain cells and acts powerfully on the lipid in the cell membrane through pores that generate channels which induces membrane permeation as explained by Refs. [18, 79] and lyses bacterial and eukaryotic cells in a nonselective pattern [80]. This mode of action for melittin is responsible for anti-fungal [81], anti-microbial [82,83], anti-tumor [84], and hemolytic properties [85]. Additionally, melittin enhances the activity of phospholipase A2 by increasing the inflow of calcium ions [86].

Table 3
Antibacterial activities of honey-BV against tested pathogenic bacteria.

Inhibition zones in mm (Mean ± SD)					
Sample	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Escherichia coli*	*Pseudomonas aeruginosa*
1 (100 mg/mL)	11.75 ± 0.5^{c}	13.00 ± 0.82^{b}	10.75 ± 0.5^{c}	11.75 ± 0.5^{c}	10.25 ± 0.5^{c}
2 (200 mg/mL)	13.25 ± 0.5^{b}	16.25 ± 1.71^{a}	12.5 ± 0.58^{b}	13.5 ± 1.0^{b}	12.25 ± 0.5^{b}
Cefetriaxone (1 mg/mL)	18.3 ± 0.58^{a}	18.00 ± 1.0^{a}	29.3 ± 1.15^{a}	30.7 ± 1.53^{a}	25.3 ± 0.53^{a}

Mean with different letters in the same column were significantly different ($P < 0.05$).

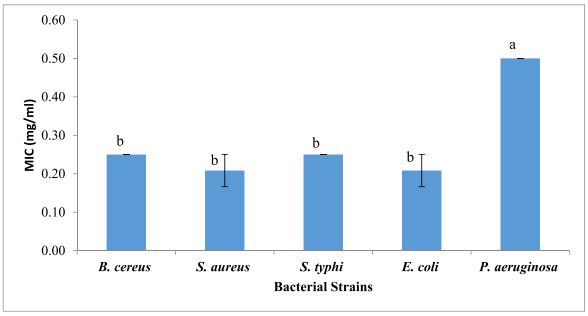

Fig. 4. Minimum inhibitory concentration for honey-BV against bacteria.

3.5. Application of honey-BV in Talaga cheese

This section constitutes the central focus of the study, wherein we delve into the application of honey-BV to Talaga cheese—serving as a representative model of soft white cheese. The central goal of this application was to evaluate the preservative effectiveness of honey-BV in maintaining the quality and freshness of the soft white cheese during the storage period. This assessment is crucial as it prepares the honey-BV for future industrial use, after a thorough health safety evaluation. The results were summarized in Table 4 and Figs. 5 and 6. Notably, both the control and honey-BV treated samples exhibited a complete absence of Enterobacteriaceae family members, including *E. coli* and *Salmonella* spp. (as shown in Fig. 5). This finding serves as a strong indicator of hygienic practices during both the manufacturing and analysis of the cheese samples, consistent with previous research [87,88].

Upon investigating the Staphylococcal growth, it was evident that the detected count of Staphylococci increased during cold storage over time (as depicted in Fig. 6). Specifically, the untreated batch (control) exhibited a significant increase in Staphylococcal count, starting from 1.3 log CFU/g at the time of manufacturing and reaching 3.7 log CFU/g after 28 days. In contrast, the honey bee venom (BV)-treated batch displayed notable differences. After 2 weeks of cold storage, there was a complete decline in Staphylococcal populations in the honey-BV-treated samples (as shown in Fig. 6).

Regarding the aerobic plate count (APC), it was observed that the APC value of the control sample increased from 4.0 to 5.3 CFU/g during the first two weeks of storage, followed by a slight decrease to 4.4 CFU/g by the end of the fourth week. Meanwhile, a steady decrease in the APC value occurred in the honey bee venom (BV)-treated sample over the storage period. Specifically, the APC number decreased from 4.0 to 2.0 CFU/g in the third week of storage and remained steady until the end of the fourth week (as shown in Fig. 4). Notably, the same pattern was noted in the results for molds and yeast counts (refer to Table 4). Importantly, there was no detected growth of either molds or yeasts during the 4 weeks of storage in the cheese samples treated with honey-BV.

The preservative effect of honey bee venom (BV) can be attributed to the inclusion of several bioactive compounds, as confirmed by HPLC and GC analyses. These bioactive materials include the polypeptides melittin and apamin, which have been reported as antibacterial and antifungal agents [9,82,83,86]. Additionally, other bioactive fatty acids, identified through GC-MS/MS analysis, contribute to the proven preservative effect of honey-BV in cheese. Notably, these active fatty acids—such as 9-Octadecenoic acid (Z)-, 9,12-Octadecadienoyl chloride, (Z,Z)-, and 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z)- are commonly found in extracts that exhibit promising antimicrobial activity [64–70,89,90].

4. Conclusion

The present investigation specifically studied the antimicrobial properties of honey-BV and their impact on various pathogens. Based on the findings, several conclusions can be drawn: All tested pathogens demonstrated susceptibility to honey-BV. Gram-positive bacteria were particularly sensitive, surpassing both gram-negative bacteria and fungal strains. The minimum inhibitory concentration (MIC) values of honey-BV against bacteria were significantly lower than those against fungi. The applied concentration of honey-BV in Talaga cheese (15 mg/g) was selected because the effective concentration of honey-BV against fungi was 13 mg/g. Overall, beyond the widespread use of honey-BV in the pharmaceutical field, the present study underscores the potential application of honey-BV as a natural antimicrobial agent for food preservation. However, further research is necessary to assess its practicality and safety as a food preservative.

CRediT authorship contribution statement

Mohamed Bedair M. Ahmed: Conceptualization, Supervision, Resources, Data curation, Writing – review & editing. **Mohamed Fathy El-ssayad:** Resources, Investigation, Data handling, Writing – original draft. **Samir Y.A. Yousef:** Resources. **Salah H. Salem:** Resources, Investigation, Data analysis, Writing –original draft.

Table 4
Influence of honey-BV treatment on the microbial growth during the storage period of Talaga cheese.

Microbiological analysis	Results (log CFU/g)									
	Zero time		7 days		14 days		21 days		28 days	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Enterobacteriaceae Count	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Escherichia coli	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella. spp.	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Staphylococci count	1.3 ± 0.04^{E}	1.3 ± 0.04^{A}	2.5 ± 0.03^{B}	2.0 ± 0.10^{P}	2.8 ± 0.13^{C}	<10	2.8 ± 0.13^{C}	<10	3.7 ± 0.13^{D}	<10
Staphylococcus aureus	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Aerobic plate count	4.0 ± 0.01^{E}	4.0 ± 0.01^{E}	4.3 ± 0.13^{F}	3.0 ± 0.03^{L}	5.3 ± 0.06^{G}	2.3 ± 0.20^{B}	4.7 ± 0.04^{H}	2.0 ± 0.00^{P}	4.4 ± 0.04^{F}	2.0 ± 0.00^{P}
Molds & Yeasts Count	<10	<10	1.8 ± 0.00^{M}	<10	3.1 ± 0.07^{L}	<10	3.4 ± 0.01^{K}	<10	3.6 ± 0.02^{D}	<10

Data expressed as Mean ± Standard error; Values in each row with different letter are significantly different ($P < 0.05$).

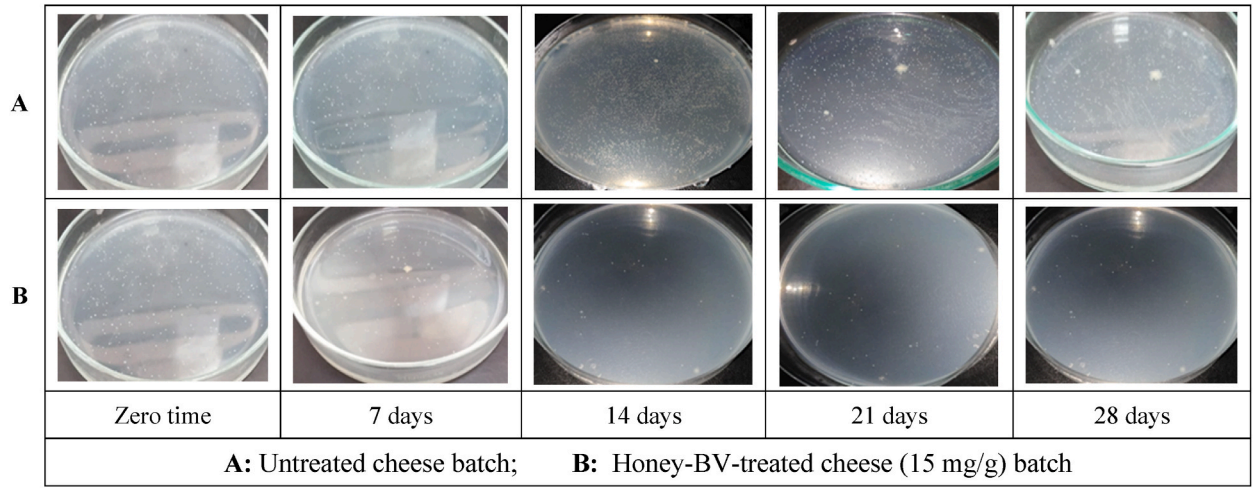

Fig. 5. Influence of honey-BV treatment on the development of aerobic plate count in Tallaga cheese.

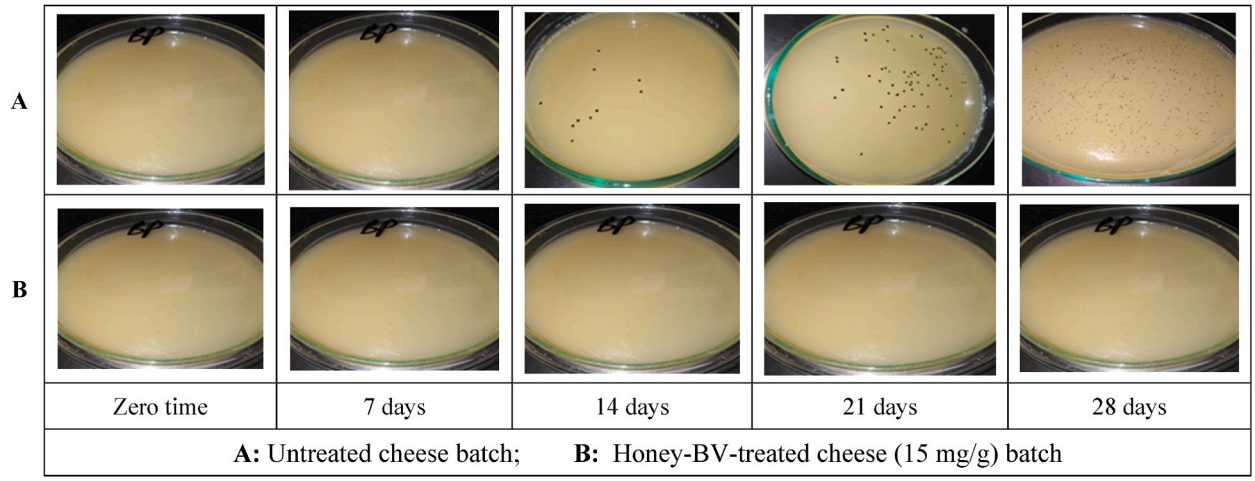

Fig. 6. Influence of honey-BV treatment on the development of Staphylococcal growth in Tallaga cheese.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28968.

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