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Research Article

Ziziphora tenuior L. Essential Oil as a Bio-Based Antimicrobial and Plant-Based Preservative for Increasing the Shelf Life of Mayonnaise

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The food industry is seeking alternatives to chemical additives and preservatives due to the increasing consumers' demand for healthier food products. *Ziziphora tenuior* L. (*Z. tenuior*) is an aromatic medicinal plant that exhibits various biological properties such as antibacterial, antifungal, antioxidant and anti-inflammatory properties. The aim of this study was to assess the impact of *Z. tenuior* essential oil (ZTEO) incorporation (700 μg/g) into mayonnaise on its stability, as well as to investigate the potential for replacing synthetic preservatives. The results demonstrated that the ZTEO extraction yield by the Soxhlet method was 3%. The GC-MS analysis of ZTEO showed the presence of 36 different components, with pulegone (27.5%), borneol (16.5%), 1, 8-cineole (9.8%), camphor (8.37%), β-pinene (5.31%), and α-pinene (4.64%) as the major compounds. Results of antimicrobial activity showed that the highest and the lowest minimum inhibitory concentration (MIC) values were obtained for *Escherichia coli* (700 μg/mL) and *Saccharomyces cerevisiae* (10 μg/mL). Also, the inhibition zone diameter increased by increasing the level of ZTEO, and *Staphylococcus aureus* (33 mm) and *S. cerevisiae* (36 mm) had the highest inhibition zone at level of 75%. Incorporation of ZTEO into mayonnaise prevented the growth of all microorganisms (bacteria, fungi, and yeast) during three months of storage. This indicates the potential of ZTEO as a natural preservative to replace synthetic preservatives. The results indicated the potential for incorporating ZTEO into mayonnaises and demonstrated the possibility of replacing commonly used preservatives with a natural alternative, offering consumers healthier food.

1. Introduction

Mayonnaise was invented in 1756 to enhance the taste of salads and sandwiches [1]. Mayonnaise is an oil-in-water (O/W) emulsion with a thick texture and a rich flavor that is widely consumed as a seasoning due to its creamy mouthfeel and special flavor [2]. It mainly contains oil, vinegar, mustard, egg yolk, salt, and sugar, flavorings, and stabilizers [3]. One of the main health concerns associated with mayonnaise is its contamination with microorganisms that come from ingredients, equipment, and air. They mainly include acid-resistant bacteria, *Bacillus* sp. (*B. subtilis* and *B. mesentericus*), yeast (*Saccharomyces* sp.), and fungi (*Aspergillus* sp.) [4]. As there is no thermal processing during mayonnaise preparation, preservatives such as sodium benzoate, potassium benzoate, potassium

sorbate, and sorbic acid are used to control the microbial safety of mayonnaise [5].

The food industry has relied on synthetic preservatives, such as sorbates and benzoates, for many years to extend the shelf life of the final product by reducing physicochemical and microbial changes [6, 7]. However, it has been reported that sodium sorbate showed adverse effects, such as genotoxicity, DNA damage, and increased micronucleus. Non-immunological (pseudoallergy) in sensitive patients and hyperactivity in children have been reported as the adverse effects of benzoate. Furthermore, urticaria, metabolic acidosis, convulsions, hyperpnoea, weak clastogenic activity, and asthma have been reported in some studies [8, 9]. In this respect, an increasing tendency of consumers for healthier lifestyle has enforced the industry to search for new natural antioxidants and antimicrobial agents [10]. One approach for increasing the mayonnaise shelf-life is biopreservation by using plant-derived substances, microbiota (mostly lactobacilli and bifidobacteria), and/or their antimicrobial metabolites [4]. Bio-based antimicrobials are safe and effective compounds derived from natural and renewable resources, such as food and agricultural byproducts. They provide a sustainable solution to antimicrobial needs and can be easily synthesized. Bio-based antimicrobials offer advantages in terms of sustainability and renewability compared to compounds synthesized from petrochemicals [11].

Natural antimicrobial substances derived from plants have been identified as a promising alternative to antibiotics. These substances are present in different parts of plants, including roots, stems, leaves, flowers, fruits, and seeds. They exhibit outstanding inhibitory effects on foodborne bacteria and fungi, as well as other useful functions such as food preservation, antiaging, antioxidant, and anticancer properties [12]. In recent years, essential oils (EOs) have gained attention as natural ingredients in foods regarding their safety and several biological activities, including antioxidant, antimicrobial, and antiviral properties [13, 14]. EOs are composed of complex mixtures of natural compounds, typically containing 20–60 components in varying concentrations [15]. They contain two or three major components that are present at high levels (20–70%) compared to other trace components and determine the biological properties of EOs [16]. EOs are known for their hydrophobic nature, which enables them to penetrate the lipids of the cell membrane, thereby increasing its permeability. This disruption of vital activities associated with the cell membrane, including the release of ions and vital compounds, ultimately leads to the cell death [17, 18]. EOs can be incorporated into a variety of foods, including fruits and vegetables, cereal-based products, dairy products, eggs, meat and meat products, and seafood [19].

Z. tenuior is a mountain plant of the *Lamiaceae* family. It is also known by other names such as narrow-leaf thyme, kakuto, regano, Quebec grass, and musk leopard stevia. The aroma of the *Z. tenuior* is very similar to thyme and slightly resembles oregano. The aerial parts (leaves and flowers) of this plant are used to obtain essential oil, which contains polgon, menthone, isomenthone, tannins, and flavonoids [20]. *Z. tenuior* is widely distributed in Iran and the phenolic and flavonoid compounds of the plant confer antioxidant and antibacterial properties, as well as gastrointestinal transit-accelerating properties [21].

Z. tenuior possesses antibacterial and expectorant properties, making it an effective treatment for colds and digestive disorders, such as diarrhea and heart spasms, and acts as an antiseptic for the respiratory and intestinal tracts. Moreover, it is a tonic for the stomach and heart and exhibits antipyretic properties. It can be used to treat headaches, and has antifungal effects. The decoction of *Z. tenuior* is a useful remedy in the treatment of rheumatism and osteoporosis. The dried and chopped leaves of *Z. tenuior*, which are similar to mint, are commonly used as a flavoring in buttermilk and as a spice in foods. Studies have demonstrated that the antimicrobial components of *Z. tenuior* essential oil can serve as natural preservatives, potentially replacing synthetic and chemical preservatives [22–24].

To the best of our knowledge, there is no study on the efficacy of *Z. tenuior* essential oil against spoilage and pathogenic microorganisms in mayonnaise. The objective of this research was to investigate the antibacterial activity of *Z. tenuior* essential oil against *S. aureus* and *E. coli*, as well as the antifungal properties of *Z. tenuior* essential oil against *S. cerevisiae* and *A. flavus*. We aimed at improving product quality and promoting consumer health with regards to food safety concerns.

2. Materials and Methods

2.1. Plant Material. The shade-dried flowers of *Z. tenuior* were obtained from the Iranian Institute of Medicinal Plants, Karaj, Iran.

2.2. Chemicals, Reagents and Medium. The chemicals and reagents (>99% purity), antibiotic, dimethyl sulfoxide (DMSO), and media: Mueller–Hinton broth, Sabouraud dextrose agar, Sabourad dextrose broth (SDB), and plate count agar were purchased from Merk (Darmstadt, Germany).

2.3. Bacteria Strains. S. aureus (ATCC25923), *E. coli* (ATCC25922), *S. cerevisiae* (ATCC60782), and *A. flavus* (ATCC5004) were provided by the culture collection at the Pasteur Institute of Iran.

2.4. Extraction of ZTEO. The dried *Z. tenuior* flowers were initially subjected to oven drying at $60 \pm 1^{\circ}C$ for 24 h until a constant weight was attained. They were then grinded to a fine powder using a hammer mill. Ethanol was used as the solvent in the Soxhlet device for 4-5 hours, in accordance with the modified method described by [25]. The brief protocol is as follows: 30 g of dried and ground *Z. tenuior* plant material were transferred into the cartridge. The filter paper was then stapled to the cartridge and transferred to the extraction chamber. The Soxhlet chamber was filled with ethanol until it reached half the volume of the flask. The water pipe should be opened and the flask placed on the refrigerator. The electric stove then turned on and the

temperature adjusted to ensure continuous boiling of the solvent inside the flask. The collected extract was separated using a rotary device under vacuum distillation with a vacuum pressure of 25 mm of mercury, speed of 1000–1200 rpm, and a temperature range of 50–60°C. It is of the utmost importance to maintain a lower temperature within the rotary device than the temperature of the solvent itself, in order to ensure a successful extraction. The solvent was completely removed, leaving behind only the extracted substance inside the balloon. Subsequently, the *Z. tenuior* essential oil (ZTEO) was placed in a sealed dark glass jar at 4 ± 1°C, where it was left until further analysis by GC-MS. The EO yield was calculated on a dry-weight basis (v/w).

2.5. Analysis of ZTEO by Gas Chromatography-Mass Spectrometry (GC-MS). To determine the chemical composition of the ZTEO, approximately 2-3 μL of the extracted ZTEO was injected into an Agilent GC-MS (Model 5975C, USA) equipped with an HP5-MS column (length 30 m × internal diameter 0.25 mm × layer thickness 25 μm). Helium gas with a purity of 99.9% was used as the carrier gas and a constant flow rate of 0.8 mL/min for the mobile phase was maintained. An electron ionization system with an ionization energy of 70 eV was employed and the temperature of the ionization source was maintained at 220°C. The initial temperature was set at 60°C and the thermal gradient was increased at a rate of 4°C/min until it reached 280–300°C. The temperature of the injection chamber was maintained at 290°C. The chromatogram produced mass spectra of the various compounds (Table 1) [26]. The identification of the spectra was based on the mass data bank, retention time, preventive index, matching of mass spectra with standards, and available information in the device library. The preventive index was calculated utilizing the normal spectrum of the Alkan series (C8–C24). Furthermore, the area under each peak's curve was determined [27].

2.6. Determination of Antimicrobial Activity of ZTEO. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of ZTEO [28]. The incorporation of EOs and broth microdilution assay was carried out in accordance with the methodology described by [29]. In order to evaluate the antibacterial and antifungal activity of ZTEO, a range of concentrations were used to assess the response of four distinct microorganisms. The disc-diffusion method was used to determine the inhibition zone diameter [30]. An inoculated agar plate was used, onto which a paper disc soaked in ZTEO was placed. The standard microbial suspension was prepared by culturing bacterial strains in plate count agar at 37°C and yeast and fungi strains on Sabouraud dextrose sgar at 25°C in aerobic conditions. The growth inhibition zone diameter was measured using an antibiogram ruler after 48 h for bacteria and 4 days for fungi. Furthermore, three reference antibiotics were used to assess the sensitivity of the tested microorganisms as positive controls. These were kanamycin for *S. aureus*, tetracycline for *E. coli*, and nystatin for *S. cerevisiae* and *A. flavus*. In addition, DMSO was used as the negative control.

Table 1: Concentration of main chemical constituents of ZTEO* by GC-MS analysis.

	Components	Conc. %	RI**
1	2-Hexenal	tr[b]	765
2	1-Nonene	tr	790
3	α-Thujone	0.24	924
4	α-Pinene	4.64	932
5	Camphene	0.75	947
6	Sabinene	1.53	972
7	β-Pinene	5.31	976
8	α-Terpinene	0.82	1016
9	*p*-Cymene	0.95	1013
10	Limonene	tr	1027
11	1, 8-Cineole (eucalyptol)	9.80	1030
12	γ-terpene	1.51	1057
13	*trans*-Sabinene	0.28	1066
14	α-Terpinolene	0.14	1088
15	*cis*-Sabinene	0.7	1098
16	Campholen	0.5	1126
17	α-Campholen aldehyde	0.63	1126
18	*trans*-Pinocarveol	2.37	1139
19	Camphor	8.37	1145
20	Pinocarvone	3.27	1163
21	Borneol	16.51	1168
22	Pino-camphene	0.34	1175
23	Terpinen-4-ol	3.23	1178
24	α-Terpineol	2.95	1191
25	Methyl chavicol	3.04	1195
26	Ethanone	tr	1198
27	Thujanol acetate	0.85	1218
28	Bornyle acetate	0.64	1285
29	Lavandulyl acetate	0.27	1289
30	Thymol	0.72	1291
31	Eugenol	tr	1357
32	*trans*-Carveol	tr	1197
33	Pulegone	27.5	1220
34	Caryophyllene oxide	tr	1575
35	Spathulenol	tr	1567
36	Germacrene	tr	1475
Total		97.86	

*ZTEO: *Ziziphora tenuior* L. essential oil. **RI: retention index; [b]tr, trace (<0.1%).

2.7. Preparation of Mayonnaise Treatments. Mayonnaise as a food model was prepared with the ingredients, including egg yolk (9%), vinegar (6%), sunflower oil (65%), water (12%), salt (1.5%), sugar (5%), xanthan and guar gum (1.1%), and mustard powder (0.3%), and different treatments were as follows:

(1) ME: containing ZTEO and microorganisms.

(2) BSM: containing 0.75 mg sodium benzoate and potassium sorbate (permitted in mayonnaise) and microorganisms.

(3) NC (negative control): without additive and with inoculated microorganism.

(4) PC (positive control): without additives and microorganisms.

ZTEO was incorporated into the ME treatment based on the maximum MIC achieved (700 μg/mL). The final concentration of each microorganism in the relevant treatments

was 1×10^8 CFU/g (0.5 McFarland Standard). The growth of microorganisms was evaluated on months 0, 1, 2, and 3 of storage.

2.8. Statistical Analysis. All experiments were performed in triplicate, and data were expressed as the mean ± standard deviation. ANOVA and Duncan multiple range tests in SPSS software (ver. 22.0, SPSS Inc., United States) were used to compare the mean values. Differences at $p < 0.05$ were considered to be significant.

3. Results and Discussion

3.1. Identification and Quantification of Chemical Components of ZTEO. The extraction yield of ZTEO by the Soxhlet method based on the dry weight of the plant was found to be 3% (v/w). The quantity of ZTEO obtained differed from the findings of Mohammadi et al. [24] and Abu-Darwish et al. [31], which can be attributed to several factors, including the difference in the part of plant selected for EO extraction, variations in the methodology used for EO extraction, and the manner, temperature, and duration of plant drying. Temperature is a critical factor that affects the quantity and quality of EO derived from the plant. The selection of the plant part for EO extraction, including the stem, leaf, or flower, is an essential factor in determining the quality and yield of the extracted oil.

The results of the compositional analysis of ZTEO revealed the presence of 36 components in the EO obtained from the flower of *Z. tenuior* representing 97.86% of the total oil (Table 1). The major component was pulegone (27.5%), in agreement with other previous studies [24, 28, 31, 32]. The second most abundant compounds in the EO were borneol (16.51%), 1, 8 cineole (9.80%), camphor (8.37%), β-pinene 95.31%), and α-pinene (4.64%) were the second major compounds in the EO. Other constituents, including α-terpinolene (0.14%), α-thujone (0.24%), lavandoleyl acetate (0.27%), and *trans*-sabinene (0.28%) were detected in small amounts in the EO [32, 33].

In a study conducted by Bakhtiar et al. [34], essential oils extracted from different types of *Ziziphora tenuior* L. cultivated in southwestern of Iran were characterized. The major compounds were pulegone (18.46–46.28%), piperitenone (0–30.61%), *p*-mentha-3-en-8-ol (0.46–28.49%), caryophyllene oxide (0.13%–14.78%), and germacrene-D (0.11–14.23%). Abu-Darwish et al. [31] assessed chemical composition of *Z. tenuior* essential oil from southern Jordan and showed that the main compounds included pulegone (46.8%), p-menth-3-en-8-ol (12.5%), isomenthone (6.6%), and 8-hydroxymenthone (6.2%). In a study by Azimi et al. [35], 42 chemical compounds were identified in the essential oil extracted from the leaves and branches of *Z. tenuior* and the major compounds included pulegone (26.65%), alpha-terpinyl acetate (9.53%), geraniol (7.11%), menthone (5.74%), thymol (5.51%), alpha terpineol (3.24%), and menthol (1.05%).

The variety of *Ziziphora*, the growing conditions of the plant, including light, temperature, and soil, are important factors influencing the composition of EO. The main components of EOs vary according to different regions in the same variety. The chemical composition of EOs is influenced by many factors, including environmental and geographical conditions, plant growth time, plant harvest time, light, humidity, physical and chemical characteristics of the soil, weather conditions, plant drying method, EO extraction method, and plant age [32, 33]. In some cases, water loss in plant organs may result in the evaporation and subsequent loss of active substances. Such changes are due to the volatility or instability of the aforementioned substances. In addition to weight loss, the drying process may cause changes in the color, odor, taste and active ingredients of medicinal and aromatic plants. It has been reported that when plants containing EO are not dried in the shade, their compounds are reduced [36].

3.2. Antimicrobial Activity of ZTEO. Antimicrobial activity of ZTEO against selected microorganisms is shown in Table 2. The highest and the lowest MIC values were recorded for *E.coli* (700 μg/mL) and *S. cerevisiae* (10 μg/mL), respectively. Furthermore, higher antibacterial activity of ZTEO against *S. aureus* compared to *E. coli* showed the sensitivity of Gram-positive bacteria [37, 38]. This is due to the presence of an impermeable outer membrane containing lipopolysaccharides and a thin peptidoglycan layer that surrounds the cell wall of Gram-negative bacteria [39, 40]. According to Table 2, by increasing the level of ZTEO, the mean inhibition zone diameter increased in all microorganisms. The most pronounced effect and the greatest diameter of the inhibition zone were observed in *S. aureus* and *S. cerevisiae* at a concentration of 75%, which were comparable to the efficacy of antibiotics including kanamycin, nystatin, and tetracycline in some EO concentrations (Table 3).

Mahboubi et al. [41] reported antibacterial activities of *Z. tenuior* essential oil against three bacterial strains, *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*. The highest inhibitory activity was observed towards *S. aureus*. Furthermore, addition of various levels of *Z. tenuior* essential oil (0.02–1.0%) to vegetable oil containing microorganisms resulted in prevention of microbial growth at 1% after two weeks. In a study by Aminzare et al. [37], antibacterial activity of *Z. tenuior* essential oil was investigated against different foodborne pathogens and the results of MIC values demonstrated that *Bacillus subtilis*, *Listeria monocytogenes*, and *Staphylococcus epidermis* were the most susceptible bacteria to the *Z. tenuior* essential oil, while *E. coli*, *Salmonella typhimurium*, and *Klebsiella pneumonia* were the most resistant bacteria. The antibacterial activity was ascribed to the high level of phenolic compounds and monoterpenes such as pulegone and the interference with lipophilic compounds of the bacterial membrane. It has been reported that pulegone causes damage after passing through the bacterial membrane and disrupts the structure of different layers of fatty acids, polysaccharides, and phospholipids [42]. In the case of 1, 8-cineole, it induced oxidative stress which resulted in the disruption of bacterial membrane via lipid peroxidation and leakage of intracellular substances leading to cell death [43].

Table 2: Antimicrobial activity of ZTEO at different concentrations against selected microorganisms.

Microorganism	MIC* (μg/mL)	Inhibition zone** (mm) ZTEO*** (%)		
		25	50	75
S. aureus	35	23	28	33
E. coli	700	10	13	18
S. cerevisiae	10	28	33	36
A. flavus	300	12	16	20

*MIC: minimum inhibitory concentration (determined by a broth microdilution technique). **Inhibition zone diameter (mm) was determined by the disc-diffusion method. ***ZTEO: *Ziziphora tenuior* L. essential oil.

Table 3: Susceptibility of microorganisms to selected antibiotics.

Antibiotics*	Microorganism	Inhibition zone (mm)
Kanamycin	*S. aureus*	25
Tetracycline	*E. coli*	23
Nystatin	*S. cerevisiae*	19
Nystatin	*A. flavus*	19

*Antimicrobial activity of antibiotics was determined by the disc-diffusion method. Antibiotics were used as the positive control and dimethyl sulfoxide (DMSO) as the negative control.

Similarly, it has been reported that *S. aureus* was the most sensitive strain to *Z. tenuior* essential oil (MIC of 0.25 μg/mL), followed by *E. coli* (MIC of 0.5 μg/mL), *S. Typhi*, and *K. pneumoniae* (MIC of 1 μg/mL) and the antibacterial activity was attributed to the high content of pulegone [44]. In a study by Shahbazi et al. [45], antimicrobial properties of *Ziziphora clinopodioides* essential oils (ZEOs) collected from four provinces in western Iran was studied. It was observed that *S. aureus*, *B. cereus*, *B. subtilis*, and *L. monocytogenes* were the most susceptible and *S. typhimurium* and *E. coli* O157:H7 were the most resistant bacteria. The antimicrobial properties of essential oil were associated with phenolic compounds particularly oxygenated monoterpenes such as carvacrol and thymol. Moghadam et al. [46] evaluated the effect of *Z. clinopodioides* essential oil at six levels (6250, 3125, 1600, 800, 400, and 196 mg/mL) on the growth and activity of *A. flavus* and *A. parasiticus*. The results showed that the EO was more effective on *A. parasiticus* than *A. flavus*. It has been highlighted that the pulegone as the major constituent of essential oil in Ziziphora species can affect the growth and morphology of fungal strains. Interference of pulegone with enzymatic reactions related to the cell wall synthesis is the main reason of antifungal activity [47].

Essential oils exhibit their antimicrobial activity through changing the cell membrane permeability, inducing granulation of cytoplasm and cytoplasmic organelles, as well as the inactivation of intracellular and extracellular enzymes that are effective on the growth of microorganisms and cause their disintegration and inactivation [48, 49]. It has been well documented that the compounds with low molecular weight such as terpenes and terpenoids present in essential oils are responsible for several biological activities, including antimicrobial and antioxidant properties [40]. The monoterpenoid compounds exhibit toxic effects on membrane structure and function. They can easily penetrate from aqueous phase to membrane structure which results in the degradation of the cell wall, increased membrane fluidity and permeability, damaging the cytoplasmic membrane, disturbance of membrane-embedded proteins, prevention of respiration, change of ion transport processes and release of intracellular materials, reducing the proton motive force, reducing the intracellular ATP pool through decreased ATP synthesis. In the case of fungi, binding to ergosterol and channel formation leads to impairment of cell membranes as well as irregularities in the mitochondria structure [50].

3.3. Biopreservation of Mayonnaise with ZTEO. Table 4 illustrates the population of *S. aureus*, *E. coli*, *S. cerevisiae*, and *A. flavus* (CFU/g) in inoculated and uninoculated high-fat mayonnaise during three months of storage at 4°C. It was observed that in mayonnaise samples without essential oil/or synthetic preservatives and containing different microorganisms, the count of *S. aureus* increased from 1.97×10^2 to 3.33×10^1 CFU/g during 3 months of storage. Also, the count of *S. cerevisiae* increased from 1.5×10^2 to more than 10^6 CFU/g during three months of storage. The population of *E. coli* decreased and no bacteria was detected after three months. Similar results have been reported by Hwang et al. [51], in which lactic acid and vinegar decreased the survival of *E. coli* O157:H7 in acidified low-fat mayonnaise. In positive control samples (without preservatives and microorganisms), only *S. cerevisiae* was present and its population increased during storage. As it is evident, no microbial growth was observed in samples containing essential oil and the samples with benzoate and sorbate during three months of storage that was an indicative of similar inhibitory activity of ZTEO and preservatives.

In a study by Moradi et al. [52], C*uminum cyminum* L. essential oil (12 mg/mL) and its nanoemulsion (6 mg/mL) were incorporated into mayonnaise and the results showed that the growth of acid-resistant bacteria, molds, yeast, and total bacterial count decreased in treated samples. Addition of oregano essential oil at levels of 0.5 and 1% to mayonnaise along with suitable acidic condition showed a bacteriostatic effect and repelled the growth of *S. entritidis* in mayonnaise stored at 30°C for 24 h [53].

Ozdemir et al. [54] incorporated basil essential oil microcapsules into mayonnaise and reported antimicrobial activity against *E. coli* and *S. typhimurium*. Similarly, the antimicrobial activity of mustard essential oil in mayonnaise during 40 days of storage demonstrated that the utilization of free and encapsulated mustard essential oil could inhibit the growth of *E. coli* and *S. entritidis* [5]. In another study, essential oils from cinnamon bark, clove, and thyme exhibited antimicrobial activity against spoilage yeasts in salad dressing. It has been reported that the most effective essential oil components was *trans*-cinnamaldehyde at 500 mg/L which inhibited *Schizosaccharomyces pombe* and *Zygosaccharomyces bailii* during 4 and 5 days storage, respectively, at 22°C [55].

Table 4: The count of *S. aureus*, *E. coli*, *S. cerevisiae*, and *A. flavus* (CFU/g) in mayonnaise during three months storage at 4°C.

Storage time (months)	Treatments*	*S. aureus*	*E. coli*	*S. cerevisiae*	*A. flavus*
0**	ME[c]	—***	—	—	—
	BSM	—	—	—	—
	NC	$1.97 \times 10^2 \pm 0.15^{c}$	$8.5 \times 10^2 \pm 0.2^{a}$	$1.5 \times 10^2 \pm 0.75^{c}$	$1.2 \times 10^2 \pm 0.3^{b}$
	PC	$4.26 \times 10^1 \pm 0.15^{d}$	—	$4.4 \times 10^1 \pm 0.15^{c}$	$5.1 \times 10^1 \pm 0.25^{c}$
1	ME	—	—	—	—
	BSM	—	—	—	—
	NC	$2.23 \times 10^2 \pm 0.15^{b}$	$5.3 \times 10^2 \pm 0.25^{b}$	$4.1 \times 10^2 \pm 0.65^{c}$	$2.1 \times 10^3 \pm 0.15^{a}$
	PC	$2.53 \times 10^1 \pm 0.30^{e}$	—	$2.9 \times 10^2 \pm 0.95^{c}$	—
2	ME	—	—	—	—
	BSM	—	—	—	—
	NC	$2.43 \times 10^2 \pm 0.15^{a}$	$1.2 \times 10^2 \pm 0.25^{c}$	$2.1 \times 10^3 \pm 0.65^{c}$	—
	PC	—	—	$9.7 \times 10^3 \pm 0.25^{b}$	—
3	ME	—	—	—	—
	BSM	—	—	—	—
	NC	$3.33 \times 10^1 \pm 0.15^{de}$	—	$^{a}10^{6}>$	—
	PC	—	—	$10^{6a}>$	—

*ME: mayonnaise containing essential oil and microorganisms, BSM: mayonnaise containing 0.75 mg sodium benzoate and potassium sorbate and microorganisms, NC (negative control): mayonnaise without additive and within oculated microorganism, PC (positive control): mayonnaise without additives and microorganisms. **The number of microorganisms after 48 h. ***Not detected. Means with different small letters in a column shows significant difference. [a-e]Means with different small letters in a column show significant difference ($P < 0.05$).

4. Conclusions

In this study, chemical composition and the effect of *Z. tenuior* L. essential oil on the growth of four microorganisms in mayonnaise was investigated during three months of storage. It was observed that the *Z. tenuior* L. essential oil are rich in pulegone, borneol, and 1, 8-cineole. The results indicated that ZTEO exhibited varying degrees of antimicrobial activity against fungal and bacterial strains. The highest and the lowest MIC values were observed in E.coli and S. cerevisiae, respectively. ZTEO showed higher antibacterial activity against S. aureus compared to E. coli. It was also observed that increasing the level of ZTEO resulted in an increased inhibition zone diameter, which was recorded for S. aureus and S. cerevisiae at a concentration of 75%. Moreover, ZTEO showed inhibitory activity against pathogenic and spoilage microorganisms in mayonnaise similar to sodium benzoate and potassium sorbate during three months of storage. Therefore, it is possible to extend the shelf-life of mayonnaise and reduce the use of chemical additives by using *Z. tenuior* L. essential oil. However, further studies should be conducted to investigate the effect of ZTEO incorporation on oxidative stability and sensory properties of mayonnaise. Furthermore, encapsulation systems can be studied for an increased stability and sustained release of *Z. tenuior* L. essential oil in mayonnaise as well as other food products.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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