Article

Inactivation of *Salmonella enterica* and *Colletotrichum gloeosporioides* on Whole Mangoes by Application of an Antimicrobial Coating Containing Oregano Essential Oil

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Abstract: Mangoes are susceptible to bacterial and fungal contamination during storage and transportation. This study investigated the effectiveness of pectin-based coatings containing oregano essential oil (OEO) to reduce *Salmonella enterica* contamination and decrease anthracnose disease on whole mangoes. A cocktail of five strains of *Salmonella* spp. and *Colletotrichum gloeosporioides* strains was spot inoculated in mangoes to verify the antibacterial and antifungal activity of OEO. The inoculated mangoes were coated with pectin-based coatings containing 0, 0.5, 0.7, and 0.9% OEO. Coated fruits were stored for 11 days at 25 °C and 90% of relative humidity. All treatments with OEO effectively inhibited the growth of *Salmonella*, causing a reduction of 2.5 CFU/cm^2 compared to the control treatment (0% OEO). In addition, coatings effectively inhibited the growth of *C. gloeosporioides* on the mango surface after 9 days of storage to the same extent as the traditional Prochloraz fungicide. The efficacy of coatings treatments was between 88.06 and 96.68% compared to the control treatment. Sensory analysis showed that the OEO did not affect the quality attributes of coated mango. Results showed the potential benefits of applying the pectin-based coatings with OEO as an alternative to control *S. enterica* and *C. gloeosporioides* in whole mangoes.

Keywords: *Mangifera indica*; antifungal; antibacterial; oregano; coating

1. Introduction

Mango fruits (*Mangifera indica* L.) have one of the world's highest consumption per capita [1]. However, as climacteric fruit, mangoes are susceptible to microbial contamination during storage [2]. The primary postharvest disease in mango is anthracnose caused by *Colletotrichum gloeosporioides*. Anthracnose disease causes about 30–60% of total production losses in mango-producing countries [3,4].

Further, fresh mangoes have been associated with foodborne diseases caused principally by *Salmonella enterica* [5,6]. Some of the problems related to mango food safety can be directly associated with the postharvest disinfestation treatments, commonly applied to prevent the incidence of agricultural pests [7]. Postharvest factors that contribute to mango contamination include the use of contaminated rinsing water, hot/cold water treatments, human manipulation, presence of animal feces, use of contaminated equipment, packing, and inadequate product exposition and storing temperatures [8,9]. All these factors have contributed to several outbreaks of foodborne illness associated with the consumption of mangoes in the United States [8,10]. Therefore, alternatives to traditional treatments are needed to improve the safety of mangoes.

Different treatments have been employed to control the postharvest development of anthracnose in mangoes [3,11] and the prevention and control of pathogenic bacteria [5,12]. Essential oils have been confirmed to be of great importance in the control of microbial

populations. The published literature indicates that essential oils possess a broad spectrum of antibacterial, antifungal, and even antiviral activity [13,14].

Oregano essential oil (OEO) is among the most effective antimicrobial and antioxidant agents. Carvacrol and thymol, constituting approximately 75–85% of OEO, are considered potent antifungal, antibacterial, and antioxidant compounds due to their structure and lipophilic characteristics [15,16]. Oregano is a source of essential oil with potentially extensive use in the food industry, especially those adopting natural alternatives to assure food safety and avoid synthetic fungicides [17].

Different techniques are being explored to facilitate the addition of essential oils as an antimicrobial food additive. The use of antimicrobial edible films and coatings could improve food safety and extend the shelf-life of food systems by controlling the release of antimicrobials on food surfaces [17]. Several investigations have evaluated the incorporation of essential oils in edible coatings with promising results in tomato [18], cantaloupes [19], strawberries [20], and papaya [21]. However, the investigations focus separately on the control of postharvest diseases or the control of foodborne outbreaks. A methodology that allows maintaining product quality has not been evaluated, both in the control of deterioration due to fungal diseases and foodborne outbreaks. The objective of this work was to evaluate the antibacterial and antifungal effects of antimicrobial coating containing OEO on whole mangoes. The findings in this study provide information on the optimal OEO concentrations that can be used to prevent *Salmonella* spp. outbreaks and control anthracnose in mangoes.

2. Materials and Methods

2.1. Materials

Mangoes (*Mangifera indica* L.) cultivar Tommy Atkins were purchased from local produce distributors. Fruits with visible physical injuries were discarded. The stage of maturity was selected according to skin color (light green to yellow) and finger feel firmness score 2 (1 = hard, 2 = slightly soft, just starting to ripen, 3 = very soft), as stated by Perumal et al. [22]. Oregano (*Origanum vulgare* L.) essential oil was purchased from Now Foods (Bloomingdale, IL, USA). Pectin from citrus peel (galacturonic acid ≥74.0% (Dry Basis)) and glycerol (99% pure) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other analytical grade chemicals and reagents were purchased from either Sigma-Aldrich or Fisher Scientific.

2.2. Oregano Essential Oil Characterization

OEO was analyzed by capillary gas chromatography-mass spectrometry (GC-MS) according to ISO 7609-1985 [23] using a certified C_6–C_{25} hydrocarbon standard as reference (AccuStandard, New Haven, CT, USA). The chromatographic analysis was performed on a chromatograph AT 6890 Series Plus (Agilent Technologies, Palo Alto, CA, USA). Mass spectra were obtained by electron ionization (EI, 70 eV) in a quadrupole mass detector in the full scan mode. The identification of the analyzed compounds was accomplished by comparing their mass spectra with computerized spectral databases and retention times.

2.3. Coatings

Pectin solutions (4% *w/v*) were prepared by dissolving 80 g of pectin powder in distilled water at 70 °C. After 2 h of stirring for complete dissolution, 0.5% *w/v* glycerol was added to the solution. Different concentrations (0, 0.5%, 0.7%, and 0.9% *w/v*) of OEO were then incorporated into the pectin-based formulation and emulsified with a high shear laboratory mixer (Fisher brand-850 homogenizer, Pittsburg, PA, USA) for 3 min at 12,500 rpm. Water was added to reach the total volume of 2 L.

Pectin, glycerol, and OEO concentrations were chosen from preliminary in vitro antifungal assays and sensory evaluation. The selected OEO concentrations were effective against *C. gloeosporioides*, did not cause damage to mango skin, and were accepted by the panelists (data not shown). The control treatment was the pectin-based coating with 0%

OEO. For the antifungal assay, commercial fungicide Prochloraz (0.12% *w/v*; TCI America, Portland, OR, USA) was used as a comparison treatment.

2.4. Pathogenic Bacteria

A cocktail of five serovars of *Salmonella* spp. was used in this study (*S. Saint Paul*, *S. Agona*, *S. Gaminara*, *S. Montevideo*, and *S. Newport*—Human clinic isolate). The pathogenic *Salmonella* spp. strains were obtained from the Department of Food Science and Technology culture collection at the University of Tennessee (Knoxville, TN, USA). Each of the strains was cultured in 10 mL of sterile tryptic soy broth (TSB; Bacto, Becton Dickinson Co., Sparks, MD, USA) for 24 h. Bacterial cultures were transferred onto tryptic soy agar plates (TSA; Difco, Becton Dickinson Co., Sparks, MD, USA), and they were kept to 37 °C for 24 h to produce bacterial lawn. Microbiological counts were done by serial dilution on TSA plates, and results were expressed as CFU/mL. The five strains were combined to yield a mixed culture containing equal proportions of each strain and diluted to 10^8 CFU/mL as the working culture. A high inoculum level was used to measure logarithmic reductions in pathogens counts during the study.

2.5. Plant Pathogenic Fungi

Colletotrichum gloeosporioides (Penz.) isolates were obtained from USDA. ARS from single anthracnose lesions in mangoes were used. All strains caused characteristic symptoms of anthracnose in mango cv. Tommy Atkins. The fungal stock cultures were maintained in filter paper at 4 °C in the dark. For antifungal assays, stock cultures were cultured in potato dextrose agar (PDA; Difco, Becton Dickinson Co., Sparks, MD, USA) for ten days at 25 °C.

Conidial suspensions were obtained by flooding the 10-day old growth of *C. gloeosporioides* with 10 mL of 0.1% (by mass) of peptone water (Fisher Scientific, Fair Lawn, NJ, USA) and scraping the surface with a sterile loop. The conidial suspensions were transferred to a test tube and were adjusted to 10^5 CFU/mL by serial dilutions in 0.1% (by mass) peptone water, spread on Dichloran Rose Bengal Chloramphenicol Agar (DRBC; Oxoid, Basingstoke, Hants, UK), and incubated at 25 °C for five days before enumeration.

2.6. Mango Inoculation

Mangoes were washed with tap water, rinsed with deionized water, and dried for 2 h at ambient conditions (21 °C, 45–50% relative humidity). Two treatments were performed separately for inoculation on the mango surface, one for *Salmonella* spp. and another for *C. gloeosporioides*. Each mango was marked with two squares (1.5 × 1.5 cm) at the equatorial region. For *Salmonella* inoculation, a total of 50 µL of *Salmonella* suspension was spot-inoculated (using 5 µL drops) on marked squares. The same procedure was used for *C. gloeosporioides* inoculation. Mangoes were dried at room temperature for 2 h to allow cell fixation. *Salmonella* spp. and *C. gloeosporioides* enumeration was assessed at 1, 2, 5, 7, 9, and 11 days. Five treatments with three biological replicates and three samples per replicate were used (n = 9) for each treatment.

2.7. Coating Treatment

After inoculation, the mangoes were immersed in the coating solution for 1 min, drained on a rack, and dried for 2 h at room temperature (21 °C) (Figure 1A). Coated fruits were stored in plastic trays in an incubator at 25 °C and 90% relative humidity for up to 9 days. Mangoes were examined at 1, 2, 5, 7, 9, and 11 days of storage for microbiological analysis.

Figure 1. Methodology for mango inoculation and microorganism's enumeration. (**A**) Inoculated marked areas on coated mango. (**B**) Mango surface samples for *Salmonella* spp. and *C. gloeosporioides* enumeration.

2.8. Salmonella Enumeration

Treated areas of mangoes rind squares were excised using a sterile scalpel and tweezers. Each sample was placed in individual centrifuge tubes containing 20 mL of sterile peptone water (Fisher Scientific, Fair Lawn, NJ, USA) and vortexed at 3100 rpm for 1 min, according to the method of Ma et al. [24] (Figure 1B). Then, each sample was transferred into a sonicator (Branson 3510, Marshall scientific, Hampton, NH, USA) for 5 min to allow the release of bacteria from the sample surface before the filtration process.

Salmonella colonies were enumerated by the membrane filtration method, according to Yin and Patel [25]. Each sample (20 mL) was filtered through a 0.45 μm (47 mm diameter) membrane filter (Merck, Saint Louis, MO, USA) using a vacuum manifold (Pall Corporation, Port Washington, NY, USA). Immediately after filtration, a membrane filter with trapped bacteria from the sample was transferred to Xylose lysine tergitol 4 agar plates (XLT4; Difco, Becton Dickinson Co., Sparks, MD, USA) to reduce or eliminate the interference of background microorganisms. The agar plates were incubated at 37 °C for 48 h for enumeration, as can be seen in Figure 2A.
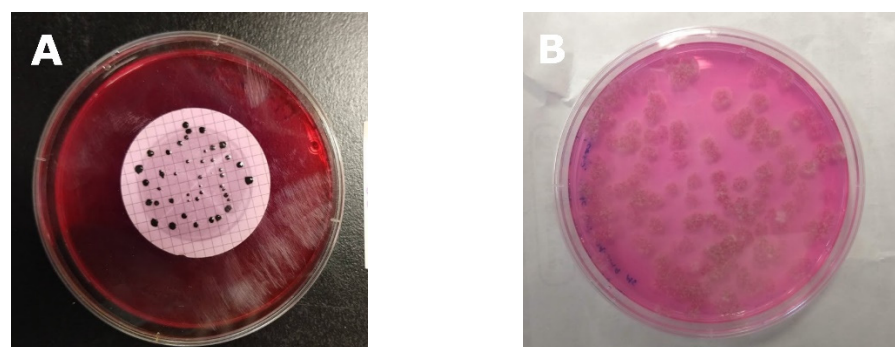

Figure 2. Enumeration of *Salmonella* spp. and *C. gloeosporioides*. (**A**) *Salmonella* spp. growth on XLT4 media after the membrane filtration method, (**B**) *C. gloeosporioides* growth on DRBC media.

2.9. C. gloeosporioides Enumeration

The inoculated areas in each mango were excised using a sterile scalpel and tweezers. Then, each sample was placed in individual centrifuge tubes containing 20 mL of sterile 10 mM phosphate-buffered saline (PBS; pH 7.4; Fisher Scientific, Fair Lawn, NJ, USA) with 0.2% (by mass) of tween 80 (Fisher Scientific, Fair Lawn, NJ, USA), and vortexed at 3100 rpm for 2 min according to the method of Ma et al. [24] (Figure 1B). The total population of the fungus was enumerated on DRBC media after a 5-day incubation at 25 °C, as seen in Figure 2B.

The efficacy of each treatment in controlling the population of *C. gloeosporioides* was calculated according to Equation (1) [26].

$$CE(\%) = \frac{N - F}{N} 100 \qquad (1)$$

where *CE* (%) is the control efficacy, *N* is the fungus population in the control treatment (CFU/cm^2), and *F* is the fungus population (CFU/cm^2) in coated fruit or with Prochloraz treatment. The results were expressed as a percentage of the fungal reduction population compared to the control treatment.

2.10. Sensory Evaluation

Sensory evaluation was performed to evaluate the effect of the maximum OEO concentration on consumer acceptance. Uninoculated mangoes treated with 0.9% (*w/v*) OEO coating and with Prochloraz fungicide as comparison treatment were used in the experiment. One hundred untrained panelists conducted the sensory evaluation, non-smokers, aged between 20–40 years. The sensory evaluation of each treatment was carried out for whole mangoes and mango pulp. Brightness, appearance, taste, aroma, and overall acceptance were evaluated. The panelists evaluated a nine-point hedonic scale where 1 corresponds to "I extremely dislike" and 9 corresponds to "I extremely like".

2.11. Statistical Analysis

Statistical analyses were performed using Minitab statistical software v.18 [27]. The one-way analysis of variance of means was performed at a significance level (*p*) of 0.05 using Tukey's test method. All results were reported as means ± standard errors.

3. Results

3.1. Oregano Essential Oil Characterization

The results of GC–MS analyses of OEO are presented in Table 1. The chemical characteristics of the compounds in essential oils influence their antimicrobial efficacy and the mechanism of action on the target organism [17].

Table 1. Constituents identified in oregano essential oil using GC–MS analyses.

Retention Time (min)	Tentative Identification	Relative Amount (%)
16.04	α-Thujene	0.2
16.41	α-Pinene	0.7
18.42	β-Pinene	0.3
18.80	β-Myrcene	0.8
19.52	ϱ-Mint-1(7),8-diene	<0.1
20.08	α-Terpinene	0.7
20.18	Cymene	<0.1
20.47	Cymene (isomer)	6.6
20.61	Limonene	0.1
20.72	β-Felandrene	<0.1
20.79	1, 8-Cineole	0.2
21.86	γ-Terpinene	4.7
22.36	ϱ-Mint-3, 8-diene	0.1
22.96	Terpinolene	0.1
23.50	Linalool	1.1
26.58	Borneol	0.4
26.86	Terpinene-4-ol	0.7
27.62	α-Terpineol	0.2
30.81	Thymol	4.9
31.43	Carvacrol	75.9
35.92	Trans-β-Caryophylene	1.4
41.24	Caryophylene Oxide	0.5

Twenty-two constituents were identified in OEO, representing 99.6% of the total oil. The phenols carvacrol (75.9%) and thymol (4.9%), as far as the monoterpene hydrocarbons γ-Terpinene (4.7%) and cymene (6.6%), were the predominant components of OEO. According to Shah et al. [3], the main constituents of OEO (carvacrol, thymol, and γ-Terpinene) are considered responsible for the antifungal activity. Due to the strong phenolics rings in their molecules structures, these components cause disintegration/dysfunction of cellular membranes, leading to depletion of substrate required for ATP production and cellular death. The terpenes (β-pinene, limonene, terpinene) found in lower amounts may also influence its antifungal properties, likely through the establishment of synergistic interactions [28].

The carvacrol precursor, ϱ-cymene, is not an efficient antimicrobial compound when used alone but can potentiate the activity of compounds such as carvacrol [29]. The antimicrobial activity of these compounds is affected by the presence of the hydroxyl group. Thymol and carvacrol, containing a free hydroxyl group, are more fungi toxic than ϱ-cymene [30,31].

3.2. Effect of Antimicrobial OEO Coating in Salmonella enterica

For the population of *Salmonella* on coated mangoes during 11 days of storage, the data revealed that strains used in this experiment grew on the mango surface when they were incubated at 25 °C (Figure 3).
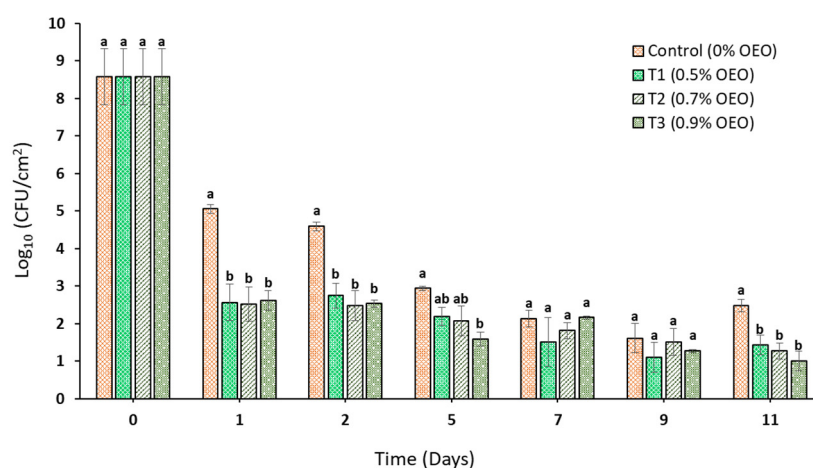

Figure 3. Growth of *Salmonella* on mango surfaces at 25 °C and 95% RH after coating treatments. Numbers at day 0 are the bacteria population before coating. Error bars represent the standard errors of the means. Different letters above bars indicate significant differences in the mean for that sampling period ($p \leq 0.05$).

The *Salmonella* population on coated mango decreased gradually for all treatments until day 5 of storage (Figure 3). A decreased survival over time for all treatments may be attributed to the epicuticular wax covering the fruit surface, which repels water and prevents microorganism attachment [6].

Coatings treatments with 0.5, 0.7, and 0.9% (*w/v*) OEO significantly reduced the viable cell counts in the first 2 days of storage compared to control treatment ($p \leq 0.05$), with about 2.0 to 2.5 log CFU/cm^2 reduction. These findings are relevant because bacterial cells that survive on mangoes for over 24 h would probably represent populations that can withstand desiccation on the fruit surface [5]. Moore-Neibel et al. [32] also observed reductions in *S. Newport* populations on the first 3 days of storage of baby spinach treated with 0.1%, 0.3%, and 0.5% oregano oil solution. Nevertheless, only the coating treatment with 0.9% OEO inhibited the viable cell counts at 5 days. At the end of storage, all the OEO coating treatments significantly reduced the bacterial population compared to the control treatment.

3.3. Effect of Antimicrobial OEO Coating in C. gloeosporioides

The population of *C. gloeosporioides* decreased on the first day of storage for all the treatments (Figure 4). A decrease in survival at day 1 may be attributed to the treatment and the natural plant defenses related to the mixture of antifungal compounds naturally in mango peel [33]. From day 2 of storage, differences between treatments were observed. Mostly coatings treatments reduced the fungal population significantly (ca. 1.5 log CFU/cm^2) compared to the control treatment.
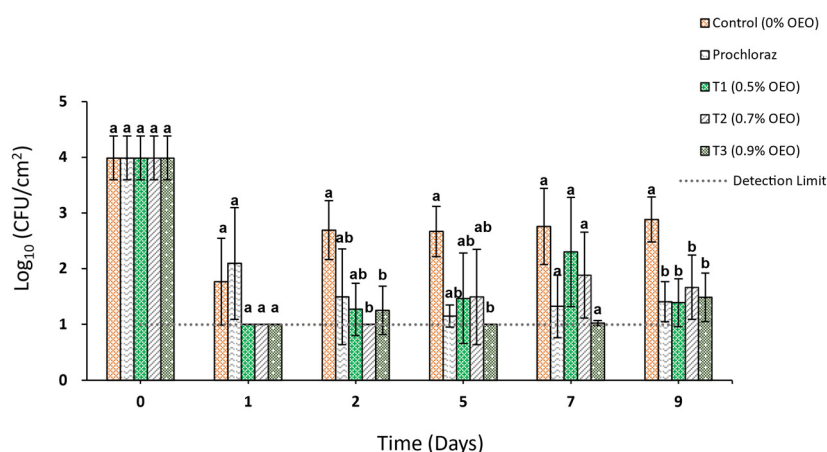

Figure 4. Populations of *C. gloeosporioides* on coated mango surfaces during storage at 25 °C and 90% RH. The detection limit was 1.0 log CFU/cm^2. Error bars represent the standard errors of the means. Different letters above bars indicate significant differences in the mean for that sampling period ($p \leq 0.05$).

The increase in fungus population at day 7 could be associated with the fruit ripening during the storage, increasing the susceptibility to pathogenic fungi [26]. On day 9, coatings with OEO and Prochloraz treatments effectively inhibited the fungus recovery, resulting in a more than 2.4 log CFU/cm^2 reduction in *C. gloeosporioides* compared to day 0 of storage (Figure 4). However, on day 11 of storage, the mangoes with the control treatment showed major damages caused by the fungus (black anthracnose lesions covering most of the fruit surface), so it was impossible to compare with the OEO coating treatments.

The efficacy of each treatment in controlling the population of *C. gloeosporioides* for coatings with OEO compared to the control treatment is presented in Table 2.

Table 2. Efficacy (%) of coatings with OEO for controlling *C. gloeosporioides* in mango cv. Tommy Atkins after 9 days of storage.

Treatment	Efficacy (%) *
Prochloraz	96.64 ± 0.53 [a]
T$_1$ (0.5% OEO)	96.68 ± 1.01 [a]
T$_2$ (0.7% OEO)	88.06 ± 7.28 [a]
T$_3$ (0.9% OEO)	93.44 ± 7.04 [a]

* Different letters in superscript indicate significant differences in the mean for that sampling period ($p \leq 0.05$).

The efficacy of coatings varies from 88.06 to 96.68% and has no significant difference with Prochloraz treatment ($p > 0.05$) (Table 2). Thus, the results show that OEO effectively inhibited *C. gloeosporioides* on the mango surface to the same extent as the traditional Prochloraz treatment.

3.4. Sensory Evaluation of Mangoes

The sensory evaluation showed that panelists approved the brightness, aroma, taste, appearance, and overall acceptance of treated mangoes (scores > 6). No significant differences were found for sensory attributes between 0.9% OEO coated mango and Prochloraz

treatment ($p > 0.05$) (Figure 5). These results suggest that OEO coating treatments can be used as an alternative to control the growth of *Salmonella* spp. and *C. gloeosporioides* without affecting the sensory characteristics of mangoes.
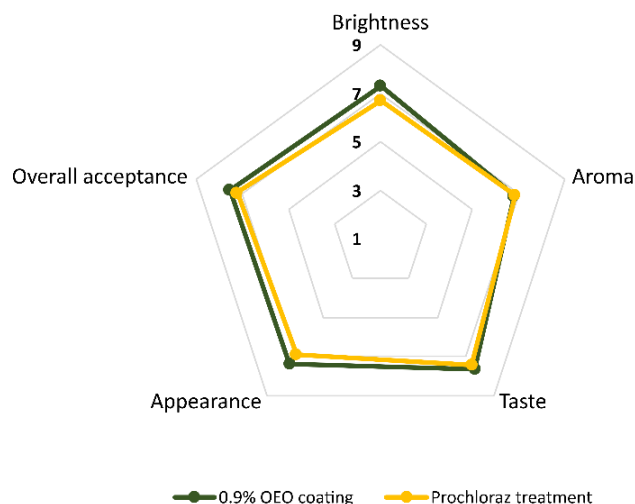

Figure 5. Sensory scores for mango samples treated with 0.9% OEO coating and Prochloraz fungicide.

4. Discussion

Although all OEO concentrations in coatings demonstrated antimicrobial effect, variations in the sensitivity of *Salmonella* to different concentrations were observed. Only the treatment T_3 (0.9% OEO) significantly inhibited the viable cell counts at day 5, and it was the treatment with the highest reduction in viable cells at the end of the storage. Moore-Neibel et al. [32] found the highest reductions (up to 4.9 log) of *Salmonella Newport* on leafy greens treated with 0.5% oregano oil over three days of storage. Gündüz et al. [34] obtained the maximum logarithmic reductions of *Salmonella typhimurium* on inoculated tomatoes treated with the highest concentrations of sumac extract (4%) and oregano oil suspensions (100 ppm). It has been suggested that carvacrol, the OEO main compound, destabilizes the cellular architecture, leading to the breakdown of membrane integrity, increasing its permeability, disrupting many cellular activities, including energy production, membrane transport, and other metabolic regulatory functions [35,36]. The cell membrane disruption by essential oils may affect various vital processes, nutrient processing, the synthesis of structural macromolecules, and the secretion of growth regulators [13,37].

All OEO coating treatments were able to reduce the recovery of *Salmonella* after 11 days of storage at 25 °C. However, none of the treatments were able to eliminate bacteria on the mango surface. Bhargava et al. [38] suggested that Gram-negative bacteria are more resistant to the essential oils treatments than Gram-positive bacteria due to the lipopolysaccharide protection from hydrophobic compounds.

OEO coatings effectively inhibited the growth and colony formation of *C. gloeosporioides* in mango peel. Previous studies found inhibition of OEO in postharvest decay. Rodriguez-Garcia et al. [16] found that concentrations between 2.59 and 3.61 g/L of OEO (*Lippia graveolens*) in pectin coatings prevented fungal decay caused by *Alternaria alternata* in tomatoes. Andrade et al. [39] found that 0.25 µL/mL of OEO in gum Arabic coating controlled Rhizopus soft rot on plums during storage at room temperature. This treatment inhibited the mycelial growth, spore germination, and sporulation of *Rhizopus stolonifer*. The efficacy of coatings with OEO is related primarily to carvacrol content. The antifungal effects of carvacrol were attributed to their ability to inhibit ergosterol biosynthesis and make the cytoplasmic membrane porous in resistant isolates [40]. Other studies suggested that carvacrol exerts its antifungal activity by disrupting calcium homeostasis and plasma membranes in the microorganism [41,42].

Essentials oils are considered low-risk targets for developing microbial resistance and can, therefore, contribute to a longer and more useful lifespan of currently used fungicides [43,44]. The antifungal activity of essential oils incorporated in coatings has shown promising results to inhibit the fungal growth of *C. gloeosporioides* in fruits. Lemongrass essential oil incorporated in coatings exhibited higher inhibitory effects on anthracnose development in mango fruit than the synthetic fungicides as thiophanate-methyl and difenoconazole [26]. In addition, edible coatings with thyme essential oil were reported to better inhibit anthracnose in avocados through decreased incidence and severity than Prochloraz treatment [44]. Studies have recently been carried out for the combined effect of essential oils on fungi and bacteria [13,14,45]. Desam et al. [14] found that *Mentha × piperita* L. essential oil shows significant antifungal and antibacterial (against Gram-positive and Gram-negative bacteria), mostly because menthol and menthone are main the chemical constituents. According to Tariq et al. [13], the action of essential oils against Gram-positive bacteria and fungi appears to be similar in sort. The essential oil compounds destroy the microorganism and fungal cell wall, which ends in a run of the protoplasm and its action. Moreover, in some fungi and Gram-positive microorganisms, which are sensitive to Imidazole and whose cell membranes are rich in unsaturated fatty acids, the membrane constituent's arrangement leads to the loss of cell viability and, eventually, lysis.

5. Conclusions

Oregano essential oil showed significant antibacterial and antifungal activity in whole mangoes. Coatings with 0.9% of OEO significantly inhibited the growth of *Salmonella enterica* cocktail on whole mangos during 11 days of storage, while coatings with 0.7 and 0.9% (*w/v*) OEO decreased *C. gloeosporioides* population during 9 days of storage at 25 °C and 90% relative humidity. Coatings with 0.9% OEO did not affect the taste, aroma, appearance, and overall acceptance of mango pulp and whole mangoes. These results could prompt the application of OEO as a valuable alternative for existing fruit protection strategies without affecting its sensory quality. Therefore, these results can be considered a method to maintain the quality of the mangoes during their commercialization.

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