Effect of some essential oils against *Aeromonas hydrophila* artificially inoculated into raw Nile Tilapia fish fillets during refrigeration storage

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Abstract

*Aeromonas* spp. is one of the emerging foodborne pathogens that gained importance during the last decades because of its zoonotic potential and as one of the specific spoilage organisms in seafood products. Agar well-diffusion assay revealed that cinnamon EO-Trans Cinnamaldehyde- (TC) and garlic Eos (GEO) showed the highest zone of inhibition against *Aeromonas* strains 18 mm for each at 0.5 % followed by thyme EO (TEO) (12 mm). While Clove EO (CEO) and onion EO (OEO) didn’t inhibit the growth of *Aeromonas* spp. Resazurin microtiter plate assay indicated that the MIC values were 3.125 mg/ml for GEO, 6.25 mg/ml for TC, 12.5 mg/ml for CEO, 50 mg/ml for TEO, and 75 mg/ml for OEO. The application of cinnamon, clove, garlic, thyme, and onion Eos against *Aeromonas* spp. on tilapia fish fillets stored at 4°C showed that among the low concentration of Eos (25 mg/ml), cinnamon and clove Eos showed a significant reduction in *Aeromonas* counts. Also, the higher concentration of CEO (25 mg/ml) caused a significant reduction rate. Counts of *A. hydrophila* in tilapia fish fillets of Eos treated samples were significantly different compared to the initial counts. EOS showed a significant reduction in the PH value of fish fillets except for the lower concentrations of TEO & OEO (alpha =0.052, P>0.05). A shelf-life extension of 2-3 days was achieved with essential oils treatment. They could be recommended as natural antimicrobial control of *A. hydrophila* in fish fillets.

Keywords: Essential oils, *A. hydrophila*, natural antimicrobials, Fish fillets

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Introduction

*Aeromonas* spp. are primarily inhabitants of the aquatic environment. They have been identified as important fish pathogens and are considered to be emerging pathogens in humans, causing enteric infections such as; gastroenteritis, traveler’s diarrhea, peritonitis, and hepatobiliary infections. Also, extraintestinal diseases such as wound infections, myositis, bacteremia, septicemia, meningitis, and soft tissue (Gilani et al., 2021; Wang et al., 2022).

Fish and other seafood are highly perishable, food additives were added to improve the shelf life of refrigerated seafood products as antimicrobial and antioxidant. Consumer awareness about the risks of preservatives and synthetically food additives grows daily (Nasab et al., 2019; Batiha et al., 2021; Huang et al., 2021). Plant-based antimicrobials are used to protect against microbial spoilage, thereby improving food safety, quality, and shelf-life. Most of these compounds are spices, like oregano, basil, cinnamon, clove, thyme, rosemary garlic, or essential oils (Eo) which are secondary oily aromatic metabolites extracted from leaves, bark, flowers, and roots of herbal plants (McClements et al., 2021).

Cinnamon, clove, and garlic Eo showed inhibitory effects against *Proteus, Aeromonas,* and *Pseudomonas* (Agha, 2020). Trans-Cinnaldehyde (TC) is a phenolic compound extracted from the bark of cinnamon that shows low toxicity and is recognized and classified as safe for addition to foods by the U.S. Food and Drug Administration (Majd et al., 2019). Cinnaldehyde from cinnamon inhibits the growth of gram-positive and gram-negative bacteria by disrupting the bacterial cell membrane (Zhang et al., 2017). While, eugenol phytocomponent found in clove prevents several metabolic processes (Davidson et al., 2020).

Thyme oil (TEO) effectively inhibited the food-borne pathogens in fish by damaging bacterial cell membranes. It inhibited the growth of both Gram-negative and Gram-positive bacteria. *A. veronii* was the most sensitive to the inhibitory activity of TEO (Ozogul et al., 2020).

Clove showed great antimicrobial activity against pathogens such as *S. aureus, Y. enterocolitica, A. hydrophila,* and *Listeria innocua* for 15 days of storage. It lowered the total volatile bases (TVB) and pH values and H₂S-producing microorganisms and extended the shelf life (da Rocha et al., 2018; Nisar et al., 2019; Batiha et al., 2020).

Garlic EO has a strong effect against *E. coli, S. aureus,* and *S. typhimurium* resulting in the destruction of bacterial cell membrane induced by the hydroxyl groups present in phenolic compounds, mainly due to sulfur-containing compounds, such as allin, diallylsulphides, and allicin. Also, onion (*Allium cepa*) possesses antimicrobial activity due to the presence of sulfur-containing compounds (Saleh et al., 2017).

This study was objected to evaluate the antibacterial effect of some essential
oils applied to Nile tilapia fish fillets experimentally contaminated with \textit{Aeromonas hydrophila}.

\section*{MATERIALS AND METHODS}

\textit{Aeromonas} medium Base (Himedia, India). Essential Oils were purchased from National Research Center, Egypt. 0.5 McFarland Standard (8.2 log10cfu/ml) (Cat. No. TM50) from Dalynn Biologicals Company. 95\% ethanol and 96-well plates and Nano-filters were purchased from Dar-ElHekma Company, Assiut City, Egypt. Mueller Hinton agar (Oxoid), Buffered Peptone Water (Oxoid).

\subsection*{Bacterial strains}

The aeromonads used in this study were isolated from fish meat and PCR identified using 16s rRNA of \textit{A. hydrophila}. All strains were stored at -80\(^\circ\)C in Brain Heart Infusion (Oxoid,) broth with 20\% (v/v) glycerol and routinely grown on \textit{Aeromonas} selective agar at 30\(^\circ\)C.

\subsection*{Essential oils}

The essential oils used in this study were purchased from the national research center, in Egypt: cinnamon (\textit{Cinnamomum zeylanicum}), clove (\textit{Ocimum gratissimum}), garlic (\textit{Allium sativum}), thyme (\textit{Thymus vulgaris}) and onion (\textit{Allium cepa}). The oils' stock solutions were prepared in 95\% (v/v) ethanol and stored at 4\(^\circ\)C until used. (Somrani et al., 2020).

\subsection*{Selection of Eos using agar well diffusion assay}

The antibacterial effect of different Eos was compared using agar well diffusion assay. Bacterial suspension of \textit{A. hydrophila} was prepared: A loopful of the stock culture was transferred to the brain heart infusion (BHI) broth and incubated at the temperature of 30 \(^\circ\)C for 24 hours. Following that, the freshly prepared suspensions were compared with 0.5 McFarland standard turbidity by using McFarland standard Apparatus (Scientific Device Laboratory, Inc., USA). Then 100 \(\mu\)l of bacterial suspension were spread onto Mueller Hinton agar plates, wells with 4 mm diameter were made by sterile cork poorer. Each well was filled with 100\(\mu\)l of different concentrations of EOs from pure (100\%) then double fold serial dilution up to 0.09 \%. One well in each plate contains 100\(\mu\)l of sterile deionized water act as a negative control. Plates allowed to set for 45 minutes at room temperature, then incubated at 30\(^{\circ}\)C for 24 h. and the diameter of inhibition zone was measured. All experiments were carried out in triplicate.

\subsection*{Determination of MIC of the essential oils by the resazurin microtiter-plate assay (Kot et al., 2019) and (Poulsen et al., 2017)}

The resazurin microtiter-plate assay allows to determine the metabolic activity of bacterial cells. The EOs were initially diluted in 96\% ethanol, and sterilized by sterile syringe with 0.45\(\mu\)m pore (Thermoscientific). First, a 100 \(\mu\)L of Mueller-Hinton broth was dispensed in each well of a plate (96-well plate), the first well was charged with 100 \(\mu\)L of the essential oil solution. Then 100 \(\mu\)L from the first well was consecutively moved into the next well, this step was repeated to the following wells to obtain two-fold serial dilutions of tested essential oil. The
obtained suspensions of the cells were diluted with PBS to obtain a final concentration of approximately $5 \times 10^5$ CFU/mL and transferred (100 µL) to the wells containing two-fold serial dilutions of tested essential oil. The final volume of mixture in each well was 200 µL in concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, 0.0976 mg/mL for each essential oil. Three wells in the last line of the plate were used as positive controls by filling 50 µL of inoculums and 150 µL of the culture media (cell suspensions without essential oil) while negative control was made with ethanol without essential oils, and 3rd control of sterility of the media (without bacterial suspension, no essential oil, nor solvent) three wells, were filled by 200 µL of the culture media as negative controls. The inoculated microplates were covered and incubated without agitation at 30°C for 24 h. after incubation, 10 µL of sterile resazurin water solution (0.01%) (Sigma-Aldrich) was added to each well. After that, the microplates were incubated for 2 h in darkness at 30°C. The presence of live bacterial cells causes the reduction of resazurin dye and the change of color from blue to pink. wells in which no resazurin color change was observed confirmed that the essential oil inhibited the bacterial metabolic activity were represented the MIC value of each EO. The previous assay was repeated three times.

**Preparation of Nile tilapia Fillets (Saleh et al.,2017)**

A piece of each fresh Nile tilapia fillet (100 g) was cut with a sterile scalpel and put under the UV light in the cabinet for 20 minutes in order to reduce the number of microorganisms attached to its surface.

**a. Preparations of Inoculate**

*A. hydrophila* strains were sub-cultured on Brain Heart Infusion (BHI) broth and incubated for 24 hours at 30°C. The inoculate was centrifugated (3000×g, 15 min), and washed twice and the bacterial suspension was prepared using saline (NaCl, 0.85%, w/v). The *Aeromonas* count was adjusted by comparing the turbidity of the prepared bacterial suspension with the 0.5 McFarland standard tube, colonies were added to the suspension to increase the bacterial concentration or the mixture was diluted using BPS to obtain a bacterial suspension equivalent to McFarland 0.5 = 1.5 x $10^8$.

**b. Inoculation of Nile tilapia fillets with tested bacteria and EOs**

1 ml of *Aeromonas hydrophila* bacterial suspension adjusted at $10^8$ was employed onto the Nile tilapia fillets. The MIC of each EO was prepared in 1x solution (0.5% v/w) and 2x solution (1% v/w).

**c. The application of Eos on Nile tilapia fillets**
The inoculated samples were divided into 7 groups; the 1st was untreated control, while the 2nd group was surface covered with 1 ml of cinnamon EO (0.5%), 3rd group was surface covered with cinnamon EO (1%), the 4th was surface covered with clove EO (0.5%), 5th group was surface covered with clove EO (1%), the 6th was mixed with garlic EO (0.5%) and the last 7th group was surface covered with garlic EO (1%). The control and treated samples were labeled and packaged as triplicates, then stored at 4°C inside the refrigerator. All groups (either control or treated) were subjected to a microbiological assessment at day zero (within 2 hours after treatment) and then Aeromonas spp. was periodically counted on the 1st, 3rd, 5th, 7th, and 9th days) or until the fillets showed signs of spoilage.

c. Enumeration of the Tested Bacteria (Erkmen, 2022)

One gram of treated samples was weighed and added to 9ml PBS, ten-fold serial dilution was made, 100μl of each dilution were surface plated using sterile bent glass rods on Aeromonas selective agar plates, then incubated at 30°C for 24 hours. Count colonies that show green with a dark green center or yellow colonies.

Sensory analysis of raw fish fillets during the storage period.

For sensory evaluation, a group of 8 staff members of the Animal Health Research Institute, Sohag branch was used for sensory evaluation of fish meat according to the method of Duman and Özpolat (2015) with minor modifications. The fillet samples were blind-coded. Panelists were asked to evaluate the texture, odor, appearance, and overall acceptability of the samples, and their mouths were washed with warm water before judging any other samples. The freshness of raw fish (color, odor, texture, and general acceptability) was evaluated using a 9point descriptive scale. A score of 8.0-9.0 indicated excellent quality, 6.0-7.9 indicated good quality, 4.0-5.9 indicated acceptable quality, and 1.0-3.9 denoted as completely spoiled, which meant the fillet had dull color, and strong stench, and flabby texture.

Reporting changes in PH measurement during storage

The pH values were measured using a Crison pH meter (Model 507, Crison, 159 Barcelona, Spain) equipped with a Crison combination electrode (Cat. no. 52, Crison, Barcelona, Spain). Measurements were carried out on the 1st, 3rd, 5th, 7th, 9th, and 12th), or until the fillets showed signs of spoilage. in triplicates.

Statistical analysis.

The obtained results were statistically evaluated by application of a one-way ANOVA test, standard deviation and standard error of the mean and the least significant difference for the mean (LSD), Turkey test, and significance at p < 0.05. using IBM-SPSS (SPSS Inc., Chicago, USA).

RESULTS

Agar well-diffusion assay revealed that cinnamon EO-Trans Cinnamaldehyde-(TC) and garlic EOs showed the highest zone of inhibition against Aeromonas strains which was18mm±1 for each at 0.5 % followed by thyme EO; 10mm±2. While Clove EO and onion didn’t inhibit the growth of Aeromonas spp.
Resazurin microtiter plate assay indicated that the MIC values were 0.003125 mg/ml for GEO, 0.0625 mg/ml for CEO, 0.125 mg/ml for TC, 0.5 mg/ml for TEO, and 0.75 mg/ml for OEO (Table 1 and Figs. 1&2).

Table 1: antibacterial effect of some Eos against *Aeromonas hydrophila* isolated from fish meat

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Resazurin microtiter plate assay (10⁵ cfu/ml)</th>
<th>Agar well diffusion assay (10⁶ cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/mL)</td>
<td>MBC (mg/mL)</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>Clove</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>Garlic</td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td>Thyme</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Onion</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

R: resistant

Among the low concentration of Eos (0.25 mg/ml), cinnamon and clove Eos showed a significant reduction in *Aeromonas* counts. Also, the higher concentration of CEO (0.5 mg/ml) caused a significant reduction rate. *Aeromonas* counts showed a significant difference in all treated samples compared with the initial count during the successive days of the experiment. While a significant reduction percentage was noticed on the 1st day of the experiment (Tables 2-3 and Figs. 3&4). Sensory scores showed a significant (P < 0.05) decline in both control and treatment groups as storage time increased (Tables 4&5 and Figs. 3&4). Samples treated with essential oils had a less fishy smell and firmer texture compared to the control.

Based on the sensory analysis, tilapia fish fillets were denoted as unacceptable quality by the sensory panel on the 5th day for control and on the 8th day for treatment groups. Thus, a shelf-life extension of 2-3 days was achieved with essential oils treatment.
EOS showed a significant reduction in the PH value of fish fillets except for the lower concentrations of TEO & OEO (alpha =0.052, P>0.05) there was no significant difference with the control.

Table 2: Log of Aeromonas counts in tilapia fish fillets treated with different essential oils compared with control.

<table>
<thead>
<tr>
<th>Storage (days)</th>
<th>Control</th>
<th>Cinnamon</th>
<th>Clove</th>
<th>Garlic</th>
<th>Onion</th>
<th>Thyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.48</td>
<td>8.48</td>
<td>8.48</td>
<td>8.48</td>
<td>8.48</td>
<td>8.48</td>
</tr>
<tr>
<td>1</td>
<td>8.48</td>
<td>6.95</td>
<td>6.84</td>
<td>5.95</td>
<td>5.60</td>
<td>6.40</td>
</tr>
<tr>
<td>3</td>
<td>8.62</td>
<td>6.48</td>
<td>6.30</td>
<td>5.54</td>
<td>5.11</td>
<td>5.95</td>
</tr>
<tr>
<td>5</td>
<td>8.72</td>
<td>5.26</td>
<td>5.08</td>
<td>5.15</td>
<td>4.95</td>
<td>5.60</td>
</tr>
<tr>
<td>7</td>
<td>9.72</td>
<td>4.89</td>
<td>4.85</td>
<td>4.60</td>
<td>4.30</td>
<td>5.18</td>
</tr>
</tbody>
</table>

ND: not done, samples showed H2S production in the agar plate on the 1st and 3rd days with marked signs of spoilage on the 3rd day of the experiment. X: the MIC of the corresponding essential oil, lowercase superscript (a) means there is a significant difference (P< 0.05) compared with the control.

Table 3: Percentage of reduction rate in Aeromonas count in tilapia fish fillets treated with different essential oils.

<table>
<thead>
<tr>
<th>EO</th>
<th>Cinnamon</th>
<th>Clove</th>
<th>Garlic</th>
<th>Onion</th>
<th>Thyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>x 2x</td>
<td>x 2x</td>
<td>x 2x</td>
<td>x 2x</td>
<td>x 2x</td>
</tr>
<tr>
<td>1</td>
<td>17.97</td>
<td>19.2</td>
<td>29.8</td>
<td>33.92</td>
<td>24.5</td>
</tr>
<tr>
<td>3</td>
<td>24.89</td>
<td>26.9</td>
<td>35.7</td>
<td>40.7</td>
<td>30.9</td>
</tr>
<tr>
<td>5</td>
<td>39.7</td>
<td>41.7</td>
<td>40.9</td>
<td>43.2</td>
<td>35.8</td>
</tr>
<tr>
<td>7</td>
<td>49.8</td>
<td>50.1</td>
<td>52.67</td>
<td>55.76</td>
<td>46.71</td>
</tr>
</tbody>
</table>
Table 4: The pH value of fish fillets treated with different Eos (1X, 2X) during refrigeration storage at 4°C.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.52 ±0.3</td>
<td>6.55±0.2</td>
<td>7±0.22</td>
<td>7.15±0.4</td>
<td>7.56±0.4</td>
<td>8±0.42</td>
<td>8.60±0.14</td>
</tr>
<tr>
<td>Cinn. (x)</td>
<td>6.16±0.3</td>
<td>6.24±0.24</td>
<td>6.38±0.22</td>
<td>6.49±0.22</td>
<td>6.88±0.32</td>
<td>7.58±0.0</td>
<td>7.76±0.14</td>
</tr>
<tr>
<td>Cinn. (2x)</td>
<td>5.86±0.0</td>
<td>5.91±0.09</td>
<td>5.98±0.1</td>
<td>6.24±0.02</td>
<td>6.32±0.02</td>
<td>6.41±0.0</td>
<td>6.96±0.06</td>
</tr>
<tr>
<td>Clove(x)</td>
<td>5.98±0.2</td>
<td>6.18±0.32</td>
<td>6.34±0.1</td>
<td>6.52±0.22</td>
<td>6.86±0.1</td>
<td>6.96±0.</td>
<td>7.21±0.3</td>
</tr>
<tr>
<td>Clove(2x)</td>
<td>5.78±0.0</td>
<td>5.88±0.01</td>
<td>5.94±0.1</td>
<td>6.12±0.1</td>
<td>6.46±0.0</td>
<td>6.84±0.</td>
<td>7.05±0.22</td>
</tr>
<tr>
<td>Thyme(x)</td>
<td>6.28±0.2</td>
<td>6.34±0.16</td>
<td>7.24±0.4</td>
<td>7.69±0.4</td>
<td>7.86±0.3</td>
<td>8.19±0.</td>
<td>nd</td>
</tr>
<tr>
<td>Thyme(2x)</td>
<td>5.88±0.1</td>
<td>5.94±0.1</td>
<td>6.04±0.1</td>
<td>6.34±0.1</td>
<td>6.86±0.2</td>
<td>7.14±0.</td>
<td>7.32±0.18</td>
</tr>
<tr>
<td>Garlic(x)</td>
<td>5.94±0.1</td>
<td>6.25±0.15</td>
<td>6.46±0.0</td>
<td>6.54±0.2</td>
<td>6.78±0.2</td>
<td>6.92±0.</td>
<td>7.26±0.25</td>
</tr>
<tr>
<td>Garlic(2x)</td>
<td>5.44±0.2</td>
<td>5.65±0.15</td>
<td>5.86±0.1</td>
<td>6.04±0.4</td>
<td>6.18±0.3</td>
<td>6.24±0.</td>
<td>6.36±0.08</td>
</tr>
<tr>
<td>Onion(x)</td>
<td>6.04±0.0</td>
<td>6.29±0.08</td>
<td>6.42±0.1</td>
<td>7.28±0.3</td>
<td>7.34±0.2</td>
<td>7.49±0.</td>
<td>7.98±0.2</td>
</tr>
<tr>
<td>Onion(2x)</td>
<td>5.64±0.0</td>
<td>5.89±0.09</td>
<td>6.12±0.1</td>
<td>6.28±0.1</td>
<td>6.34±0.1</td>
<td>6.46±0.</td>
<td>6.98±0.12</td>
</tr>
</tbody>
</table>

Values followed by similar small lowercase superscript letters (a) within the same row are significantly different (P<0.05). Differences between the treatments evaluated by the turkey test at 5% significance, there was no significant difference in pH values between thyme EO and control samples as alpha =0.052 which is greater than P>0.05.
Figure 3: Aeromonads reduction rate % achieved by cinnamon, clove, garlic, onion and thyme Eos during storage at 4°C compared to the untreated fish fillets control.
DISCUSSION

As shown in Table 1 and Figure 1, Cinnamon EO- Trans Cinnaldehyde (TC) had the highest zone of inhibition against Aeromonas strains. On the other hand, the CEO did not inhibit the growth of Aeromonas spp. on agar well-diffusion assay, a similar result was found by Agha (2020).

Table 2 and figure 2 showed that the MIC values were 3.125mg/ml for GEO, 6.25 mg/ml for CEO, 12.5 mg/ml for TC, 50 mg/ml for TEO, and 75 mg/ml for OEO. Lower MIC results for TC were obtained by Kluga et al. (2021), Agha (2020), Abdelhamed et al. (2019), Bokhari (2017) 3.12 mg/ml, 0.09mg/ml, 0.008 mg/ml and 0.412 mg/ml, respectively. In contrast, Kot et al. (2019) TC concentrations between 0.19 and 0.780 mg/ml were necessary to inhibit the growth of 5 different A. hydrophila strains. Bokhari (2017) estimated that 2.08 mg/ml of CEO inhibited Aeromonas spp.

Our findings revealed that 50-100 mg/ml of TEO was able to inhibit the growth of tested isolates, while 100 mg/ml had a bactericidal effect. Also, Kot et al. (2019) found a lower results. While Junior et al. (2018) and Quendera et al. (2019) revealed that growth of both A. hydrophila ATCC 7966 and A. hydrophila MF 372510 was inhibited at 25 mg/ml and 47-190 mg/ml of TEO. The MBC values of TEO were equal to MIC (25-50 mg/ml) in 9 Aeromonas tested strains. Somrani et al. (2020) found MIC of garlic and onion EOs was 10 mg /ml for each.

The differences in MICs could have resulted from different methods and different culture media used during the
determination of MIC (Kot et al., 2019), the composition of the used EO (Nieto et al., 2017), or the origin and characteristics of the tested strains (Junior et al., 2018). In this study, the determination of the MIC values of the tested agents was carried out by the resazurin microtiter-plate assay which allowed to determine the metabolic activity of bacterial cells. This assay is effective for reliable assessment of the antibacterial activity of phytochemicals against bacteria (Kot et al., 2018).

Table 3 and Figure 3 showed that cinnamon and clove at 25 mg/ml had the highest reduction rate on *Aeromonas* counts on 1st day of treatment followed by garlic(25mg/ml) and onion showed a 24.23% reduction rate, while thyme essential oil at a concentration (75 mg/ml) failed to reduce *Aeromonas* counts.

Plant extracts’ effect against microbial contamination could be evaluated in laboratory and food systems (Olszewska et al., 2020). The variation could be because of the interaction of other constituents/compounds with the food matrix or based on differences in the source and processing of raw material (Davidson et al., 2020). However, the activity of plant extracts varies, based on the concentration of active ingredients present, method of extract preparation, and method of application (Bora et al., 2020).

High concentrations of EOs have strong odors and may cause organoleptic changes in food. In our experiment, the upper acceptable concentration of essential oils used in tilapia fish fillets was 0.5% (v/v) as judged by the sensory panel. All panelists considered the level of 0.25% (v/v) acceptable and did not have negative effects on the organoleptic quality of fillets. EOs can effectively decrease the fishy odor. Which correlated well with (0.1-0.4% and 0.1%(v/v) that were previously reported (Haute et al., 2016) and (Huang et al., 2018), respectively.

Sensory scores showed a significant (P < 0.05) decline in both control and treatment groups as storage time increased (Table 7). Samples treated with essential oils had a less fishy smell and firmer texture compared to the control, and this could be attributed to the antimicrobial and antioxidative properties of essential oils by inhibiting bacterial growth and lipid oxidation, which are the main reasons of the offensive odors of spoiled products.

Table 7 showed that the best organoleptic quality of tilapia fish fillets was observed in the cinnamon essential oil (TC) treated group, which was due to the high antimicrobial properties of cinnamon essential oil. This result agrees with (Vital et al., 2018) who concluded that fillets treated with TC had shown higher sensory acceptability regarding odor, color, and weight among treated samples.

Based on the sensory analysis, tilapia fish fillets were denoted as unacceptable quality by the sensory panel on the 5th day for control and on the 8th day for treatment groups. Thus, a shelf-life extension of 2-3 days was achieved with essential oils treatment. Similarly, Ozogul et al. (2017) also found that the application of essential oils nanoemulsions extended the shelf-life of rainbow trout (*Oncorhynchus mykiss*) by approximately 3 days, and Huang et al. (2018) reported that TEO had extended the
shelf life of grass carp fillet by 2 days compared with untreated control. It can be explained by the fact that samples treated with essential oils show less H2S production than the untreated control sample leading to delayed spoilage. The same result was reported by

EOS showed a significant reduction in the PH value of fish fillets except for the lower concentrations of TEO & OEO (alpha =0.052, P>0.05) there was no significant difference with the control. Typically, the pH of live fish is just above 7.0 about 7.3, while, the post-mortem pH is between 6.0 and 6.8 this marked fall induced by rigor mortis where glycogen is converted to lactic acid. Differences among the initial pH values may be due to the species, diet, season, level of stress during the catch as well as the type of muscle (Gerges et al.,2016).

CONCLUSION

Essential oils (cinnamon, clove, garlic, thyme, and onion) have been investigated as a natural antimicrobial against *Aeromonas hydrophilla* experimentally inoculated in Nile tilapia fish fillets during chilled storage at 4° C. It could be concluded that essential oils treatment effectively inhibited the growth of *A. hydrophilla* and extended the shelf-life of tilapia fish fillets by 2-3 days compared to the control. Cinnamon, clove, and garlic essential oils showed a significant reduction of *Aeromonas* counts without significant changes in the organoleptic parameters. While thyme and onion Eos revealed nonsignificant inhibition compared with the control.

REFERENCES


International Journal of Food Microbiology, 266 (7): 52-59.


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