SVU- International Journal of Veterinary Sciences, 5(4): 88-102, 2022
Print ISSN: 2535-1826 **Online ISSN: 2535-1877**

Research Article Open Access

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^{o}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

DOI: 10.21608/svu.2022.149625.1217 Received: July 27, 2022 Accepted: November 10, 2022 Published: December 12, 2022 *Corresponding Author: Tawfik Esmat Abd-Elhafeez Tawfik
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Citation: Tawfik et al., Effect of some essential oils against *Aeromonas hydrophila* artificially inoculated into raw Nile Tilapia fish fillets during refrigeration storage. SVU-IJVS 2022, 5(4): 88-102.

Competing interest: The authors have declared that no competing interest exists.

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 (EOs) which are secondary oily aromatic metabolites extracted from leaves, bark, flowers, and roots of herbal plants **(McClements et al., 2021)**.

Cinnamon, clove, and garlic Eos 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)**. Cinnamaldehyde 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 H_2S-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 *Aeromonas hydrophila*.

MATERIALS AND METHODS

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 (Oxiod), Buffered Peptone Water (Oxiod).

Bacterial strains

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

Essential oils

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

Selection of Eos using agar well diffusion assay

The antibacterial effect of different Eos was compared using agar well diffusion assay. Bacterial suspension of *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 ºC 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 µl 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 100µl of different concentrations of EOs from pure (100%) then double fold serial dilution up to 0.09 %. One well in each plate contains 100µl of sterile deionized water act as a negative control. Plates allowed to set for 45 minutes at room temperature, then incubated at 30^{o}C for 24 h. and the diameter of inhibition zone was measured. All experiments were carried out in triplicate.

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 0.45µm pore (Thermoscientific). First, a 100 µL of Mueller-Hinton broth was dispensed in each well of a plate (96-well plate), the first well was charged with 100 µL of the essential oil solution. Then 100 µL 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 x 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.

Determination of MBC (accordance with the guidelines of the CLSI, document VET04-A2 (CLSI 2014)

Mixtures (100 µL) from wells showing no metabolic activity of bacterial cells were sub-cultured and incubated at 30 ° C for 24 h. After incubation, the MBCs were defined as the lowest concentrations of the essential oil, at which no growth or less than 5 colonies were observed on the MH agar plate. Each test 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 0.003125mg/ml for GEO, 0.0625 mg/ml for CEO, 0.125 mg/ml for TC, 0.5mg/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

Essential oil	Resazurin microtiter plate assay (10^5cfu/ml)		Agar well diffusion assay (10^6cfu/ml)
	MIC (mg/ mL)	**MBC (mg/ mL)**	**Inhibitory zone (mm)**
Cinnamon	6.25	12.5	18 ±1mm
Clove	12.5	25	R (not diffused into the agar)
Garlic	3.125	6.25	18±1 mm
Thyme	50	75	8±2 mm
Onion	75	100	R (7±2mm)

R: resistant

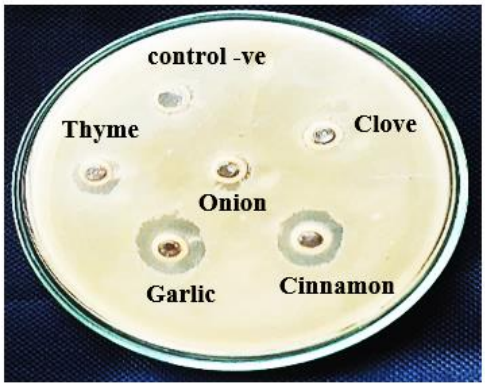


Figure 1: Zone of inhibition formed by different Eos using agar well diffusion method
Control -ve (ethanol 96%+DW similar to the ratio used with Eos), TEO 75 mg/ml; 7mm, GEO 25 mg/ml; 18mm; TC 25 mg/ml: 14mm; CEO 25 mg/ml: 9mm; OEO 75 mg/ml: 7mm.

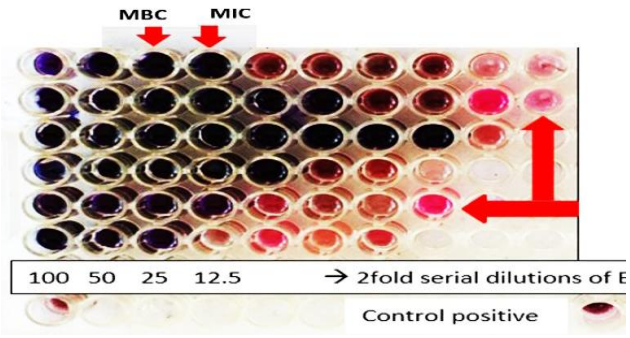


Figure 2: Determination of minimal inhibitory concentrations (MIC) of Eos by using the resazurin microtiter-plate assay.
Control +ve: bacterial suspension + resazurin + DW (without Eo)→pink color, Control -ve: Eo+ resazurin + DW (without bacterial suspension)→ blue color. MIC is indicated by the first dilution at which the color change from purple to pink.

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 (0.5mg/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.

Storage (days)	Control	Cinnamon		Clove		Garlic		Onion		Thyme	
		x	2x	x	2x	x	2x	x	2x	x	2x
0	8.48	8.48	8.48	8.48	8.48	8.48	8.48	8.48	8.48	8.48	8.48
1	8.48 [a]	6.95	6.84	5.95	5.60	6.40	6.34	6.65	6.40	7.3	7.00
3	8.62 [a]	6.48	6.30	5.54	5.11	5.95	5.52	6.60	6.00	7.54	6.85
5	8.72 [a]	5.26	5.08	5.15	4.95	5.60	5.26	5.48	5.00	ND	5.32
7	9.72 [a]	4.89	4.85	4.60	4.30	5.18	4.8	4.91	4.71	ND	3.91

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.

EO	Cinnamon		Clove		Garlic		Onion		Thyme	
Days	x	2x	x	2x	x	2x	x	2x	x	2x
1	17.97	19.2	29.8	33.92	24.5	25.2	24.5	33.31	16.80	17.42
3	24.89	26.9	35.7	40.7	30.9	35.9	30.4	42.01	19.32	20.5
5	39.7	41.7	40.9	43.2	35.8	39.8	37.2	47.2	Nd	38.9
7	49.8	50.1	52.67	55.76	46.71	50.10	45.85	49.49	Nd	49.77

Table4: The pH value of fish fillets treated with different Eos (1X, 2X) during refrigeration storage at 4°C.

Groups	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 12
days							
Control	6.52 ±0.3 [a]	6.55±0.2 [a]	7±0.22 [a]	7.15±0.4 [a]	7.56±0.4 [a]	8±0.42 [a]	8.60±0.14 [a]
Cinn. (x)	6.16±0.3 [a]	6.24±0.24 [a]	6.38±0.22 [a]	6.49±0.21 [a]	6.88±0.36 [a]	7.58±0.12 [a]	7.76±0.14 [a]
Cinn. (2x)	5.86±0.04 [a]	5.91±0.09 [a]	5.98±0.02 [a]	6.24±0.08 [a]	6.32±0.06 [a]	6.41±0.05 [a]	6.96±0.06 [a]
Clove(x)	5.98±0.22 [a]	6.18±0.32 [a]	6.34±0.42 [a]	6.52±0.23 [a]	6.86±0.16 [a]	6.96±0.14 [a]	7.21±0.3 [a]
Clove(2x)	5.78±0.02 [a]	5.88±0.01 [a]	5.94±0.14 [a]	6.12±0.12 [a]	6.46±0.06 [a]	6.84±0.24 [a]	7.05±0.22 [a]
Thyme(x)	6.28±0.22	6.34±0.16	7.24±0.42	7.69±0.42	7.86±0.32	8.19±0.41	nd
Thyme(2x)	5.88±0.11	5.94±0.1	6.04±0.14	6.34±0.12	6.86±0.22	7.14±0.24	7.32±0.18
Garlic(x)	5.94±0.16 [a]	6.25±0.15 [a]	6.46±0.19 [a]	6.54±0.28 [a]	6.78±0.22 [a]	6.92±0.14 [a]	7.26±0.25 [a]
Garlic(2x)	5.44±0.26 [a]	5.65±0.15 [a]	5.86±0.29 [a]	6.04±0.48 [a]	6.18±0.37 [a]	6.24±0.04 [a]	6.36±0.08 [a]
Onion(x)	6.04±0.08 [a]	6.29±0.08 [a]	6.42±0.12 [a]	7.28±0.3 [a]	7.34±0.22 [a]	7.49±0.26 [a]	7.98±0.2[a]
Onion(2x)	5.64±0.09 [a]	5.89±0.09 [a]	6.12±0.12 [a]	6.28±0.1 [a]	6.34±0.12 [a]	6.46±0.06 [a]	6.98±0.12 [a]

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.

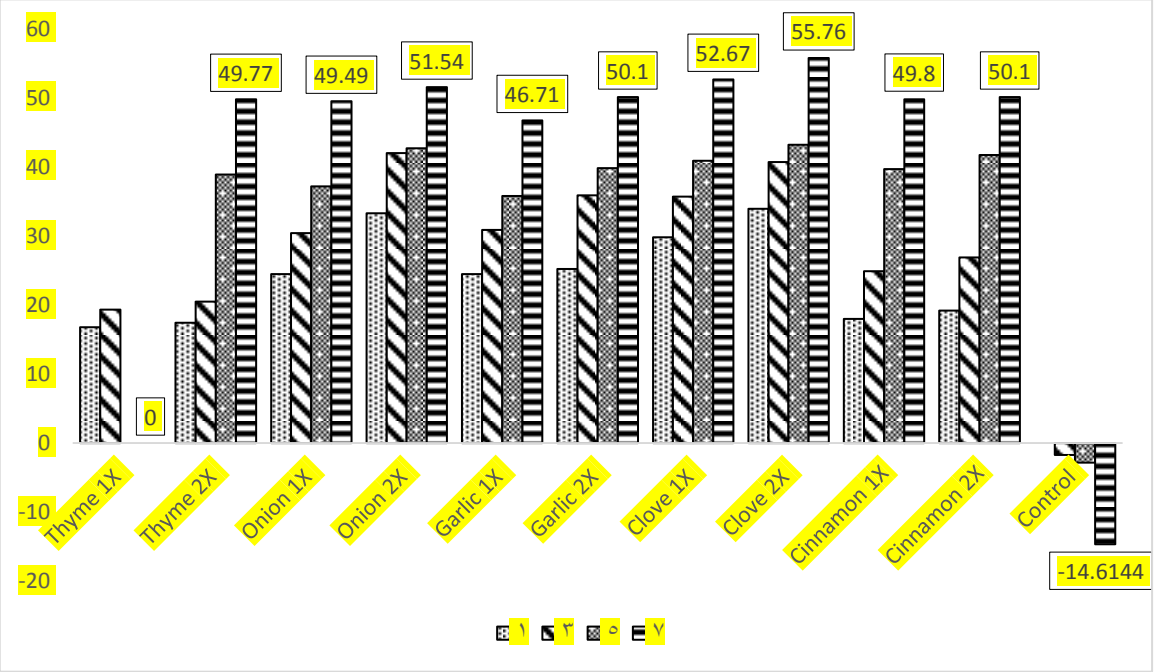
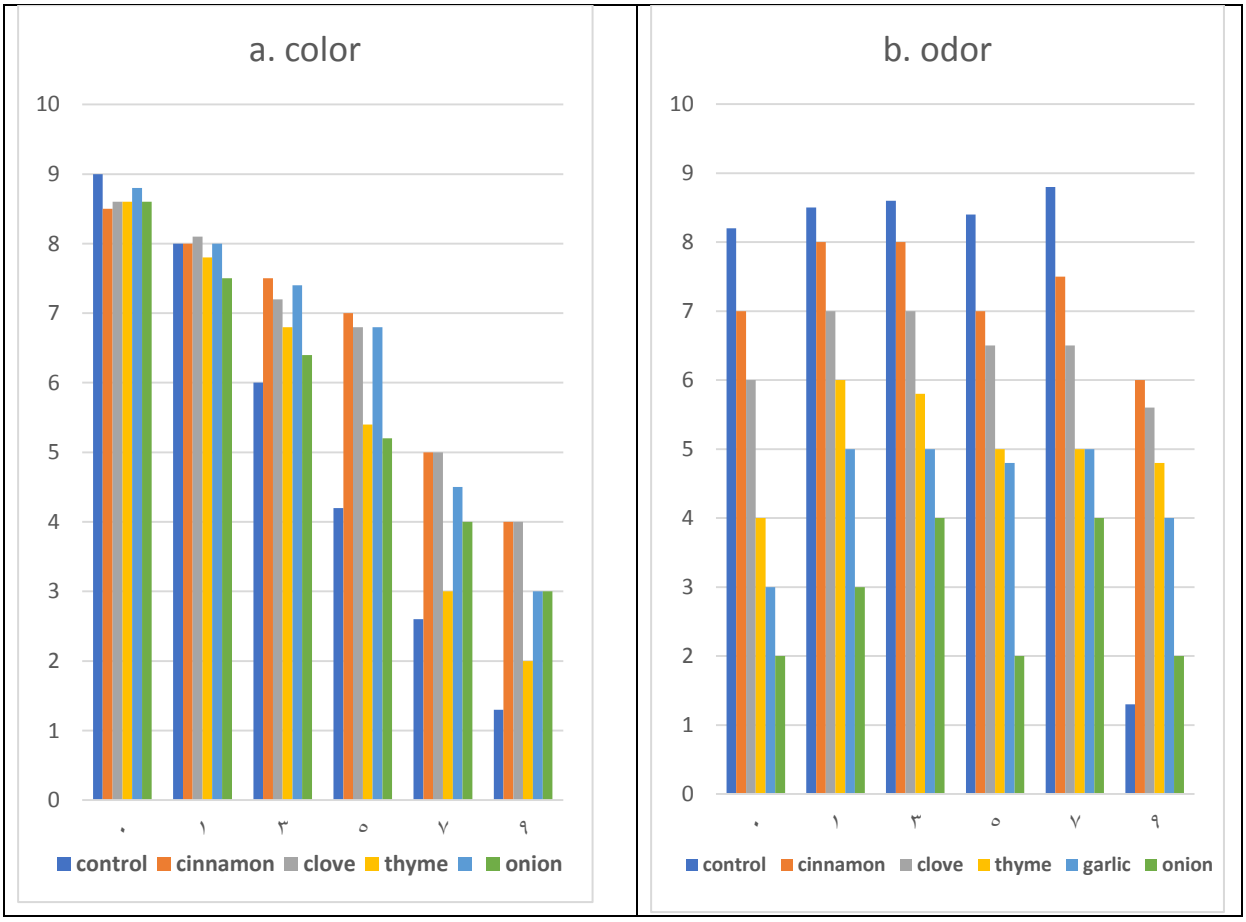


Figure 3: Aeromonads reduction rate % achieved by cinnamon, clove, garlic, onion and thyme Eos during storage at 4°C compared to the untreated fish fillets control.



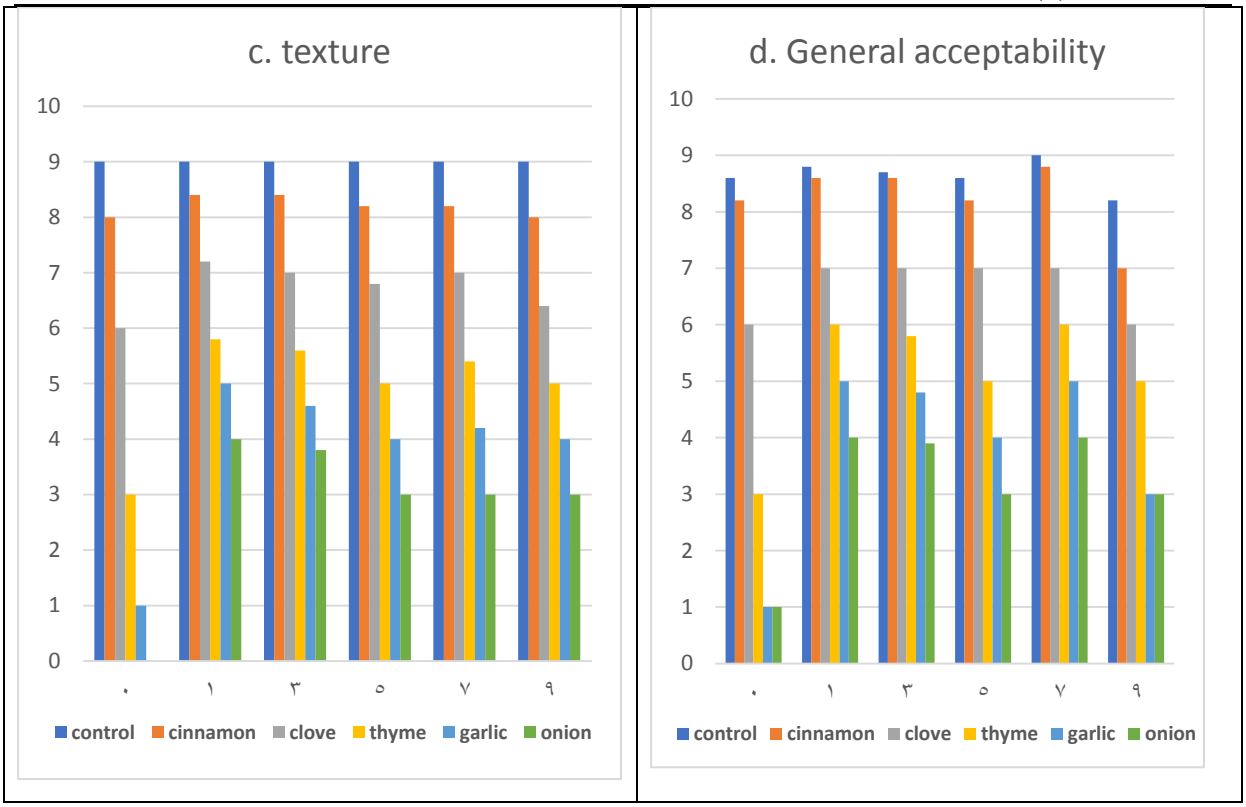


Figure 4: Changes in sensory scores of raw tilapia fish fillets during chilled storage(2X).

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^{o} 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

Abdelhamed H, Ozdemir O, Ibrahim I, Lawrence M, Karsi A (2019). Antibacterial activities of trans-cinnamaldehyde, caprylic acid, and β-resorcylic acid against catfish pathogens. Aquaculture, 504: 334–344.

Agha YY (2020). Effect of some plant oils on swarming motility and Biofilm formation in *Proteus, Aeromonas,* and *Pseudomonas*. Journal of Advanced Pharmacological and Educational Research, 10(4): 64-71.

Batiha E, Hussein DE, Algammal AM, George TT, Jeandet P, Al-Snafi AE, Tiwari A, Pagnossa JP, Lima CM, Thorat ND, Zahoor M, El-Esawi M, Dey A, Alghamdi S, Hetta HF and Cruz-Martins N(2021). Application of natural antimicrobials in food preservation: Recent views. Food Control, 126: 66- 80.

Bokhari SMR (2017). The effect of essential oils on the growth of bacteria from municipal wastewater treatment. Master thesis of Applied Science, Environmental Applied Science, and Management, 2017, Ryerson University.

Bora H, Kamle M, Mahato DK, Tiwari P and Kumar P (2020). Citrus essential oils (CEOs) and their applications in food: An overview. Plants, 9(3): 357.

da Rocha M, Viviane AA, Romani P, Caballero EL, Guillen CG, Montero P and Prentice C (2018). Effects of agar films incorporated with fish protein hydrolysate or clove essential oil on flounder (Paralichthys orbignyanus) fillets shelf-life. Food Hydrocolloids, 81:351-363.

CLSI (2014). Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals; approved guideline, 2nd edn. Wayne, PA: Clinical and Laboratory Standards Institute.

Davidson PM, Taylor TM and David J R (Eds.). (2020). Antimicrobials in food. CRC Press.

Duman M and Özpolat E (2015). Effects of water extract of propolis on fresh shibuta (Barbus grypus) fillets during chilled storage. Food Chemistry. 189: 80-85.

Erkmen O(2022). Isolation and counting of *Aeromonas hydrophila*. Microbiological analysis of foods and food processing environments, chapter: Practice 26, Academic Press: 285-290.

Gerges T, Selim A and Osman M (2016). Improvement of the Shelf Life of Tilapia Fillets Stored at Chilling Condition. Benha Veterinary Medical Journal, 31 (2): 45-55. https://doi.org/10.21608/bvmj.2016.31260

Gilani JT, Goudarztalejerdi A, Yavari M and Kalourazi MN (2021). Isolation and identification of *Aeromonas hydrophila* from *Cyprinidae* suspected with hemorrhagic septicemia in pools of warm water fishes in Gilan Province. International Journal of Nutritional Sciences, 6 (1): 52-58.

Huang Z, Liu X, Jia S, Zhang L, and Luo Y (2018). The effect of essential oils on microbial composition and quality of grass carp (*Ctenopharyngodon idellus*) fillets during chilled storage. International Journal of Food Microbiology, 266 (7): 52-59.

Huang X, Lao Y, Pan Y, Chen Y, Zhao H, Gong L, Xie N and Mo CH (2021). Synergistic antimicrobial effectiveness of plant essential oil and its application in seafood preservation: A review. Molecules (Basel, Switzerland), 26(2):1-13.

Haute SV, Raes K, Meeren PVD and Sampers I, (2016). The effect of cinnamon, oregano, and thyme essential oils in marinade on the microbial shelf life of fish and meat products. Food Control 68: 30-39.

Ibrahim M, Ahmad F, Yaqub B, Ramzan A, Imran A, Afzaal, M and Akram Q (2020): Current trends of antimicrobials used in food animals and aquaculture. In M. Z. Hashmi (Ed.), Antibiotics and antimicrobial resistance genes in the environment. Amsterdam, the Netherlands: Elsevier: 39-69.

Junior GB, Sutili FJ, Gressler LT, Ely VL, Silveira BP, Tasca C, Reghelin M, Matter LB, Vargas APC and Baldisserotto B (2018). Antibacterial potential of phytochemicals alone or in combination with antimicrobials against fish pathogenic bacteria. Journal of Applied Microbiology 125: 655- 665.

Kluga A, Terentjeva M, Vukovic NL and Kaˇcániová M (2021). Antimicrobial activity and chemical composition of essential oils against pathogenic microorganisms of freshwater fish. Plants, 10: 1265.

Kot B, Kwiatek K, Janiuk J, Witeska M and Pękala-Safińska A (2019).

Antibacterial activity of commercial phytochemicals against *Aeromonas* species isolated from fish. Pathogens, 8(3): 1–12. https://doi.org/10.3390/pathogens8030142

Kot B, Wierzchowska K, Gru˙zewska A and Lohinau D (2018). The effects of selected phytochemicals on biofilm formed by five methicillin-resistant *Staphylococcus aureus*. Natural Product Research, 32: 1299–1302.

Majd M H, Ghanbarzade, B, Noghabi MS, Najafi MA and Hosseini M (2019). A new active nanocomposite film based on PLA/ZnO nanoparticle/essential oils for the preservation of refrigerated Otolithes ruber fillets. Food Packaging and Shelf Life, 19: 94-103.

Poulsen AD, Mahdavi B, Yaacob WA and Din LB (2017). Asian Pacific Journal of Tropical Medicine. Asian Pacific Journal of Tropical Medicine, 10(8): 819–826. https://doi.org/10.1016/j.apjtm.2017.08.006

McClements DJ, Das AK, Dhar P, Nanda P K, and Chatterjee N (2021). Nanoemulsion-Based Technologies for Delivering Natural Plant-Based Antimicrobials in Foods. Frontiers in Sustainable Food Systems, 5(2): 1-23. https://doi.org/10.3389/fsufs.2021.643208

Nasab MM, Kouhdasht AM and Oliyaei N (2019). Application of Essential Oils for Shelf-Life Extension of Seafood Products, In "Essential Oils - Oils of Nature", Hany A. El-Shemy, In tech Open, 1-13.

Nieto G (2017): Biological activities of three essential oils of the Lamiaceae family. Medicines, 4: 63.

Nisar T, Yang X, Alim A, Iqbal M, Wang ZC and Guo Y(2019). Physicochemical responses and microbiological changes of bream (Megalobrama ambycephala) to pectin-based coatings enriched with clove essential oil during refrigeration. International Journal of Biological Macromolecules, 124:1156-1166

Olszewska M A, Gędas A, and Simões M (2020). Antimicrobial polyphenol-rich extracts: Applications and limitations in the food industry. Food Research International, 134,109214.

Omonijo FA, Ni L, Gong J, Wang Q, Lahaye L and Yang C (2018). Essential oils as alternatives to antibiotics in swine production. Animal Nutrition, 4, 126-136.

Ozogul Y, Boğa EK, Akyol I, Durmus M, Ucar Y, Regenstein J M and Köşker AR (2020). Antimicrobial activity of thyme essential oil nano-emulsions on spoilage bacteria of fish and food-borne pathogens. Food Bioscience, 36:635.

Quendera AP, Barreto AS and Lemsaddek TS (2019). Antimicrobial activity of essential oils against foodborne multidrug-resistant enterococci and aeromonads in planktonic and biofilm state. Food Sciences and Technology International journal, 25, 101–108.

Saleh EA, Morshdy AM.A, Mohamed, AM and El-sobary BF (2017). Prevalence of *Aeromonas* Species and Their Herbal Control in Fish. Global Veterinaria 18 (4): 286-293.

Somrani M, Debbabi H and Abidi F (2020). *Garlic, Onion, and Cinnamon* essential oil anti-biofilms' effect against *Listeria monocytogenes*. Foods journal, 9: 567: 1-12.

Vital ACP, Guerrero A, Ornaghi MG, Kempinski EMBC, Sary C, de Oliveira Monteschio J, Matumoto-Pintro PT, Ribeiro RP, do Prado IV (2018). Quality and sensory acceptability of fish fillet (*Oreochromis niloticus*) with alginate-based coating containing essential oils. Journal of Food Sciences and Technology, 55: 4945-4955.

Wang X, Pan J, Chen L, Han Y, Di Z, Ling B, Ahmad A, Yang N, Fan L, Li Q, Zeng J, Guo G and Zheng J (2022). Prevalence, virulence-related genes, and antimicrobial resistance of *Aeromonas spp.* from loach *Misgurnus anguillicaudatus* with skin ulcer and healthy controls in Southern China. Aquaculture, 8486(22):156-209.

Zhang Y, Li D, Lv J, Luo Y and Kong C (2017). Effect of cinnamon essential oil on bacterial diversity and shelf-life in vacuum-packaged common carp (Cyprinus carpio) during refrigerated storage. International Journal of Food Microbiology, 249:1-8