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Mitigating Pathogenic *Vibrio* Species Impact in Refrigerated Basa Fillets Using *Moringa oleifera* Seed Extract: A Natural, Clean-Label Bio-Preservative

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Abstract

THE rapid proliferation of *Vibrio* spp. in seafood during refrigeration poses a serious challenge to public health and product shelf-life, especially under rising global temperatures. The purpose of this work was to conduct a potential decontamination strategy for controlling this opportunistic zoonotic pathogen using a natural bio preservative such as *Moringa oleifera* seed extract. Additionally, the study aimed to investigate the antimicrobial activity of *M. oleifera* seed *extract* against *Vibrio parahaemolyticus* and *Vibrio cholera* that were inoculated in basa fillets stored under refrigeration conditions (4 ± 1 °C) for seven days. The inoculated fillets were dipped in the extract for 15, 30, or 45 minutes, and the microbial counts were monitored throughout storage. The extract's effect on the sensory and bacteriological quality of fillets was also evaluated. Treated samples showed significantly lower *Vibrio* counts ($p < 0.05$) compared to controls, with inhibition increasing in a dose- and time-dependent manner. Notably, complete suppression of *V. parahaemolyticus* was achieved by day 3 in the 45-minute group. Total aerobic plate counts were also significantly reduced, indicating better keeping quality. Treated fillets maintained favourable sensory attributes. These results highlight the efficacy of *M. oleifera* extract as a clean-label antimicrobial agent for improving the safety and shelf-life of chilled fish products, providing a sustainable alternative to synthetic preservatives in seafood preservation.

Keywords: Fish fillet, *Moringa* seed extract, antibacterial activity, *Vibrio* Species, sensory quality, shelf life.

Introduction

Fish and fish-derived products play a crucial role in the global food system, offering significant benefits in terms of food security and nutritional value. However, the growing demand for seafood has also raised serious public health concerns [1]**.** Over the recent decades, Basa fish fillet has become one of the most popular and consumable seafood items, particularly in developing countries, not only due to its low cost but also their tremendous sensory attributes. Even though fish fillets are considered highly perishable food items with a limited shelf life because of their high digestible protein and moisture levels [2]. Consequently, concerns about the safety of seafood are primarily associated with microbial contamination, particularly by pathogenic bacteria, which render these products unsafe for human consumption. Among these pathogenic bacteria, *Vibrio parahaemolyticus* and *Vibrio cholerae* are associated with severe foodborne illnesses characterized by acute gastroenteritis, as well as their related serious human foodborne outbreaks, particularly in the context of the growing presence of these pathogenic *Vibrio* species in marine ecosystems due to global warming [3].

Modern seafood processing demands, and intervention strategies should not only ensure microbial safety but also maintain the physicochemical and sensory quality of the product

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throughout storage [4]. Various novel approaches have been introduced to maintain seafood quality and safety. However, these methods are either expensive or lead to health issues. Moreover, they don't guarantee that the product is free from pathogenic bacteria [5]**.** Accordingly, natural antimicrobials offer safer, eco-friendly, and affordable alternatives to synthetic chemicals [6,7]

Among the strong natural plant bio-preservatives are the members of the genus *Moringa*, particularly *Moringa oleifera* seeds, owing to their high total phenolic and flavonoid, fatty acids, and glycosides contents [8,9]. These bioactive components as glycosides, as well as 4-(α-L-rhamnosyloxy)-benzyl isothiocyanate and 4-(α-L-rhamnosyloxy)-phenyl-acetonitrile, act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or the synthesis of essential enzymes, leading to inactivation of pathogenic bacteria and oxidative spoilage retardation [10,11].

Despite the extensive research on *Moringa oleifera* in the food sector, particularly due to its antimicrobial properties against a wide range of foodborne pathogens and bacterial infections, there is still limited data regarding the efficient utilization of *M. oleifera* seed extract as a potential decontamination strategy for fish and fish product preservation, with special emphasis on its effect on pathogenic *Vibrio* spp. in fish meat.

Therefore, the current study was conducted to investigate the inhibitory efficacy of a successive ethyl acetate extract of *M. oleifera* seeds against *V. parahaemolyticus* and *V. cholerae* through dipping of fish fillets in the seed extract for various durations. Additionally, the study aimed to evaluate the impact of *M. oleifera* seed extract dipping on the microbial quality and sensory attributes of fish fillets during chilled storage at 4 °C for 7 days.

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Material and Methods

Preparation of Moringa Seed Extract

Moringa seeds were purchased from the National Research Centre, Dokki, Giza, Egypt. The seeds were peeled and opened by hand to get the kernels, which were then sorted. Sound Kernels were chosen and dried in an oven at 103 °C for 3 hours to remove moisture. The dried kernels were then ground into a fine powder using a blender (Moulinex, France). Moreover, *Moringa oleifera* seed extract was prepared following the method outlined by [12].

Seed powder was soaked in ethyl acetate with a ratio of 1:5 (w/v), then mixed for 24 hours at 30°C. The mixture was then filtered using Whatman No. 1 filter paper. The filtered material was re-extracted with the same amount of ethyl acetate for 48 hours, or until the solvent was clear. Finally, the liquid extracts were evaporated at 40°C using a rotary evaporator.

In vitro determination of antibacterial activity of Moringa seed extract

Bacterial Strains and Inoculum Preparation

The two isolates of *V. cholera* and *V. parahaemolyticus* have already been characterized biochemically and molecularly identified by [1] with emphasis on their prevalence and virulence gene profiles. Each isolate was refreshed twice in tryptone soy broth (CM0129; Oxoid Ltd., Basingstoke, UK) with 2.5% NaCl. The bacterial suspension was incubated overnight at 37°C, then each culture's turbidity was adjusted using a saline solution compared to the McFarland tube No. 0.5 for reaching 10^8 CFU/ml count before being inoculated on MH (Mueller Hinton broth, Himedia-1084), incubated for 24 h at 37 °C. Subsequently, 1 mL of the original suspension from each fresh bacterial culture was aseptically transferred into a tube containing 9 mL of sterile alkaline peptone water (CM0009; Oxoid Ltd., Basingstoke, UK) to prepare a 10-fold serial dilution. Thereafter, 100 µL from each dilution was spread onto sterile Petri dishes containing thiosulfate–citrate–bile salts–sucrose (TCBS) agar to obtain the desired inoculum density, targeting approximately 10^8 CFU/mL [13].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) CLSI [14].

Moringa seed extract stock solutions were prepared in 1.5% DMSO to ensure complete solubilization. Serial dilutions were then prepared down to 0.19 mg/mL. The MIC was determined using a sterile 96-well microtiter plate. Each well from 1 to 12 of columns was filled with 100 µL of sterile Mueller-Hinton broth supplemented with 2.5% NaCl. Next, 100 µL of plant extract at a concentration of 10 mg/mL was added to well No.1 in column A, bringing the total volume to 200 µL and the concentration to 5 mg/mL. A two-fold serial dilution was carried out across the wells. Following this, 100 µL of the bacterial suspension (adjusted to the McFarland tube No. 0.5) was added to each well, adjusting the starting concentration of the plant extract to 2.5 mg/mL. Well No.11 was designated as the negative control (containing only plant extract and sterile broth). In contrast, well No.12 served as the positive control (containing broth and bacterial inoculum without plant extract). MIC values were recorded as the lowest concentration at which no visible bacterial growth was observed. Following the identification of the Minimum Inhibitory Concentration (MIC), 100 µL from each well showing no visible growth was subcultured onto TCBS agar plates and incubated at 37 °C for 24 hours. The lowest concentration of the extract that showed no bacterial growth on the agar plate was recorded as the MBC value.

In vivo determination of antibacterial activity of M. oleifera seed extract against the inoculated strains.

Inoculation of Basa fillets

The inoculation process adhered to the methodology described by [15] by dropping the inoculum on the fillets with gentle pressure and shaking to ensure tissue uptake. Finally, the fillet samples were held at room temperature for 30 minutes to facilitate bacterial attachment and absorption.

A total of five kg of frozen white Basa fish fillets (*Pangasius boccorti)* were purchased from a local fish market in Giza, Egypt, and immediately transferred to the Microbiology laboratory of Food Hygiene, Faculty of Veterinary Medicine, Cairo University, in a sterile ice box containing crushed ice. After preparation, they were exposed to surface sterilization under a UV cabinet (Cole-Parmer 9818 Series-Darkroom) at a wavelength of 254 nm for one hour from both sides (30 min for each side) according to the procedures pronounced by [16] with some modifications to ensure the fillets are free from *Vibrio* spp. and that the total aerobic bacterial count is below the permissible limits. Fillets were segregated into 5 groups in duplicate for each strain: Group 1 (control-ve group) was dipped in distilled water), group2(control +ve group) was only inoculated with the previously prepared *V. parahemolyticus* and *V. cholera* strains separately, with a final concentration of 10^7 cfu/g for both *Vibrio* strains in fillet samples), group 3, group 4 and group 5 were inoculated with10^7 cfu/ml of each strain and dipped in *Moringa* seed extract for 15 min, 30 min., and 45 min respectively). At the end of dipping periods, the fillets were left to drain and dry under laminar air flow for 15 min before being stored at 4°C for 7 days in sterile polyethylene bags. Throughout the storage duration, enumeration of the count of both *Vibrio* strains was conducted at regular intervals on the 1st day, 3rd day, 5th day, and 7th day. The experiment was repeated for three trials at separate times.

Enumeration of V. parahaemolyticus and V. cholerae count in chilled Basa fillets

Ten grams of treated fillets were homogenized in a stomacher bag containing 90 mL of sterile peptone water (1 g/kg) using a stomacher (Lab Blender 400, Seward Lab, Model No. AB 6021) at high speed for 2 min. Using the spread plate method, homogenates were exposed to serial dilutions and 100 µl of each dilution was surface plated onto Thiosulfate Citrate

Bile Salts Sucrose (TCBS Oxoid Ltd., Basingstoke, England) agar. All inoculated plates were incubated at 37°C for 24 h. Colony-forming units (CFUs) were then counted, and bacterial loads were calculated by multiplying the number of CFUs by the dilution factor [17,18]

Effect of Moringa seed extract dipping on bacteriological and sensory quality of Basa fillets during chilled storage

To investigate the effect of immersion of Basa fillets in *Moringa oleifera* seeds ethyl acetate extract on the bacteriological quality of Basa fish fillets. Four groups of Basa fillets, away from the inoculated groups (control negative group, which was dipped in distilled water and treated fillets groups were dipped in MOS extract for 15 min, 30 min, 45 min respectively). Bacteriological quality was determined by the enumeration of total Aerobic mesophilic bacteria count according to the procedures guided by [19] during the storage period**.** Fillet homogenate was prepared by the same above-mentioned procedure, followed by serial dilution and spreading 0.1 ml from each dilution over the surface of double sets of Plate Count Agar (PCA Oxoid CM0463B, Hampshire, England) and incubated at 35 ºC for 48 hours.

Sensory quality evaluation of chilled Basa fillets

The sensory examination was performed on a previously prepared group of fillets which was dipped in *Moringa* seed extract for 45 min. besides, the control negative group, which was dipped in distilled water, the two groups underwent sensory analysis based on the methods pronounced by [20]. The samples were assessed using the nine-point hedonic scale, where 9 refers to extremely like and 1 refers to extremely unlike for the following characteristics: appearance, color, odor, texture, and overall acceptability. This evaluation was conducted by twelve well-trained panellists from the Food Hygiene and Control Department staff members throughout the storage period. All trials of the experiment were repeated three times independently

Statistical analysis

Each measurement was performed three times; the mean ± SE was used to represent the findings. Data were statistically analyzed by one-way ANOVA using SPSS 23 for Windows (SPSS, 2017). Results were considered statistically significant at *p*-values $P < 0.001$

Results

In vitro determination of antibacterial activity of Moringa seed extract

Minimum Inhibitory and Bactericidal Concentrations (MIC & MBC) of Moringa Seed Extract on Vibrio spp.

The minimum inhibitory concentration (MIC) of *Moringa oleifera* seed extract was determined as 1.25 mg/ml for *V. parahaemolyticus* and 2.5 mg/ml for *V. cholerae*. The minimum bactericidal concentration (MBC) was twofold higher than the MIC for both species, confirming a bactericidal effect.

In vivo determination of antibacterial activity of Moringa seed extract in Inoculated Basa Fillets

Antibacterial activity against V. parahaemolyticus

Fig. 1 and Table 1 showed a significant ($P < 0.001$) reduction in *V. parahaemolyticus* log count across all treated groups. Complete inhibition (100% reduction) was observed by day 5 in all treatment groups, with the highest reduction in bacterial load was observed in the 45-minute dipping group, continuing through day 7.

Antibacterial activity against V. cholerae

Fig. 2 and Table 2 indicated a significant decrease ($p < 0.05$) in *V. cholerae* counts across treatments. The 45-minute dipped group showed the highest reduction (99.99%) by day 7, though complete inhibition was not achieved throughout the storage period.

Total Aerobic Plate Count (APC)

As shown in Fig. 3 and Table 3, the total aerobic mesophilic bacteria count was significantly reduced ($p < 0.05$) in all treated groups. The 45-minute treated group had the lowest bacterial load, especially on days 3 and 5, indicating improved microbial shelf stability.

Sensory Evaluation

Table 4 and Fig. 4 summarize the sensory scores. Dipping in *M. oleifera* extract improved appearance, color, texture, and overall acceptability compared to the control group ($p < 0.05$).

Treated samples maintained higher sensory scores up to day 7, although odor was slightly less intense than the control.

Reduction of Vibrio spp. in Moringa-Treated Basa Fillets

The antimicrobial efficacy of *Moringa oleifera* seed extract against *Vibrio parahaemolyticus* and *Vibrio cholerae* inoculated into Basa fillet samples was evaluated and is summarized in Table 5 and Table 6, respectively.

As shown in Table 5, fillets treated with the extract for 15 minutes exhibited a *V. parahaemolyticus* reduction ranging from 99.95% on day 1 to 100% from day 3 onwards. Treatments of 30 and 45 minutes were more effective, consistently achieving reductions between 99.99% and 100% throughout the 7-day storage period.

Table 6 presents the reduction percentages for *V. cholerae* under the same treatment conditions. A 15-minute dip resulted in a reduction of 98.58% to 99.99%**,** with greater variability in early storage. However, fillets treated for 30 and 45 minutes showed more consistent antimicrobial activity, reaching 99.99% by day 5 and maintaining that level through day 7.

Overall, both tables demonstrate that increased dipping time in *Moringa oleifera* seed extract leads to enhanced and sustained bacterial reduction, with near-complete inactivation achieved by longer treatments.

Discussion

From a public health perspective, *Vibrio* species are of considerable concern due to their association with foodborne illnesses, particularly through the consumption of raw or undercooked seafood. Along with the ability of *Vibrio* spp. to colonize a wide range of marine hosts, including fish, mollusks, and crustaceans, increases their opportunity for human exposure. Therefore, the implementation of an effective inhibitory and decontamination strategy against this pathogen has become imperative, considering health, environmental, and economic considerations [21]

The determined MIC and MBC values indicate the strong inhibitory and bactericidal effects of *M. oleifera* seed extract against *V. parahaemolyticus* and *V. cholerae*. The comparatively lower MIC observed for *V. parahaemolyticus* suggests a higher susceptibility to the phytochemical constituents of *M. oleifera*, which may be attributed to variations in cell membrane composition or stress response mechanisms [22,23]. The MBC was confirmed by subculturing samples from clear MIC wells onto agar plates, where the absence of colony growth at concentrations twice the MIC indicated a bactericidal effect. These results are consistent with earlier reports demonstrating the broad-spectrum antimicrobial properties of *M. oleifera* seed extracts, primarily associated with isothiocyanates, flavonoids, and other bioactive metabolites like saponin ,alkaloid,tanins,and phenolic acid [24,25].The demonstrated efficacy against clinically significant *Vibrio* species supports the extract's potential as a natural, clean-label bio-preservative for enhancing seafood safety and extending refrigerated shelf-life, particularly in light of the global demand for alternative plant-based antimicrobial agents [26] .

As shown in Fig. (1), the application of *Moringa oleifera* seed extract exhibited a pronounced inhibitory effect against *V. parahaemolyticus* experimentally inoculated into Basa fish fillets during chilled storage. A significant reduction ($p < 0.05$) in bacterial load was observed, with up to a 5-log decrease compared with the positive control group. Complete inhibition was achieved in fillets dipped for the longest treatment duration (45 min), resulting in 100% reduction in vibrio growth and survival by the 5th day, an effect that persisted throughout the 7 days of refrigerated storage (99.99–100% reduction). With respect to the antimicrobial activity against *V. cholerae* (Fig. 2; Table 2), although a slight residual bacterial count was detected, the log values decreased significantly ($p < 0.05$) compared with the positive control. The reduction percentages ranged from 99.28% to 99.99% by the 7th day, particularly in samples treated for 45 min; however, complete inhibition of *V. cholerae* was not achieved during the storage period.

The strong antimicrobial activity of *M. oleifera* seed extract may be attributed to its bioactive constituents, including the antibiotic pterygospermin and various phytochemicals, notably a short polypeptide present in the seeds. These compounds are known to confer broad-spectrum antimicrobial potential against diverse foodborne pathogens and spoilage microorganisms [27,28,29] .

In addition, Anzano *et al*. [30] declared compatible results demonstrating significant antimicrobial activity of *Moringa* seed polar extracts against antibiotic-resistant strains of *S. aureus* and *S. epidermidis*, representing concentration-dependent activity owing to their specific fatty acid content. Meanwhile Pagadala and Shankar [31] attribute the antimicrobial action of *Moringa* seed to the presence of Lectin, which hinders bacterial growth and survival, besides cell permeability disruption against a wide range of bacterial pathogens. On the other hand, several authors reported disagreeable findings with the current study, which reported a limited antibacterial efficacy of *M. oleifera* seed and stem extracts against some foodborne pathogens [32,33,34] which is attributed to variance in the extraction method that may affect the amount of bioactive constituent in the extract. Together with [35], who claimed that the inherent characteristics of *Vibrio spp*. can reduce or neutralize the activity of the bioactive chemicals found in *M. oleifera* seeds and stems, resulting in reduced antimicrobial efficiency.

The aerobic plate count (APC), or the Total Plate Count, is described as a fundamental index for assessing the bacteriological integrity of meat products. This quantitative measure provides an absolute indication of the aerobic bacterial load as an important shelf-life indicator. Therefore, it's used as a standard test to monitor the bacteriological quality of meat [36]. Altogether, the present study findings are shown in Fig. 3 pronounced a clear significant reduction in log counts of total aerobic mesophilic bacteria, which was more obvious on the 3rd day and the 5th day of storage, particularly in the fillet group dipped for 45 min. Consequently, it indicates significant shelf stability throughout the chilled

storage period ($p < 0.05$) when compared to the control group. The results of [37] are consistent with the current findings as they emphasized that treated chicken fillets with *Moringa* seed oil extract nano-emulsions revealed significantly lower total viable counts (TVC) and *Staphylococcus aureus* count until the end of the 10th day of storage at 4°c. He also explained that a lot of secondary metabolites present in the *Moringa* seed extract disrupt the cell wall and cell membrane of *S. aureus*, resulting in loss of intracellular contents, besides blocking cellular energy metabolism.

As a consequence of the findings of the first experiment in the current study, the effect of *Moringa* seed extract dipped for 45 min on the sensory quality of bass fish fillets was examined. Result were presented in Fig. 4 revealed that the dipping treatment did not adversely affect all sensory parameters scores but improves sensory attributes of fish fillets, particularly appearance, color, texture which differed significantly ($p < 0.05$) from the untreated control group over the chilled storage period till the end of the 7th day with a great enhancement in the product's overall acceptability by the 3rd day of storage while there were low scores of odor of the treated group rather than the control one. In this context,[38] reported slightly similar results for enhancing sensory quality, safety, and extending the shelf life of tilapia fillets during refrigerated storage by the use of the lemon extract, which has a potent antibacterial effect against Gram-negative bacteria, particularly *Vibrio* species. Another compatible result was reported by [39] who stated that despite of addition of *Moringa* flower extract, the nuggets retained their desirable sensory attributes and remained of good quality and palatable throughout a 15-day storage period. On contrary, [40] disclaimed the current findings in beef burger formulated with *Moringa* seed flour, they observed that the burger recorded significantly lower sensory properties which was owed to the moisture absorbent nature of *Moringa* seed flour used in the study that negatively affects the sensory attributes despite their desirable effect on delaying the product deterioration and shelf-life extension.

Conclusion

The current study confirms the antimicrobial, shelf-life-extending, and sensory-preserving potential of *M. oleifera* seed extract against foodborne *Vibrio* species, supporting its application as a clean-label preservative in seafood. The study highlighted the pronounced efficacy of dipping for 30 min and 45 min of Basa fillets in *M. oleifera* seed extract. The extract was obtained by successive solvent extraction method as an effective natural decontamination strategy for combating serious human pathogens like *V. parahemolyticus* and *V. cholera* which may contaminate fish fillets. The fillets were stored under chilling condition for 7 days without adversely affecting their sensory characteristics as well as providing an extended shelf stability due to highly acceptable sensory scores and elevated microbial quality. Accordingly, the application of this strategy is recommended in other seafood and fish products to control the growth and survival of severe foodborne pathogens while ensuring safety and improving quality along their shelf life.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Not applicable

TABLE 1. Inhibitory activity of *M. oleifera* seed extract treatment against *V. parahaemolyticus* count (CFU/g) of Chilled Basa Fish Fillets.

Storage day	Treatment	Count (log CFU/g ± SE)
1st day	Control Positive	6.50 ± 0.50^{a}
	15 min	3.50 ± 0.50^{b}
	30 min	2.50 ± 0.50^{c}
	45 min	2.00 ± 0.00^{c}
3rd day	Control Positive	8.21 ± 0.27^{a}
	15 min	3.52 ± 0.14^{b}
	30 min	3.29 ± 0.06^{b}
	45 min	0.00 ± 0.00^{c}
5th day	Control Positive	7.71 ± 0.45^{a}
	15 min	0.00 ± 0.00^{b}
	30 min	0.00 ± 0.00^{b}
	45 min	0.00 ± 0.00^{b}
7th day	Control Positive	9.30 ± 0.30^{a}
	15 min	0.00 ± 0.00^{b}
	30 min	0.00 ± 0.00^{b}
	45 min	0.00 ± 0.00^{b}

TABLE 2. Inhibitory activity of *M. oleifera* seed extract treatment against *V. cholerae* count (CFU/g) of Chilled Basa Fish Fillets.

Storage day	Treatment	Count (log CFU/g ± SE)
1st day	Control Positive	6.00 ± 0.00^{a}
	15 min	5.00 ± 0.00^{b}
	30 min	4.00 ± 0.00^{c}
	45 min	4.00 ± 0.00^{c}
3rd day	Control Positive	7.29 ± 0.18^{a}
	15 min	4.95 ± 0.01^{b}
	30 min	4.71 ± 0.02^{bc}
	45 min	4.51 ± 0.05^{c}
5th day	Control Positive	8.23 ± 0.03^{a}
	15 min	4.45 ± 0.04^{b}
	30 min	3.29 ± 0.01^{c}
	45 min	2.98 ± 0.13^{d}
7th day	Control Positive	8.92 ± 0.01^{a}
	15 min	3.37 ± 0.03^{b}
	30 min	3.09 ± 0.05^{c}
	45 min	2.63 ± 0.15^{d}

TABLE 3. Effect of *M. oleifera* seed extract on bacteriological quality of Chilled Basa Fish Fillets.

Storage day	Treatment	Count (log CFU/g ± SE)
1st day	Control Positive	8.61 ± 0.05^{a}
	15 min	5.35 ± 0.05^{b}
	30 min	4.22 ± 0.04^{c}
	45 min	3.77 ± 0.07^{d}
3rd day	Control Positive	9.59 ± 0.03^{a}
	15 min	4.62 ± 0.04^{b}
	30 min	3.20 ± 0.03^{c}
	45 min	2.89 ± 0.05^{d}
5th day	Control Positive	9.98 ± 0.01^{a}
	15 min	5.73 ± 0.01^{b}
	30 min	4.65 ± 0.03^{c}
	45 min	3.45 ± 0.25^{d}
7th day	Control Positive	10.94 ± 0.01^{a}
	15 min	6.57 ± 0.03^{b}
	30 min	5.45 ± 0.04^{c}

TABLE 4. Effect of *Moringa oleifera* Seed Extract Treatment on Sensory Attributes of Chilled Basa Fish Fillets.

Day	Group	Appearance	Color	Odor	Texture	Overall Acceptability
0 day	Control	6.67 ± 0.33^{a}	7.33 ± 0.33^{b}	5.33 ± 0.33^{b}	7.67 ± 0.33^{a}	7.00 ± 0.00^{a}
	Treated	7.33 ± 0.33^{a}	8.67 ± 0.33^{a}	6.33 ± 0.33^{a}	6.67 ± 0.33^{b}	7.33 ± 0.33^{a}
3rd day	Control	6.33 ± 0.33^{a}	6.67 ± 0.33^{b}	6.67 ± 0.33^{a}	6.33 ± 0.33^{b}	6.33 ± 0.33^{b}
	Treated	7.33 ± 0.33^{a}	7.67 ± 0.33^{a}	6.33 ± 0.33^{a}	7.33 ± 0.33^{a}	7.33 ± 0.33^{a}
5th day	Control	5.67 ± 0.33^{a}	5.33 ± 0.33^{b}	5.67 ± 0.33^{b}	5.33 ± 0.33^{b}	5.33 ± 0.33^{b}
	Treated	6.67 ± 0.33^{a}	7.67 ± 0.33^{a}	6.33 ± 0.33^{a}	6.33 ± 0.33^{a}	7.00 ± 0.00^{a}
7th day	Control	5.33 ± 0.33^{b}	4.67 ± 0.67^{b}	5.33 ± 0.33^{b}	4.33 ± 0.33^{b}	5.00 ± 0.00^{b}
	Treated	5.67 ± 0.33^{a}	7.33 ± 0.33^{a}	6.33 ± 0.33^{a}	6.33 ± 0.33^{a}	6.67 ± 0.33^{a}

Different superscript letters (a, b) in the same row indicate a significant difference between treatments for the same attribute and day ($p < 0.05$).
SE: Standard Error, n = 3 per group.
Treatment = *Moringa* seed extract (MSE).

TABLE 5. Reduction % of *V. parahaemolyticus* count experimentally inoculated into Basa fillet samples dipped in *Moringa* seed extract for different durations (15 min, 30 min, and 45 min)

Groups	1st day	3rd day	5th day	7th day
Fillets dipped for 15 min	99.95	99.99	100	100
Fillets dipped for 30 min	99.99	99.99	100	100
Fillets dipped for 45 min	99.99	100	100	100

TABLE 6. Reduction % of *V. cholerae* count experimentally inoculated into Basa fillet samples dipped in *Moringa* seed extract for different durations (15 min, 30 min, and 45 min).

Groups	1st day	3rd day	5th day	7th day
Fillets dipped for 15 min	99.71	98.58	99.98	99.99
Fillets dipped for 30 min	99.28	99.76	99.99	99.99
Fillets dipped for 45 min	99.45	99.84	99.99	99.99

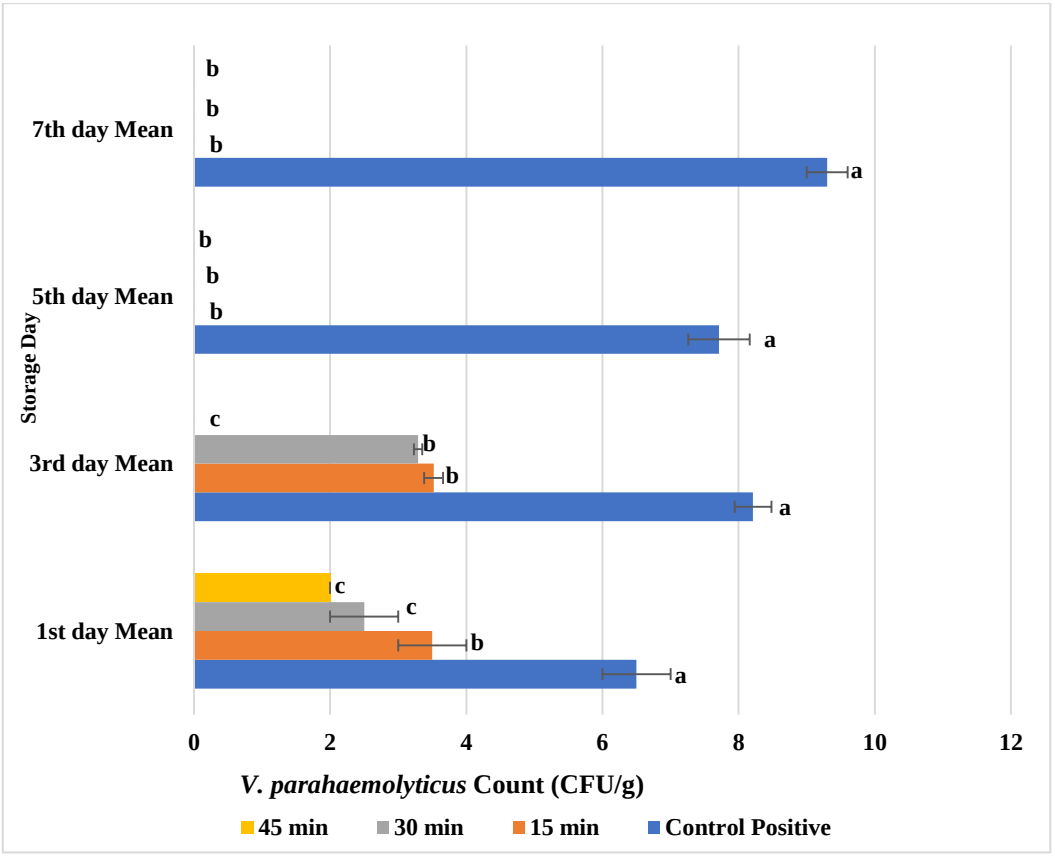

Fig.1. Inhibitory Activity of *M. oleifera* Seed Extract Treatment against *V. parahaemolyticus* Count (CFU/g) of Chilled Basa Fish Fillets.

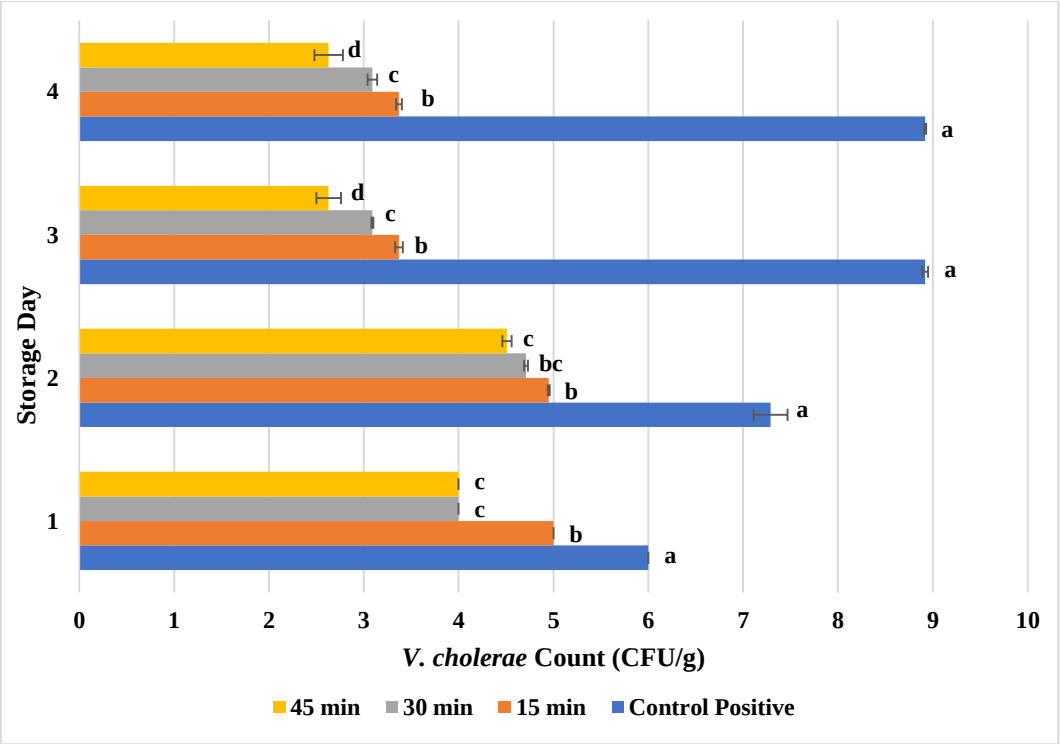

Fig. 2. Inhibitory activity of *M. oleifera* seed extract treatment against *V. cholerae* count (CFU/g) in chilled Basa fish fillets.

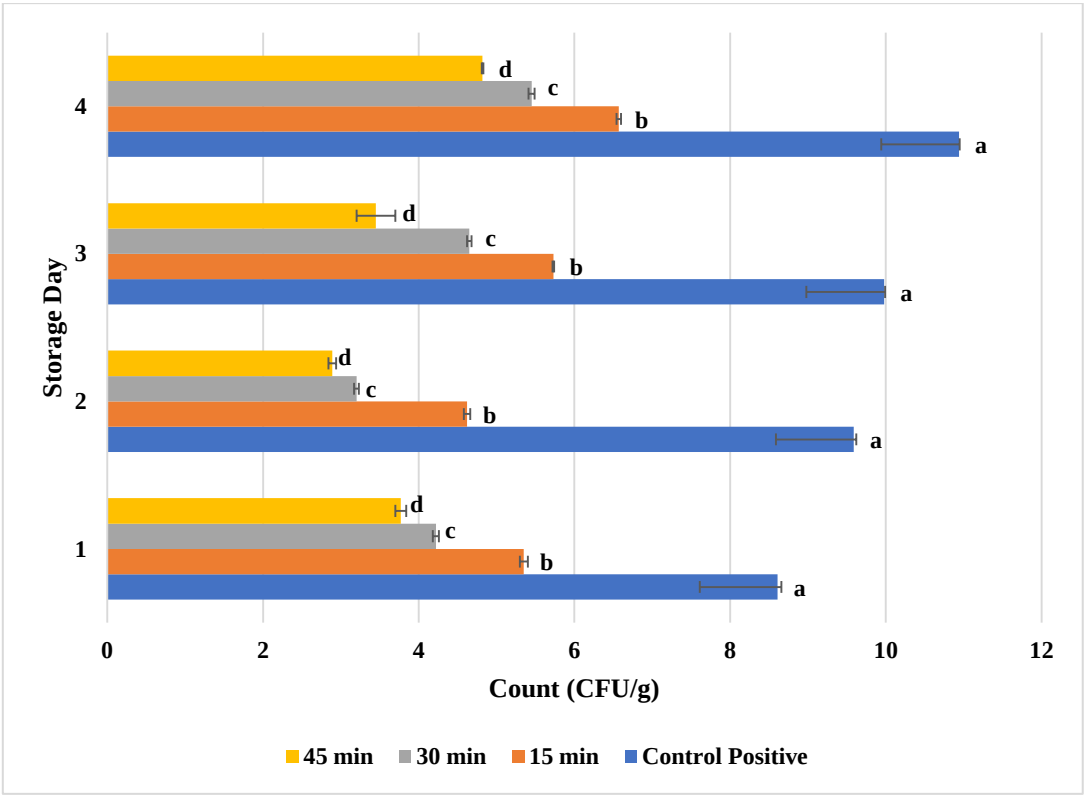

Fig. 3. Effect of *Moringa oleifera* seed extract treatment on aerobic plate count of chilled Basa fish fillets.

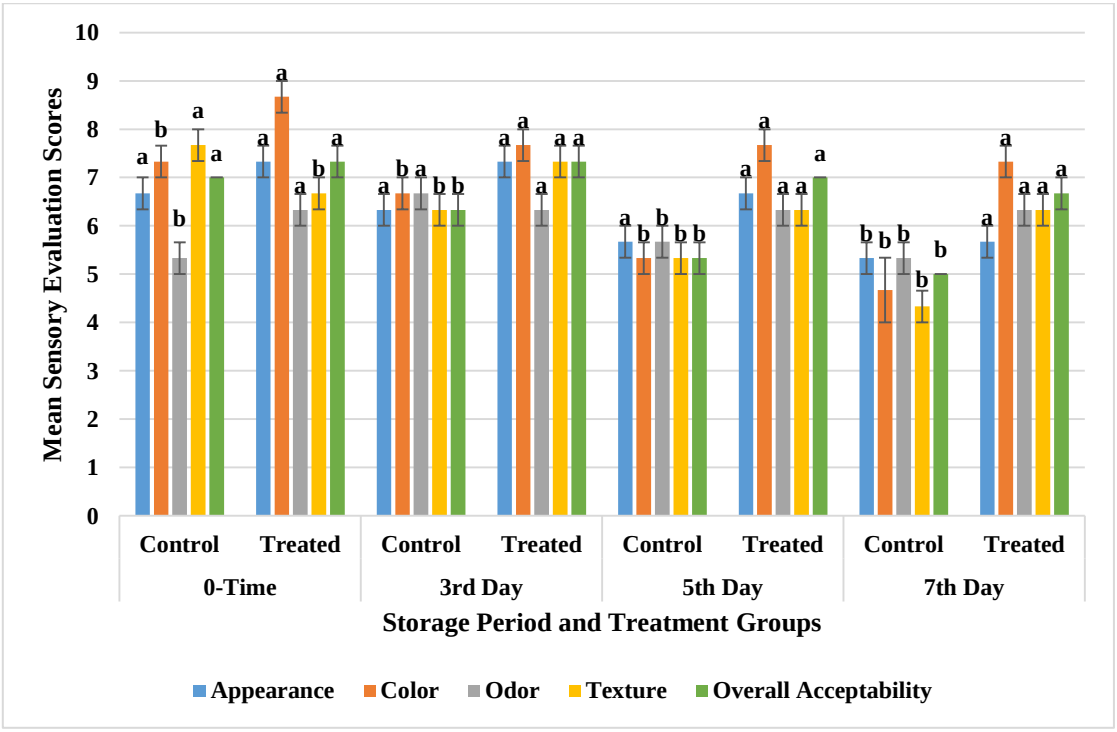

Fig. 4. Effect of *Moringa oleifera* Seed Extract Treatment on Sensory Attributes of Chilled Basa Fish Fillets.

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التخفيف من تأثير أنواع الـفيبريو الممرضة في شرائح سمك الباسا المبردة باستخدام مستخلص بذور المورينجا أوليفيرا :مادة حافظة بيولوجية طبيعية ونظيفة

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الملخص

يشكّل التكاثر السريع لأنواع الـفيبريو في المأكولات البحرية أثناء التبريد تهديدًا كبيرًا للصحة العامة ومدة صلاحية المنتجات، وهو تحدٍ يتفاقم مع ارتفاع درجات الحرارة عالميًا. هدفت هذه الدراسة إلى تقييم فعالية مستخلص بذور المورينجا أوليفيرا كمادة حافظة طبيعية للسيطرة على هذه المسببات المرضية الانتهازية المشتركة بين الإنسان والحيوان. جرى اختبار نشاطه المضاد للميكروبات ضد *Vibrio parahaemolyticus* و *Vibrio cholerae* التي أُقِحت في شرائح الباسا المخزنة عند (4 ± 1 °م) لمدة سبعة أيام. تم غمر الشرائح الملقحة في المستخلص لمدة 15 أو 30 أو 45 دقيقة، مع متابعة العدّ الميكروبي خلال فترة التخزين. كما تم تقييم تأثير المستخلص على الجودة الحسية والبكتريولوجية للشرائح. أظهرت النتائج انخفاضًا معنويًا ($p < 0.05$) في أعداد الـ *Vibrio* بالعينات المعالجة مقارنةً بالضوابط، مع ازدياد التثبيط تبعًا للجرعة ومدة الغمر. ومن اللافت أنه تم تحقيق تثبيط كامل لـ *V. parahaemolyticus* بحلول اليوم الثالث في مجموعة الـ 45 دقيقة. كما انخفضت أعداد الأحياء الدقيقة الهوائية الكلية بشكل ملحوظ، مما يشير إلى تحسن مدة الصلاحية. وأكد التقييم الحسي احتفاظ الشرائح المعالجة بخصائص مقبولة. تُبرز هذه النتائج فعالية مستخلص بذور *M. oleifera* كمضاد ميكروبي طبيعي نظيف لتحسين سلامة وعُمر تخزين منتجات الأسماك المبردة، مما يوفر بديلًا مستدامًا للمواد الحافظة الصناعية في حفظ المأكولات البحرية.

الكلمات الدالة: شرائح السمك، مستخلص بذور المورينجا، النشاط المضاد للبكتيريا، أنواع بكتيريا الفيبريو، الجودة الحسية، مدة الصلاحية.