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Insights on Vibriosis in white shrimp (*Metapenaeus stebbingi*): prevalence, virulence genes, and potential limitations of Existence in the meat

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ABSTRACT

Vibrios are the most widespread species of all crustaceans, including shrimp, which are susceptible. Numerous *Vibrio* species are linked to food-borne illnesses and are harmful to humans. The present investigation was performed to identify the prevalence and virulence genes of *Vibrio* species isolated from white shrimp (*Metapenaeus stebbingi*) and test the efficacy of some organic acids on its survival with improving shrimp shelf life. Samples of white shrimp (*M. stebbingi*) were collected from fishermen in Ismailia City, Egypt, between July and November 2023. *Vibrio* species were isolated and identified from the shrimp's hepatopancreas and musculature using its specific media (Thiosulfate-Citrate-Bile-Sucrose Agar) and biochemical tests. Then, the identification was confirmed and their virulence genes were detected by using PCR. Moreover, trials were performed by using acetic and citric acids treatments for decreasing *Vibrio parahaemolyticus* counts in artificially inoculated shrimp at different treatment durations, as well as for extending shelf life of chilled shrimp at refrigeration storage (4°C). The sensory attributes, pH values, total aerobic plate counts and lipid oxidation were evaluated under refrigeration at interval 0, 3, 6, 9 and 12 days. Results revealed that *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were found to be present in naturally infected white shrimp (*M. stebbingi*), with a total prevalence of 14%, where *V. alginolyticus* was the most prevalent, accounting for 9.6 ٪followed by *V. parahaemolyticus* at 4.4 .%The trails of organic acids treatments revealed significant gradual reductions in *V. parahaemolyticus* counts. As the organic acids concentration and immersion time increased, the count decreased. Additionally, there were improvements in all sensory characters, pH values, total aerobic plate counts and lipid oxidation of shrimp samples under refrigeration with extending shelf life up to 9 days by treating with acetic acid either 3% or 5%. Totally, it can be concluded that white shrimps were found to be naturally vectors for different *Vibrio* species and organic acids offer a safe, cost-effective solution for decontamination of shrimp as well as extending its shelf life.

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INTRODUCTION

Crustaceans, including crabs, clawed and spiny lobsters, and penaeid shrimp, have recently gained significant importance due to their high demand in global markets **(Mehanna and El-Gammal, 2008)**. These aquatic species are rich in nutrients of global importance as animal protein of super quality, essential amino acids, as well as omega-3 fatty acids **(Morshdy et al. 2022)**. According to **Sadek et al. (2002)**, *Penaeus semisulcatus, P. japonicas,* and *Metapenaeus stebingi* are widely found species in Egypt. *Metapenaeus stebbingi* is a target species for fisheries, accounting for approximately 90% of shrimp catches. White shrimp (*M. stebbingi*) originated exclusively in the Indo-West Pacific, migrated through the Suez Canal and became well established along the Egyptian Mediterranean coast and Suez Canal lakes **(Mehanna and El-Gammal, 2008)**.

Vibriosis in shrimp is one of the dangerous bacterial diseases that often affects many species of shrimp and is caused by many species of Vibrio **(Abdel-Latif et al. 2022)**. *Vibrio* species are common bacteria in aquatic systems, especially marine ecosystems, and belong to the class Gammaproteobacteria, the most diverse gram-negative, motile and facultatively anaerobic bacteria in the family Vibrionaceae **(Sampaio et al. 2022)**. Among the various *Vibrio* species that have already been isolated from diseased shrimp, *V. parahaemolyticus, V. alginolyticus, V. campbellii, V. vulnificus, V. anguillarum,* and *V. harveyi* are the most common **(Chatterjee and Haldar, 2012)**.

Species of Vibrio are found in both wild and farmed shrimps' natural microbiota **(Bamel et al. 2022)** and, when their natural defenses are undermined, turn into opportunistic pathogens **(Brock and Lightner, 1990)**. Vibriosis is promoted by a large number of virulence factors that enable pathogen infection and host damage **(Schroeder et al. 2017)**. Molecular methods (PCR) eliminate the need for laborious traditional methods by providing sensitive, fast and accurate data for the identification of particular bacterial pathogens **(Abdelsalam et al. 2022)**.

Many specific species within the Vibrio genus serve as the most prevalent pathogens transmitted through seafood consumption in humans **(Stratev et al. 2023)**. *Vibrio parahaemolyticus, V. vulnificus,* and *V. alginolyticus* are the most commonly found species in seafood-borne infections **(Fadel and El-Lamie, 2019)**. These *Vibrio* spp. are foodborne pathogens mainly present in various types of seafood that increase susceptibility of humans to gastroenteritis as a serious public health concern **(Morshdy et al. 2022)**. Moreover, instances of food poisoning cases caused by *Vibrio* species may result from the practice of eating raw or insufficiently cooked shrimp or through cross contamination **(Salem and Amin, 2012)**. Additionally, several factors at the retail time could lead to the high levels of *Vibrio* species in shrimp as contaminated ice, improper storage or unsanitary practices by sellers of fish **(Seham and Naglaa, 2021)**. Furthermore, due to their highly perishable nature, fresh shrimps can easily deteriorate during processing and storage, compromising food safety **(Afify et al. 2023)**, as well as their high susceptibility to spoilage during transportation and marketing, which limits their shelf-life resulted in experience quality loss **(Ye et al. 2020)**. The demand for natural antimicrobials is gaining the growing popularity as a method of preserving meat products as well as be safe and free of harmful chemicals **(Noordin et al. 2018)**.

In this line, ensuring food safety by preventing the growth of harmful microorganisms while simultaneously maintaining desirable sensory characteristics poses a significant challenge. For achieving these goals with adhering to modern demands of consumer and meat regulations natural preservation, certain organic acids considered good choices **(Ben Braïek and Smaoui, 2021)**. These organic acids can be utilized as preservatives and additives in non-heating modern processing technologies because of their antimicrobial activities to prolong shelf life **(Morshdy et al. 2022)**.

The current investigation's goal was to identify the clinical picture of Vibriosis in

white shrimp (*M. stebbingi*); the isolation and molecular identification of the *Vibrio* species; Identify the virulence genes; Determine the prevalence of the Vibrio isolates in the examined shrimp and finally evaluate the efficacy of acetic and citric acids with different concentrations on reducing *V. parahaemolyticus* growth in artificially contaminated shrimp as well as their efficacy in prolonging shrimp shelf life at refrigeration storage (4°C).

MATERIALS and METHODS

Sampling (Naturally infected shrimp)

Between July and November of 2023, a total of 250 moribund or freshly dead white shrimp (*Metapenaeus stebbingi*) weighing 22±3g, were bought from fishermen in Ismailia City, Egypt. These gathered shrimp were shipped in an ice box to the wet lab of the Fish Diseases and Management Department of Animal Health Research Institute Ismailia branch for clinical and bacteriological testing, using the procedures outlined by **Noga (2010)**.

Preparation of samples

The hepatopancreas and musculature were sampled using the techniques outlined by **Fadel and El-Lamie (2019)** after the shrimps were dissected in an aseptic condition.

Hepatopancreas

Sterile forceps were used to lift the carapace, and sterile cotton swabs were used to remove the inoculum from the inside of the hepatopancreas. The inoculum was then inoculated in tubes with 9 milliliters of sterile alkaline peptone water supplemented with 3% sodium chloride (NaCl).

Musculature

The surface was scorched with hot scissors, and a muscle sample (5 g) was aseptically removed and put into clean polyethylene sacs that contained 45 milliliters of alkaline peptone water supplemented with 3% NaCl.

Bacterial isolation and identification

Alkaline peptone water tubes have been incubated at 37°C for a duration of 24 hours. The method of **Markey et al. (2013)** involved streaking a loopful of incubated cultured broth onto thiosulfate-citrate-bile salts-sucrose agar (TCBS, Oxoid) plates and incubating them for 24 hours at 37°C. Following incubation, colonies that were yellow or green were collected, purified and used to identify the colonies phenotypically using the motility test, gram staining and biochemical testing through various sets of tests such as urease test, indole test, methyl red, Voges Proskauer, catalase and cytochrome oxidase (Biomerieux, Marcy-l'Étoile, France), as well as sensitivity against varying concentrations of sodium chloride (0-6.5%), according to **Austin and Austin (2012)**. PCR was utilized to confirm the identity and find the virulence genes of the isolated Vibrio bacteria, as stated by **El Zlitne et al. (2022)**.

Identification of *Vibrio* Species and Their Virulence Genes Using Polymerase Chain Reaction (PCR)

PCR is used to verify the identification of biochemically recognized *Vibrio* species and identify their virulence genes.

Extraction of DNA. The QIAamp DNA Mini kit (Qiagen, Germany, GmbH) changed into used to extract DNA from samples, with a few adjustments made according to the manufacturer's instructions. In summary, 10 µl of proteinase K and 200 µl of lysis buffer were added to 200 µl of the sample suspension and incubated for 10 minutes at 56°C. Following the incubation period, the lysate was mixed with 200 µl of 100% ethanol. The manufacturer's instructions were then followed for washing and centrifuging the sample. 100 µl of the elution buffer included in the kit was used to elute the nucleic acid.

Primer for oligonucleotides. The primers utilized, which are mentioned in **table (1),** were provided by Metabion (Germany).

Amplification by PCR. Primers were utilized in a 25-µl reaction for PCR, which included 5µl of DNA template, 1µl of each primer at a 20 pmol concentration, 5.5 µl of water, and 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan). A 2720 thermal cycler from Applied Biosystems was used to perform the reaction.

Analysis of PCR Products. At room temperature, the PCR products were separated via way of means of electrophoresis using gradients of 5V/cm on a 1 percent agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer. The PCR products were loaded into each gel slot with 20 µl for the gel analysis. Using Fermentas, Thermo Scientific, Germany, the Generuler 100 bp ladder was utilized to calculate the fragment sizes. Data was analyzed using computer software after the gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

Table 1. Primers sequences an PCR conditions for detection of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* and some virulence genes.

Target bacteria	Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
Vibrio parahaemolyticus	*toxR*	GTCTTCTGACGCA ATCGTTG CGTGCTGGCAACA AAGGACAG	685	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Kim *et al.*, (1999)
	Trh	CACAGCCAATATGT CGGTGAAG GTCACCTTCTCGC TCAGGC	326	94˚C 5 min.	94˚C 30 sec.	30˚C 40 sec.	72˚C 30 sec.	72˚C 7 min.	Mustapha *et al.*, (2013)
	Tdh	CCATCTGTCCCTT TTCCTGC CCAAATACATTTT ACTTGG	592	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Mustapha *et al.*, (2013)
Vibrio alginolyticus	*Collagenase*	CGAGTACAGTCAC TTGAAAGCC ATACGAGTGGTTG CTGTCATG	737	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Abu-Elala *et al.*, (2016)
	Trh	GGCTCAAAATGGT TAAGCG CATTTCCGCTCAT ATGC	326	94˚C 5 min.	94˚C 30 sec.	30˚C 40 sec.	72˚C 30 sec.	72˚C 7 min.	Mustapha *et al.*, (2013)
	Tdh	CCATCTGTCCCTT TTCCTGC CCAAATACATTTT ACTTGG	373	94˚C 5 min	94˚C 30 sec	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min	Mustapha *et al.*, (2013)

Experimental Part:

First experiment: evaluating the efficacy of organic acids treatments on the limitation of *V. para-haemolyticus* counts in artificially contaminated unpeeled shrimp:

Preparation of shrimp samples:

Two kilos of fresh unpeeled shrimp samples (50-60 per kilo) were bought directly from local fishermen. The shrimp were immediately transported to the lab in an icebox, using sterile procedures. Upon arrival, they were rinsed with sterilized water, disinfected with alcohol and left to drain on sterile metal mesh.

Preparation of bacterial strain inoculum:

A loopful of previously isolated *V. parahaemolyticus* was inoculated aseptically into 10 ml sterile Alkaline Peptone Water **(APW: Merck, Germany)** solution containing 3% NaCl and incubated at 37°C for 24 hrs. After incubation, *V. parahaemolyticus* was counted by spread plate method by using TCBS agar plates and incubated at 37°C for 24 h. Plates showing 30-300 colonies were counted **(ISO,**

8914/1990). Then the initial count ~10^5 CFU/ml was selected as initial inoculums load used to contaminate the fresh shrimp.

Preparation of organic acid solutions:

About 500 ml solutions each of citric acid (CA) of 4% and 6%, and acetic acid (AA) of 3% and 5% were prepared to test their effectiveness against *V. parahaemolyticus* growth.

Artificial contamination of shrimp samples with *V. parahaemolyticus*:

Careful surface inoculation of previously prepared *V. parahaemolyticus* inoculum (10^5cfu/ml) in shrimp samples was performed. They were left for 30 minutes at room temperature with shaking every 5 min **(Shirazinejad and Ismail, 2010)**. Then, *V. parahaemolyticus* were counted according to standard methods of **ISO (8914/1990)** to determine the initial load before treatments addition.

Treatment with citric and acetic acids:

The contaminated shrimp samples were divided into 5 equal groups. Then, each group was subdivided into three subgroups to be kept in the treatments for different immersion times 5, 10 and 15 minutes at room temperature 25° C±1°C. The groups represented as control group: dipped in sterile distilled water, the second group: were dipped in citric acid solution 4%, the third group: were dipped in citric acid solution 6%, the fourth group: were dipped in acetic acid solution 3% and the fifth group: were dipped in acetic acid solution 5%. Solutions completely submerged the entire surface of the shrimp, including the heads. After each designated emmersion time, the shrimp were carefully removed and allowed to drain.

Enumeration of Vibrio in shrimp samples:

Following standard methods outlined by **ISO (8914/1990)** *V. parahaemolyticus* were counted in each group of samples. The counts were reported as log CFU/ml.

Second experiment: evaluating the efficacy of organic acids treatments on extending the shelf life of unpeeled shrimp at refrigeration storage (4^0C):

Preparation of shrimp samples:

Three kilos of freshly caught unpeeled shrimp samples (50-60 per kilo) were divided into 5 equal groups and each group was subdivided into three subgroups for emmersion times 5, 10 and 15 minutes at room temperature 25°C±1°C. The first group served as control, were dipped in sterile distilled water. The second and third groups were immerged in citric acid solutions 4% and 6%, respectively. The fourth and fifth groups were immerged in acetic acid solutions 3% and 5%, respectively. Then, the shrimp were carefully removed and allowed to drain. After that all treated shrimp samples groups were stored under refrigeration at 4°C and examined at intervals of 0, 3, 6, 9 and 12 days or until spoilage for the following:

Sensory Evaluation:

Sensory evaluation was assessed by seven trained panelists on a 5-point hedonic scale according to **Pelin-Can and Arslan (2011)** (5 for excellent, 4 for good, 3 for fair, 2 for poor, 1 for unfit). The evaluation parameters were indicators of color, odor and texture.

Bacteriological examination:

Total aerobic plate counts of shrimp samples were performed according to the standard procedures according to **ISO (2013)**.

pH Measurement:

Ten gram of shrimp from each group were homogenized and mixed with 100 mL of distilled water for measuring of pH using a pH meter at room temperature **(AOAC, 1990)**.

Determination of Thiobarbituric Acid Reactive Substances (TBARS):

TBARS were determined according to the method described by **Thepnuan *et al.* (2008)**.

Statistical analysis

Microbial counts were converted into logarithms values (log_{10} CFU/g). Log_{10} reduction and reduction percentages were calculated and

all data were subjected to One Way Analysis of Variance (ANOVA) using **SPSS Version 19.0 (SPSS Inc., Chicago, IL, USA)**, followed by comparison of means using Duncan's test. Significance was defined at a level of $P<0.05$.

REULTS

Clinical picture

The majority of naturally infected white shrimps (*M. stebbingi*) had black spots on their cuticles and carapaces. Some of them also had black patches on their cuticles (**Fig. 1a, 1b, 1d, and 1e**). In the majority of cases, the body appendages, the telsons, the uropods, the pleopods, the pereopods, (**Fig. 1a, 1b, 1c, 1d and 1e**) and the gills showed black coloration (**Fig. 1a, and 1b**). A few cases showed reddish coloration on pereopods and pleopods (**Fig. 1d and 1e**). In some cases, the hepatopancreas seemed to be congested and soft (**Fig. 1e**) (**Plate,1**).

Plate. (1): Naturally infected white shrimps (*M. stebbingi*) with Vibriosis showing black spots and patches on their carapaces and cuticles, and black coloring was present on the body's appendages, telsons, uropods, pleopods, and pereopods (Fig. 1a, 1b, , 1c, 1d and 1e). The gills had black coloration (Fig. 1a, and 1b), reddish coloration on pereopods and pleopods (Fig. 1d and 1e) and the hepatopancreas seemed congested (Fig. 1e).

Bacteriological examination

The morphological, cultural and biochemical characteristics of isolated *V. parahaemolyticus* and *V. alginolyticus* from naturally infected *M. stebbingi* shrimps were presented in **Table (2)**. The Vibrio isolates appeared as motile, gram-negative curved rods, forming circular, green/yellow colonies on TCBS agar. Moreover, these bacteria exhibited sensitivity to 6.5 % sodium chloride.

Table 2. Biochemical and Cultural Characteristics of Isolated *Vibrio* spp. from Naturally Infected *M.stebbingi* shrimp

Test	*Vibrio parahaemolyticus*	*Vibrio alginolyticus*
Growth on TCBS	Green	Yellow
Gram-stain	-ve	-ve
Shape	Curved rod	Curved rod
Motility	+	+
Cytochrom oxidase	+	+
Catalase	+	+
H_2S on triple sugar iron (TSI)	K/A	- A/A
Indole	+	+
Citrate	-	-
Methyl red	-	+
Vogaus Proskauer	-	+
Urease production	-	-
Growth at 0 % Nacl	-	-
Growth at 1 % Nacl	+	+
Growth at 6.5 % Nacl	+	+

= Negative; + = Positive, H_2S (TSI) = production of H_2S from triple sugar iron, A/A= Acid/ Acid, K/A= Alkaline/ Acid

Result of Molecular Identification of *Vibrio* species Isolates by Polymerase Chain Reaction (PCR).

Molecular identification of *V. parahaemolyticus*

Figure 1 showed the presence of the toxR gene in the four selected *V. parahaemolyticus* isolates and the detection of two virulence genes Tdh and Trh, which were detected only in two of the four isolates of *V. parahaemolyticus*.

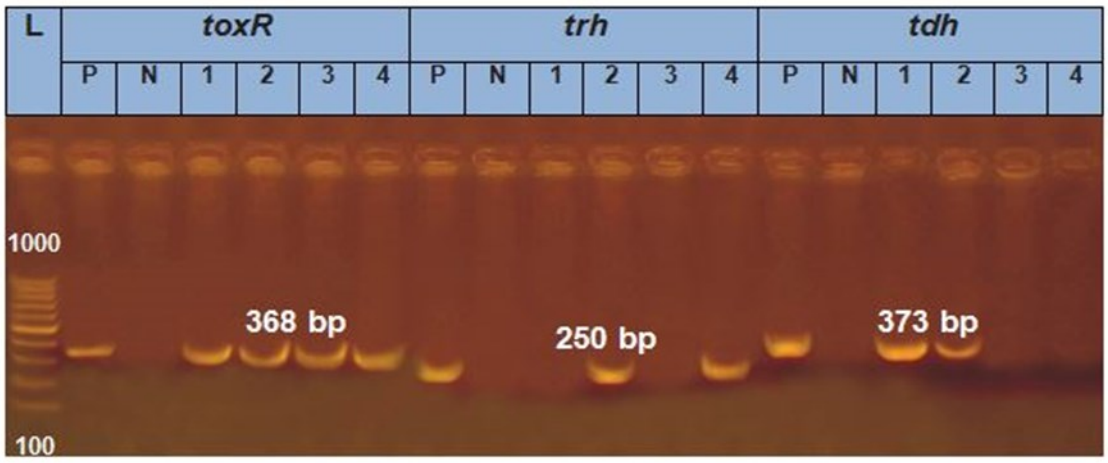


Figure (1): Detection of toxR (368 bp), and virulence genes trh (250 bp), and tdh (373 bp) for characterization of *V. parahaemolyticus* using PCR. Lane N: negative control. Lanes 1–4: toxR gene-positive *V. parahaemolyticus* strains. Lanes 2 and 4: trh gene positive strain. Lanes 1 and 2: tdh gene positive strain

Molecular identification of *Vibrio alginolyticus*

Figure (2) demonstrated the presence of collagenase gene in 5 out of 6 selected isolates of *Vibrio alginoltyticus*, whereas **Plate. (2)** demonstrated the presence of tdh and trh virulence genes in the selected 5 isolates of *V. alginoltyticus*, with tdh gene being present in 5 isolates and trh gene being present in 4 out of 5 isolates

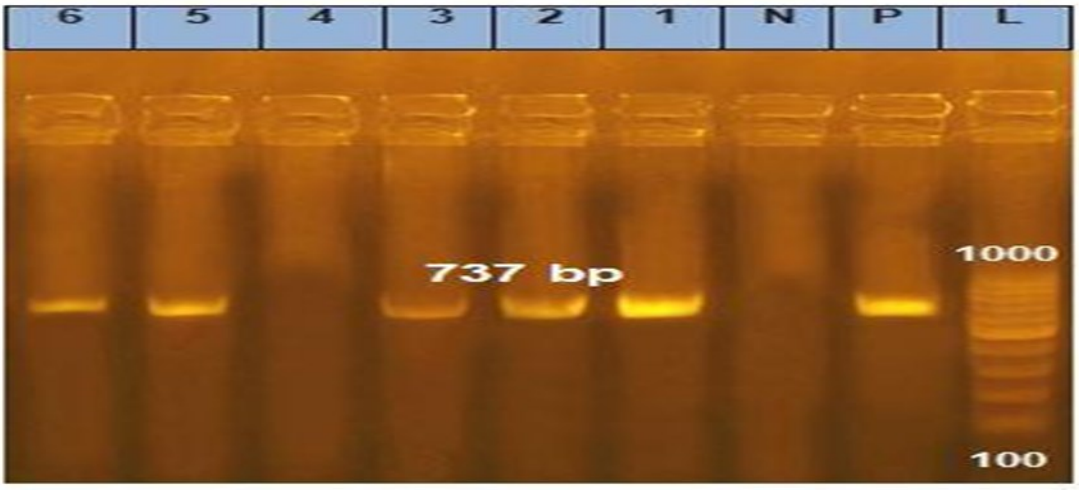


Figure (2): PCR detection of the collagenase (737 bp) gene from *Vibrio alginolyticus*. *V. alginolyticus* is visible in lanes 1, 2, 3, 5, and 6 with bands at 737 bp; Lane 4: negative sample and Lane N is the negative control**.**

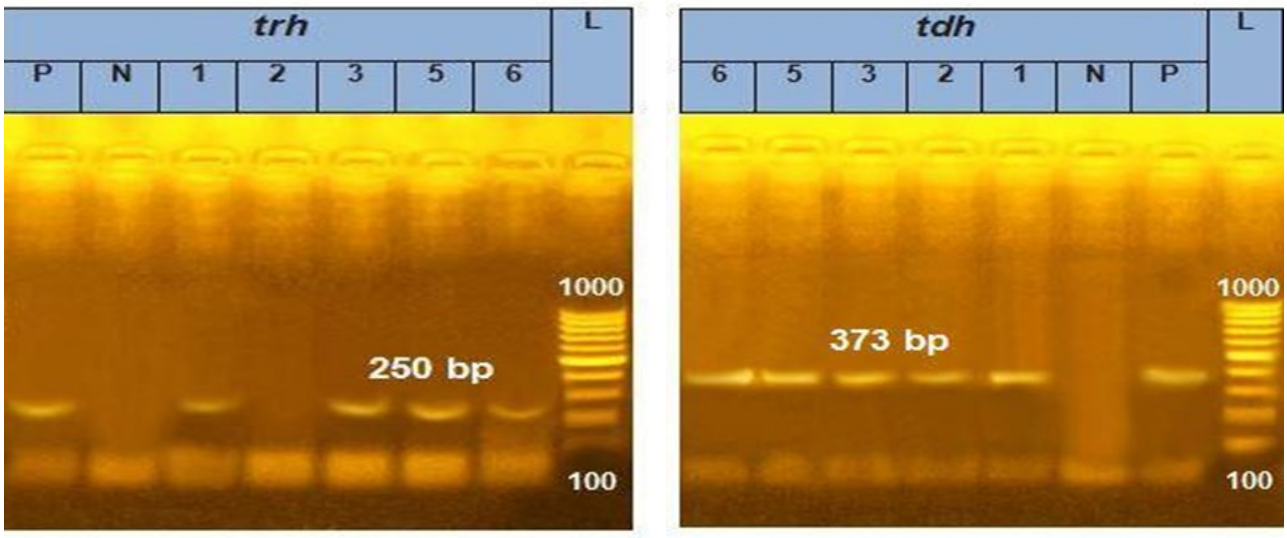


Plate (2): Detection of the *Vibrio alginolyticus* virulence genes trh (250 bp) and tdh (373 bp) using PCR. Lane N: negative control. Lanes 1, 3, 5 and 6: trh gene-positive strains of *V. alginolyticus*. Lanes 1, 2, 3, 5 and 6: tdh gene-positive strain

Prevalence of *Vibrio* Species from Naturally Infected White Shrimp (*Metapenaeus stebbingi*):

The total prevalence of *Vibrio* spp. in white shrimp (*M. stebbingi*) from July to November 2023 was 14%, with *V. alginolyticus* being the maximum wide-spread at 9.6%, followed by *V. parahaemolyticus* at 4.4% **(Table. 3)**. During the summer season, there was a greater presence of *Vibrio* spp., *V. alginolyticus* and *V.parahaemolyticus*, compared to the observations in autumn, (July 6,12%, August 8,18%, September 4,8%, October 2,6% and November 2,4% respectively) **(Fig. 3)**

Table 3. Prevalence of Bacterial Isolates in Naturally Infected White Shrimp (*M. stebbingi*)

Type of bacterial pathogen	No. of examined shrimp samples	infected shrimp samples	
		No.	%
Vibrio parahaemolyticus	250	11	4.4
Vibrio alginolyticus		24	9.6
Total		35	14

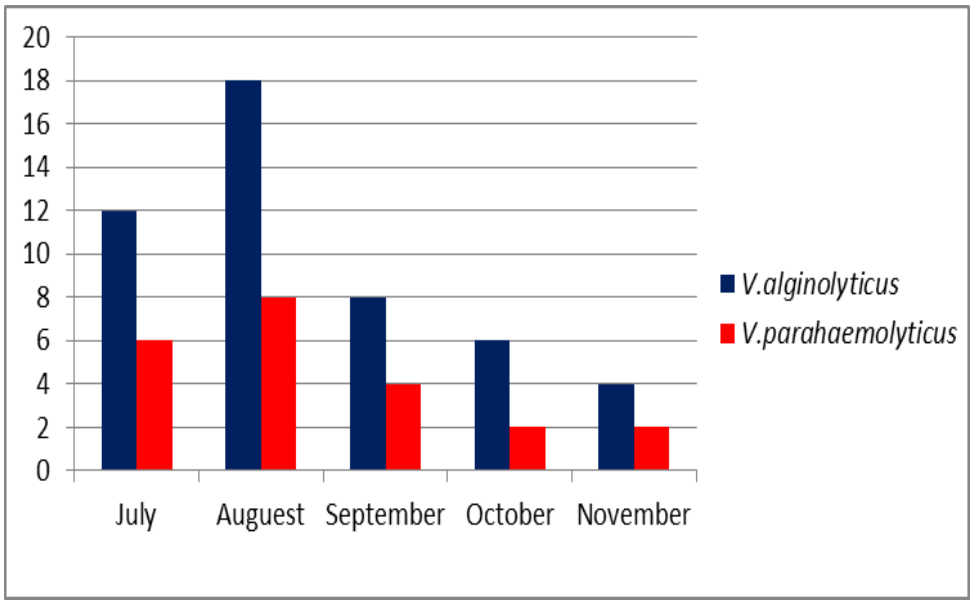


Figure (3) Prevalence of *Vibrio* spp. Isolates in different months of the study

Prevalence of *Vibrio* Species from the Hepatopancreas and Musculature of Naturally Infected White Shrimp (*Metapenaeus stebbingi*):

In **Figure (4),** it was demonstrated that *Vibrio* spp. as *V. alginolyticus* and *V. parahaemolyticus* were found in the hepatopancreas and musculature of naturally infected white shrimp (*M. stebbingi*) at high rates, with prevalence in the hepatopancreas at 70.83% and 72.72%, and in the musculature at 29.16% and 27.27% respectively.

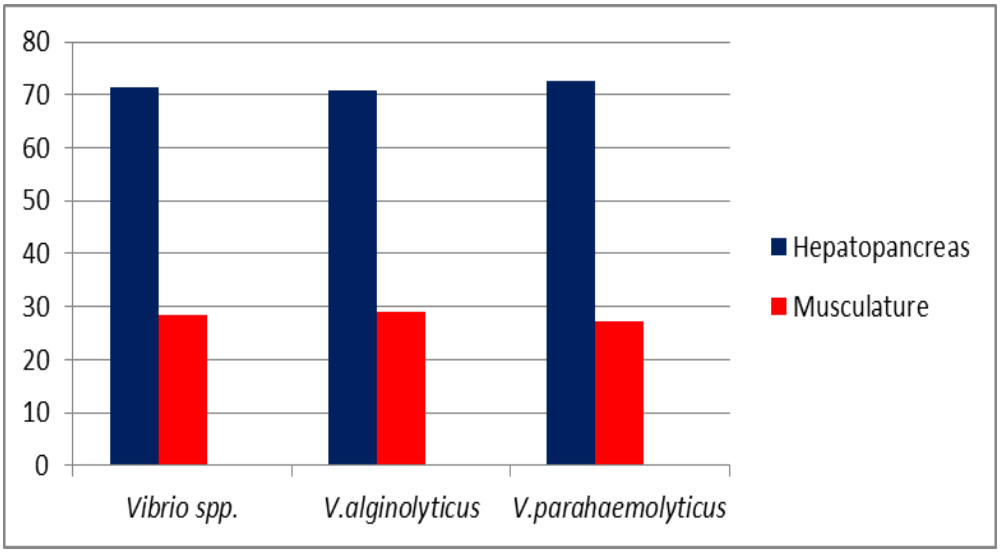


Figure (4) Prevalence of *Vibrio* spp. Isolates from the hepatopancreas and musculature

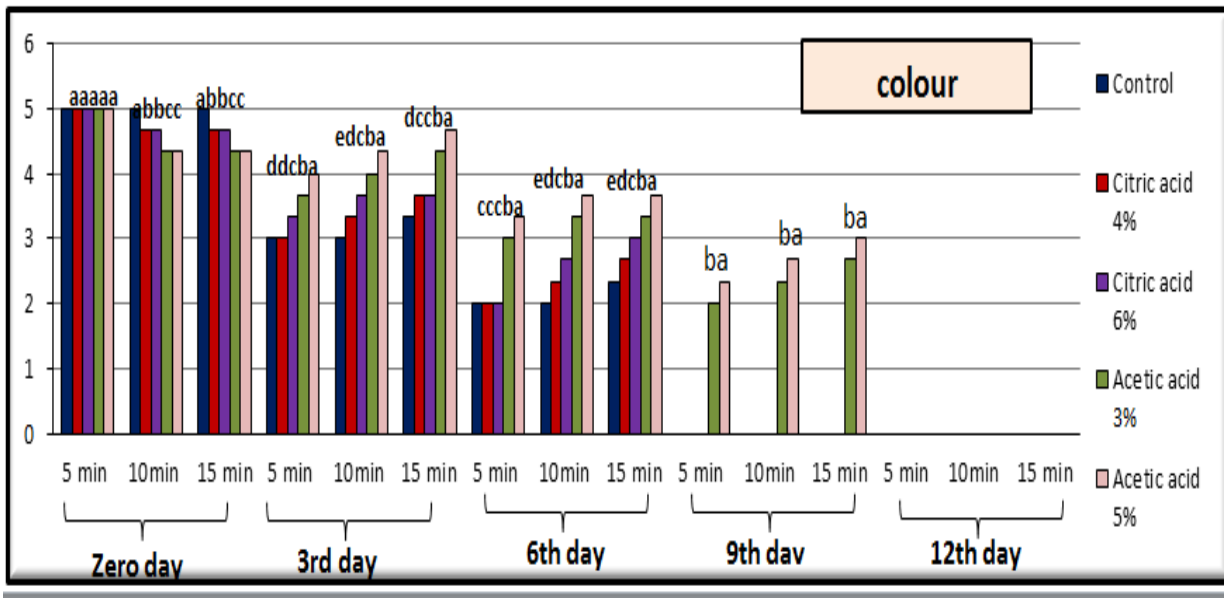
Effect of organic acids treatments on growth of *V. Parahaemolyticus* in artificially contaminated shrimp:

Table 4. Effects of different concentrations of citric and acetic acid treatments on *V. parahaemolyticus* log counts and reduction percentages in artificially contaminated unpeeled shrimp samples:

Treatment Groups	Immersion Times					
	5 minutes		10 minutes		15 minutes	
	V. parahaemolyti-cus Counts	Reduction Percentages	*V. parahaemolyti-cus* Counts	Reduction Percentages	*V. parahaemo-lyticus* Counts	Reduction Percentages
Control (Distilled water)	6.48±0.11[a]	--- (0%)	6.52±0.23[a]	--- (0%)	6.71±0.11[a]	--- (0%)
Citric acid 4%	5.60±0.17[b]	0.86 (13.3%)	5.27±0.11[b]	1.19 (18.4%)	4.79±0.13[b]	1.67 (25.8%)
Citric acid 6%	5.21±0.11[b]	1.25 (19.3%)	4.68±0.17[c]	1.78 (27.5%)	4.02±0.29[c]	2.44 (37.8%)
Acetic acid 3%	3.89±0.06[c]	2.57 (39.7%)	3.19±0.06[d]	3.27 (50.6%)	2.26±0.09[d]	4.3 (66.6%)
Acetic acid 5%	3.34±0.17[d]	3.12 (48.3%)	2.09±0.05[e]	4.37 (67.6%)	1.57±0.23[e]	4.89 (75.5%)

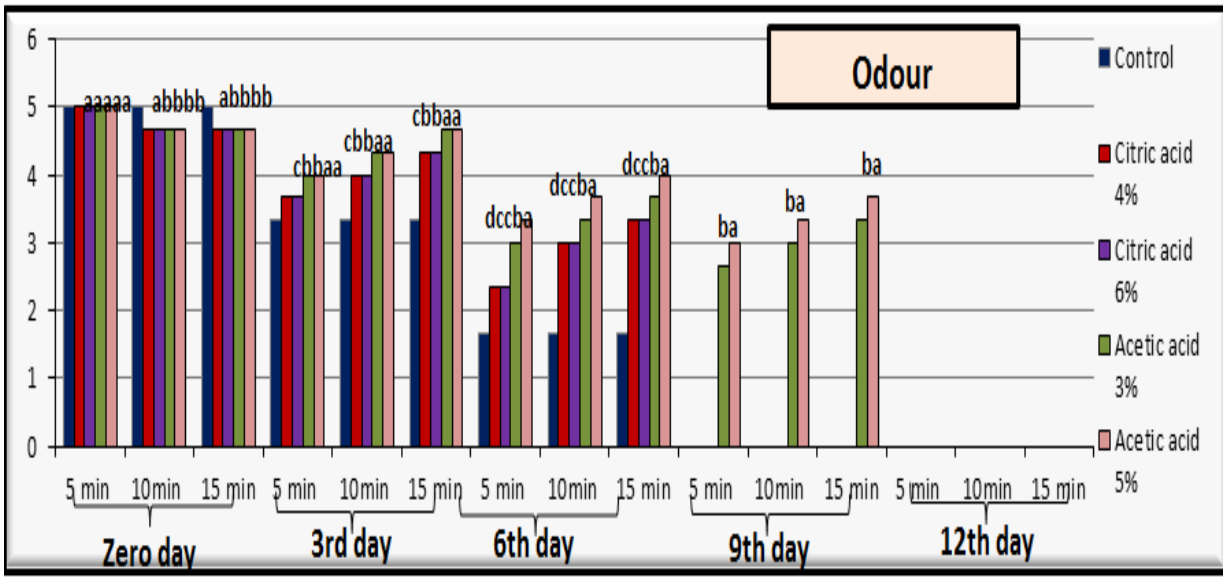
The Initial Load after contamination and before treatment was 6.46 Log CFU/g.
The values are represented as means±SD of three replicates.
Different letters on the same column show significant differences ($p < 0.05$).

Effect of organic acids treatments on unpeeled shrimp samples shelf life:



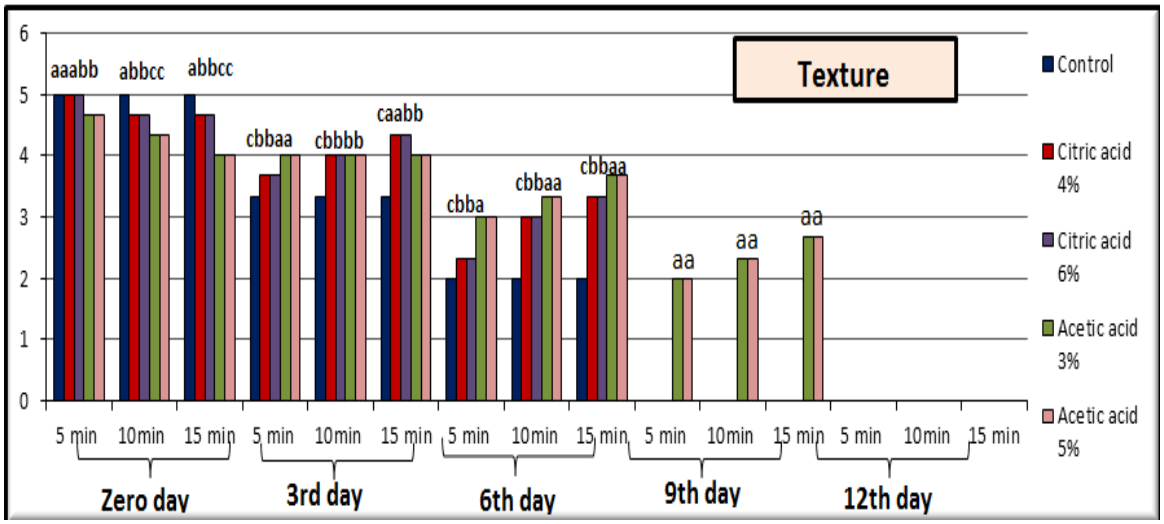
Coloums with different letters in the same duration time show significant differences ($p < 0.05$).

Figure 5. Effect of citric and acetic acids treatments on colour of unpeeled shrimp samples under refrigeration storage at 4°C at interval 0, 3, 6, 9 and 12 days



Coloums with different letters in the same duration time show significant differences ($p < 0.05$).

Figure 6. Effect of citric and acetic acids treatments on odour of unpeeled shrimp samples under refrigeration storage at 4°C at interval 0, 3, 6, 9 and 12 days



Coloums with different letters in the same duration time show significant differences ($p < 0.05$).

Figure 7. Effect of citric and acetic acids treatments on texture of unpeeled shrimp samples under refrigeration storage at 4°C at interval 0, 3, 6, 9 and 12 days:

Table 5. Effect of citric and acetic acids treatments on pH values of unpeeled shrimp samples under refrigeration storage at 4°C at interval 0, 3, 6, 9 and 12 days:

Treatment groups	Immersion times	Storage time				
		0 days	3days	6days	9 days	12 days
Control (Distilled water)	5 minutes	6.81±0.02 ^{a}	7.28±0.03 ^{a}	7.91±0.02 ^{a}	8.62±0.03 ^{a}(s)	8.89±0.01 ^{a}(s)
	10 minutes	6.83±0.01 ^{a}	7.25±0.01 ^{a}	7.90±0.03 ^{a}	8.61±0.01 ^{a}(s)	8.91±0.03 ^{a}(s)
	15 minutes	6.82±0.04 ^{a}	7.23±0.04 ^{a}	7.90±0.01 ^{a}	8.62±0.04 ^{a}(s)	8.90±0.02 ^{a}(s)
Citric acid 4%	5 minutes	6.78±0.01 ^{a}	7.02±0.02 ^{b}	7.87±0.04 ^{ab}	8.41±0.03 ^{b}(s)	8.67±0.05 ^{b}(s)
	10 minutes	6.68±0.03 ^{a}	6.97±0.01 ^{b}	7.84±0.03 ^{ab}	8.16±0.01 ^{b}(s)	8.53±0.01 ^{b}(s)
	15 minutes	6.62±0.02 ^{a}	6.84±0.01 ^{b}	7.76±0.01 ^{ab}	8.05±0.05 ^{b}(s)	8.38±0.02 ^{b}(s)
Citric acid 6%	5 minutes	6.71±0.02 ^{b}	7.36±0.01 ^{c}	7.83±0.04 ^{b}	8.06±0.02 ^{c}(s)	8.57±0.02 ^{c}(s)
	10 minutes	6.65±0.02 ^{b}	7.19±0.03 ^{c}	7.78±0.08 ^{b}	7.98±0.01 ^{c}(s)	8.48±0.03 ^{c}(s)
	15 minutes	6.60±0.04 ^{b}	7.08±0.04 ^{c}	7.72±0.02 ^{b}	7.96±0.04 ^{c}(s)	8.41±0.01 ^{c}(s)
Acetic acid 3%	5 minutes	5.98±0.01^{c}	6.68±0.03 ^{d}	7.56±0.03^{c}	7.89±0.03^{d}	8.36±0.06^{d}(s)
	10 minutes	5.76±0.03^{c}	6.47±0.01 ^{d}	7.50±0.01^{c}	7.85±0.03^{d}	8.12±0.01^{d}(s)
	15 minutes	5.54±0.01^{c}	6.31±0.02 ^{d}	7.35±0.04^{c}	7.81±0.01 ^{d}	7.98±0.02^{d}(s)
Acetic acid 5%	5 minutes	5.84±0.04 ^{d}	6.42±0.01 ^{e}	7.35±0.02^{d}	7.78±0.06^{e}	8.25±0.03^{e}(s)
	10 minutes	5.61±0.01^{d}	6.35±0.03 ^{e}	7.20±0.01^{d}	7.72±0.02^{e}	8.02±0.02^{e}(s)
	15 minutes	5.58±0.03^{d}	6.19±0.01^{e}	7.06±0.02^{d}	7.70±0.01^{e}	7.97±0.02^{e}(s)

Means with different letters on the same column show significant differences ($p < 0.05$).
(S) means spoiled sample.

Table 6. Effect of citric and acetic acids treatments on total aerobic plate counts (log cfu/ml) of unpeeled shrimp samples under refrigeration storage at 4°C at interval 0, 3, 6, 9 and 12 days:

Treatment groups	Immersion time	Storage time 0 days	3 days	6 days	9 days	12 days	MPL
Control (Distilled water)	5 minutes	4.78±0.05 ^{a}	5.34±0.11 ^{a}	6.03±0.07 ^{a}	8.03±0.09^{a}(S)	9.23±0.11 ^{a}(S)	Not exceeded 10^{6} cfu/ml
	10 minutes	4.79±0.09 ^{a}	5.42±0.08 ^{a}	6.02±0.11 ^{a}	8.02±0.17 ^{a}(S)	9.22±0.07 ^{a}(S)	
	15 minutes	4.75±0.13 ^{a}	5.47±0.17^{a}	6.08±0.09^{a}	8.08±0.04 ^{a}(S)	9.27±0.04 ^{a}(S)	
Citric acid 4%	5 minutes	3.72±0.10 ^{b}	4.85±0.07 ^{b}	5.71±0.13 ^{b}	7.64±0.07 ^{b}(S)	8.84±0.05 ^{b}(S)	
	10 minutes	3.59±0.08 ^{b}	4.65±0.11 ^{b}	5.54±0.18 ^{b}	7.44±0.10 ^{b}(S)	8.61±0.11 ^{b}(S)	
	15 minutes	3.41±0.07 ^{b}	4.38±0.12 ^{b}	5.21±0.08 ^{b}	7.17±0.13 ^{b}(S)	8.35±0.17 ^{b}(S)	
Citric acid 6%	5 minutes	3.24±0.07 ^{b}	4.64±0.06^{b}	5.98±0.06^{b}	7.53±0.05 ^{b}(S)	8.75±0.09 ^{b}(S)	
	10 minutes	3.14±0.05 ^{b}	4.36±0.09 ^{b}	5.74±0.17 ^{b}	7.26±0.09 ^{b}(S)	8.42±0.14 ^{b}(S)	
	15 minutes	3.02±0.14 ^{b}	4.17±0.17^{b}	5.69±0.09 ^{b}	7.10±0.15 ^{b}(S)	8.31±0.11^{b}(S)	
Acetic acid 3%	5 minutes	2.84±0.07 ^{c}	3.76±0.16 ^{c}	4.71±0.05 ^{c}	5.95±0.05 ^{c}	6.95±0.07 ^{c}(S)	
	10 minutes	2.23±0.06 ^{c}	3.48±0.07 ^{c}	4.56±0.12 ^{c}	5.73±0.11 ^{c}	6.86±0.07 ^{c}(S)	
	15 minutes	2.03±0.12 ^{c}	3.39±0.11 ^{c}	4.35±0.11 ^{c}	5.67±0.09 ^{c}	6.81±0.07 ^{c}(S)	
Acetic acid 5%	5 minutes	2.14±0.05 ^{cd}	3.54±0.13 ^{c}	4.63±0.09^{c}	5.87±0.04 ^{c}	6.74±0.07 ^{c}(S)	
	10 minutes	1.89±0.07 ^{d}	3.31±0.05 ^{c}	4.37±0.13^{c}	5.64±0.11 ^{c}	6.69±0.07 ^{c}(S)	
	15 minutes	1.78±0.07 ^{d}	3.26±0.05 ^{c}	4.20±0.05 ^{c}	5.59±0.07 ^{c}	6.62±0.07 ^{c}(S)	

Different letters on the same column show significant differences ($p < 0.05$).(S) means spoiled sample.
MPL is maximum permissible limits stipulated by **Egyptian standards (5021/ 2005)**.

Table 7. Effect of citric and acetic acids treatments on TBARS values (mg/kg) of unpeeled shrimp samples under refrigeration storage at 4°C at interval 0, 3, 6, 9 and 12 days:

Treatment groups	Immersion time	Storage time 0 days	3 days	6 days	9 days	12 days	MPL
Control (Distilled water)	5 minutes	0.78±0.01^{a}	1.56±0.02^{a}	2.87±0.03^{a}	4.83±0.01^{a}(S)	5.94±0.02^{a}(S)	4.5mg/kg
	10 minutes	0.77±0.01^{a}	1.55±0.01^{a}	2.89±0.01^{a}	4.82±0.02^{a}(S)	5.96±0.03^{a}(S)	
	15 minutes	0.78±0.02^{a}	1.56±0.00^{a}	2.89±0.01^{a}	4.82±0.01^{a}(S)	5.97±0.01^{a}(S)	
Citric acid 4%	5 minutes	0.63±0.02^{b}	1.46±0.03^{b}	2.65±0.02^{b}	4.56±0.01^{b}(S)	5.51±0.01^{bA} (S)	
	10 minutes	0.62±0.01^{b}	1.46±0.01^{b}	2.66±0.03^{b}	4.54±0.01^{b}(S)	5.50±0.02^{b}(S)	
	15 minutes	0.61±0.02^{b}	1.45±0.02^{b}	2.64±0.01^{b}	4.53±0.01^{b}(S)	5.49±0.01^{b}(S)	
Citric acid 6%	5 minutes	0.61±0.01^{b}	1.45±0.02^{b}	2.64±0.02^{b}	4.52±0.01^{b}(S)	5.49±0.01^{b}(S)	
	10 minutes	0.61±0.01^{b}	1.44±0.01^{b}	2.63±0.03^{b}	4.50±0.03^{b}(S)	5.49±0.01^{b}(S)	
	15 minutes	0.62±0.02^{b}	1.43±0.02^{b}	2.61±0.01^{b}	4.50±0.01^{b}(S)	5.48±0.02^{b}(S)	
Acetic acid 3%	5 minutes	0.62±0.02^{b}	1.43±0.03^{c}	2.15±0.02^{c}	3.19±0.02^{c}	4.59±0.01^{c}(S)	
	10 minutes	0.62±0.01^{b}	1.41±0.01^{c}	2.16±0.03^{c}	3.19±0.01^{c}	4.58±0.03^{c}(S)	
	15 minutes	0.61±0.02^{b}	1.41±0.02^{c}	2.14±0.01^{c}	3.18±0.02^{c}	4.50±0.01^{c}(S)	
Acetic acid 5%	5 minutes	0.61±0.01^{b}	1.42±0.02^{c}	2.15±0.02^{c}	3.16±0.02^{c}	4.52±0.01^{c}(S)	
	10 minutes	0.60±0.01^{b}	1.40±0.01^{c}	2.16±0.03^{c}	3.17±0.01^{c}	4.50±0.03^{c}(S)	
	15 minutes	0.60±0.02^{b}	1.40±0.02^{c}	2.14±0.01^{c}	3.16±0.02^{c}	4.50±0.01^{c}(S)	

Different letters on the same column show significant differences ($p < 0.05$).
(S) means spoiled sample.
MPL is maximum permissible limits stipulated by **Egyptian standards (5021/ 2005)**.

DISCUSSION

Vibriosis is one of the dangerous bacterial diseases that often affects many species of shrimp **(Abdel-Latif et al. 2022)**. This time of the year from July to November was chosen for the research due to the availability of fresh white shrimp (*M. stebbingi*) in the Ismailia governorate with high amounts, different sizes, and prices. It represents the golden period of purchasing and the demand for eating. The majority of naturally infected white shrimps (*M. stebbingi*) had black spots on their cuticles and carapaces. Some of them also had black patches on their cuticles. In the majority of cases, the body appendages, the telsons, the uropods, the pleopods, the pereopods, and the gills showed black coloration. In a few cases, a reddish coloration of the pereopods and pleopods could be detected. In some cases, the hepatopancreas seemed to be congested and soft. The present findings were almost identical to those reported by **El Zlitne et al. (2022)** and **Hoa et al. (2023)**. The disease in shrimp caused by *Vibrio* species is likewise acknowledged as a "brown spot" or "black spot lesion/ disease". This condition begins when the exoskeleton cuticle is damaged mechanically or chemically, allowing entry of opportunistic micro-organisms **(Radhakrishnan and Kizhakudan, 2019)**. *Vibrio* species that are chitinolytic may pierce the damaged cuticle, causing the cuticle layer to be lost and causing erosion, pitting, inflammation, lesions (which start small and get larger), and necrosis. If the damage is deep, the disease develops and the bacteria travel to the hemolymph and spread throughout the body. Furthermore, melanization occurs onthe exoskeleton of freshwater and marine crustaceans **(Cuéllar-Anjel et al. 2014)**. Some highly pathogenic and virulent strains of *Vibrio* species produce lethal exotoxins, such as protease, cysteine, and hemolysins, which can damage the lining of intestinal epithelial cells, facilitating the invasion of other body tissues and organs by opportunistic bacteria **(Soonthornchai et al. 2010)**.

In this study, bacteriological examination showed that the most common isolates from naturally infected *M. stebbingi* shrimp belonged to gram-negative, motile, halophilic, curved rod-shaped bacteria. *V. alginolyticus* and *V. parahaemolyti*cus were identified at the species level using TCBS (Thiosulphate Citrate Bile Salt agar) and biochemical testing. All Vibrio isolates were positive for oxidase, catalase, and indole production tests and negative for urease production and citrate test. All *V. alginolyticus* isolates formed yellow colonies on TCBS because they could ferment sucrose present in the medium (sucrose positive), positive for the methyl red and the Vogues-Proskauer tests and gave an acid/acid reaction in TSI, while all *V. parahaemolyticus* isolates formed green colonies on TCBS as they were considered non-sucrose fermentative bacteria, negative for the methyl red and Vogues-Proskauer tests and gave an alkaline/acid reaction in TSI. These findings concurred with those of **El Zlitne et al. (2022); Shimaa and Walaa (2023); Yousef et al. (2023)** and **Zobayda et al. (2023)**.

Vibrio spp., including *V. alginolyticus* and *V. parahaemolyticus*, were isolated from naturally infected *M. stebbingi* shrimp in the current investigation, and their virulence genes were determined by PCR. These outcomes resembled by **Zobayda et al. (2023)** found that all Vibrio bacteria isolated from shrimp samples were analyzed using simplex PCR (molecular detection) for the detection of *V. cholerae, V. parahaemolyticus, V. vulnifcus ,* and *V. alginolyticus.* The study's findings demonstrate that the identification of *V. alginolyticus* by detecting the collagenase gene in the six selected isolates that were chosen for analysis resulted in bands of 737 bp. in five of the six isolates of *V. alginolyticus*. The identification of two virulence genes, tdh and trh, was observed in the form of bands appearing at 373bp. in all five strains and at 250bp. in four out of the five positive strains of *V. alginolyticus*. These outcomes resembled those of **Shimaa and Walaa (2023)** who used PCR to analyze pathogenic *V. algynolyticus* using the collagenase gene and reported that a virulence gene (Tdh) was also detected and **Gobarah et al. (2022)** who reported PCR results showed the collagenase gene in *V. alginolyticus* isolates became found in all five isolates of *V.*

alginolyticus with an occurrence of 100% giving bands at 737bp. On the other hand, The PCR evaluation on this take a look at confirmed that *V. parahaemolyticus* was identified by the detection of toxR (368 bp) in the four selected isolates and its virulence genes trh (250 bp) and tdh (373 bp) were detected in two of the four *V. parahaemolyticus* isolates. These results resembled those of **Patel et al. (2018)** reported that the toxR (368 bp) gene unique to *V. parahaemolyticus* from shrimp was amplified in all 7 isolates. After PCR ruled out the isolates' pathogenicity, 1 out of 7 (14.28%) isolates showed amplification of the virulent tdh (269 bp) gene and **Gobarah et al. (2022)** reported PCR analysis revealed that in *V. parahaemolyticus* isolates, the tlh gene become observed in all five isolates at a frequency of 100% of the strains and the tdh gene become observed in three of the five isolates, representing 60% of the strains.

In the present study, the total prevalence of *Vibrio* spp. in white shrimp (*M. stebbingi*) from July to November 2023 which isolated from the hepatopancreas (71.43%) and musculature (28.57%) was 14%, with *V. alginolyticus* being the most prevalent at 9.6%, followed by *V. parahaemolyticus* at 4.4%. A higher observation was noted by **Hirshfeld et al. (2023)** reported that the prevalence of *Vibrio* and *Enterococcus* species isolated from retail shrimp was 60.25% and 89.75%, respectively and **Zobayda et al. (2023)** found that the occurrence of *Vibrio* spp. in shrimp samples was 54%, with *V. parahaemolyticus* accounting for 24% and *V. alginolyticus* accounting for 10%. In addition, **Yu et al. (2023)** reported that the prevalence of *V. alginolyticus* in shrimp was 28.6% while the percentage of *V. parahaemolyticus* was 20.6%, and **Ibrahim et al. (2018b)** who reported that *Vibrio* species prevalence in shrimp samples was 52%, of which 4 (16%) *V. parahaemolyticus* and 2 (8%) *V. alginolyticus.* While a lower level of observation was noted by **Fadel and El-Lamie (2019)** reported that among 170 shrimp samples (*Metapenaeus monoceros*) tested, the overall prevalence of Vibriosis was 7/170, or 4.12%, and exhibited an identical occurrence of Vibriosis (4.7%) in both the hepatopancreas and musculature and **Patel et al. (2018)** reported that 5 (3.33%) *V. parahaemolyticus* isolates were recovered from 150 shrimp samples, including 3 (4.28%, 3/70) from marine shrimp samples and 2 (2.5%, 2/80) fresh water shrimp samples. Additionally, In the summer, there were more *Vibrio* spp., like *V. alginolyticus* and *V. parahaemolyticus*, than in the autumn. Several factors such as location, fish immunity, types of fish, water quality, and sample size could explain the variations in prevalence rates. It was suggested that the increase in *Vibrio* spp. during summer is linked to warmer water temperatures, which weaken fish defenses and make them more vulnerable to infections (**Ismail et al. 2024)**.

A large proportion of food poisonings are caused by *V. parahaemolyticus*, a foodborne pathogen. The infection is characterized by severe gastroenteritis, which is associated with consuming raw or undercooked seafood **(Almagro-Moreno et al. 2023)**.

Concerning the counts of *V. parahaemolyticus* in artificially contaminated unpeeled shrimp samples showed in **table 4**, the initial load count was 6.46 log CFU/g. Significant gradual reductions ($P<0.05$) were obtained in the counts of all treatments with different concentrations at immersion time of 5, 10 and 15 minutes, as the organic acid concentration increased, the counts decreased. The overall results revealed that using organic acids could be an effective way to reduce *V. parahaemolyticus* growth. Prior researches have documented the effectiveness of organic acids in combating microbial contamination by various foodborne pathogens in food production and processing **Mohan and Pohlman, (2016); Tsai et al. (2021)** and **Didem et al. (2023)**).

Moreover, according to the reduction percentages of *V. parahaemolyticus* counts, the citric and acetic acid effectiveness increased consistently with higher concentrations and longer immersion times **(Salem and Amin, 2012)**. The acetic acid had higher reduction effects than citric acid, reached up to 75.5% at immersion time 15 minutes. Similar studies on *Vibrio* spp inhibition were previously performed by **Ibrahim et al. (2018a); Fadel and**

El-Lamie (2019); Morshdy et al. (2022) and **Yousef et al. (2023)**. Acetic acid stands out as the most widely accepted organic acid for food products decontamination, it is food acidulate. It exhibits a stronger antibacterial effect at room temperature than when used at refrigerator temperature **(Fadel and El-Lamie, 2019)**.

While citric acid, the main acid in lemons, is commonly used in cooking as a flavoring agent and to add a tart taste, has partial antibacterial activity **(Ibrahim et al. 2018a)**.

Furthermore, organic acid treatments could be a valuable solution to the rapid spoilage of seafood include shrimp, particularly those relying solely on refrigeration for preservation. Improvements in products shelf-life play a crucial economic role by reducing spoilage and allowing the products to be sent in new and further markets **(Ibrahim et al. 2018a)**.

Organic acids were previously used for a long time in food industry, both as flavorings and preservatives under various processing conditions **(Salem and Amin, 2012)**.

Concerning the results of the effects of citric and acetic acids on sensory attributes of treated shrimp under refrigeration storage **(figure 5, 6, and 7)**, the shrimp samples in control groups showed noticeable and steady decrease in their sensory qualities (odor, smell, and texture) over storage time. While, all treated shrimp groups had significantly higher sensory scores when compared to the control group, as well as had significant differences between each other. However, only acetic acid with 3% and 5% concentration could extend shelf life up to 9 days. Using acetic acid can improve shrimp shelf life and safety, while maintaining an acceptable taste, smell and texture for consumers at a reasonable cost. This approach also avoid financial losses caused by food spoilage **(Fadel and El-Lamie, 2019)**. Additionally using citric acid, lead to acid taste production by increase the acidity give a characteristic texture and aroma **(Ibrahim et al. 2018a)**.

The previous findings suggested that all used organic acids treatments helped shrimp retain their sensory quality. The main objective of sensory analysis is to predict consumer's acceptance, it is now widely used in marketing research, quality control and product development **(Ibrahim et al. 2018a)**. Similar previous studies were reported by **Khodanazary (2019); Tolba et al. (2020); Sabu et al. (2020)** and **Şen Yılmaz (2023)**.

Concerning changes of pH values of shrimp samples treated with citric and acetic acids showed in **table 5**, all pH values of treated shrimp were significantly ($P<0.05$) decreased immediately after treatment with organic acids than control shrimp dipped in distilled water. Moreover, all values including control and treated groups continued to increase throughout the refrigeration storage, with minimum significant rate in acetic acid 3% and 5% treatments. The acidity level (pH) of shrimp is considered a reliable indicator of its quality. Shrimp with a pH of 7.7 or below are considered of the highest quality. While, pH between 7.7 and 7.95, shrimp are still acceptable but of lower quality, while those with a pH of 7.95 or higher are deemed unacceptable for consumption **(Gökoğlu, 2004)**. Accordingly, treated samples with acetic acid 3% and 5% considered good quality till the 9th day of storage, whereas quality of control and citric acid groups samples were accepted up to 6th day.

Organic acids effects depend on decreasing pH level of shrimp as an inhibition mechanism to the growth of microbes **(Ben Braïek and Smaoui, 2021)**. Likewise, the highest treatments showing antimicrobial effect lead to the highest pH reduction. This is because the growth and survival of bacteria are greatly affected by acidity, in addition to other external factors, as initial flora and temperature **(Didem et al. 2023)**. Similar studies were previously performed by **Noordin et al. (2020); Sabu et al. (2020); Tolba et al. (2020); Didem et al. (2023)** and **Şen Yılmaz (2023)**.

Concerning the results of **table 6**, total aerobic plate counts of shrimp samples were decreased by citric and acetic acids treatments than the control group. However, along the

storage periods for all groups there were continuous increase in counts with different rates. The control shrimp showed higher significant (P<0.05) rates than all treated ones. By comparing the results with the maximum permissible limit stipulated by the Egyptian standards **(ES: 5021/2005)**, the control and citric acids groups remained within the permissible limits till the 6th day of storage, while acetic acid groups 3% and 5% extended till 9th day of storage. On Contrary, **Smyth et al. (2018)** stated that there were no significance between total aerobic counts in cod fillets treated with 5% citric acid. Similar studies were performed previously by **Khodanazary (2019); Noordin et al. (2020); Sabu et al. (2020); Tolba et al. (2020)** and **Şen Yılmaz (2023)**.

The organic acids antimicrobial efficacy is occurred by penetrating the bacterial cell lipid membrane, entering its cytoplasm, dissociates into anions and protons, forcing bacteria to use up more energy to maintain their internal balance. When energy is depleted, bacteria stopped growing and died **(Fadel and El-Lamie, 2019)**. The growth and activity of microbes are a major reason for food spoilage producing biogenic amines, alcohols, sulfides, aldehydes and ketones, give off unpleasant and undesirable odors and flavors **(Ibrahim et al. 2018a)**.

According to the results of **table 7**, there were significant decrease in TBARS values of shrimp in all treated groups than the control group at the first day. However, there were continuous increase in all groups along the period of storage with different rates. By comparing the results with the maximum permissible limit stipulated by the Egyptian standards **(ES: 5021/2005)** which recommended that TBARS of chilled shrimp should not exceed 4.5 mg/Kg, the control and citric acids groups remained within the permissible limits till the 6th day of storage, while acetic acid groups 3% and 5% extended till 9th day of storage.

On contrary, **Noordin et al. (2020)** reported no significant (p<0.05) differences occurred by organic acids treatments. Measuring TBARS is a reliable way to determine how much fat in seafood has gone rancid during storage **(Khodanazary, 2019)**. Malondialdehyde resulted from oxidation of lipid, is badly influencing the quality, as well as has harmful effects on the human health, criticized as carcinogenic factor **(Djenane and Roncalés, 2018)**. Increasing the level of TBARS in shrimp over time resulted from partial dehydration of shrimp tissue, which is rich in polyunsaturated fatty acids, as well as exposure to oxygen leading to lipid oxidation which damages the tissues, producing off-odors and off-flavors, resulted in shortening the shelf-life **(Noordin et al. 2020)**. Similar studies were previously performed by **Khodanazary (2019); Sabu et al. (2020)** and **Tolba et al. (2020)**.

CONCLUSION

Vibriosis is one of the dangerous bacterial diseases that often affects many species of shrimp caused by many species of Vibrio, *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were found to be present in naturally infected white shrimp (*M. stebbingi*), where *V. alginolyticus* was the most prevalent, followed by *V. parahaemolyticus*. Citric and acetic acid treatments with different concentrations offer a safe, economic and effective way to control the presence of those harmful *Vibrio* spp. in shrimp, as well as acetic acid can act as alternative preservatives that can extend shrimp shelf life under refrigeration storage.

Recommendations

Practical application of citric and acetic acids during washing and processing in the seafood industry could improve preservation methods, reduce foodborne illness risk for consumers, and specifically inhibit the growth of harmful bacteria like *V. parahaemolyticus* commonly found in shrimp. This approach has the potential to improve food safety not only commercially but also for individual consumers who can use these acids at home.

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