Global Veterinaria 18 (4): 286-293, 2017
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2017.286.293

Prevalence of *Aeromonas* Species and Their Herbal Control in Fish

[1]*Ebeed, A. Saleh,* [2]*Alaa Eldin M.A. Morshdy,* [3]*A.M. Mohamed and* [4]*Basma F. El-sobary*

[1]Food Hygiene Department, Faculty of Veterinary Medicine, Damanhour University, Egypt
[2]Food Hygiene Department, Faculty of Veterinary Medicine, Zagazig University, Egypt
[3]Food Hygiene Department, Benha University, Egypt
[4]Veterinary Medicine Directorate in Kafr El-sheikh, Egypt

Abstract: *Aeromonas* species are ubiquitous in aquatic ecosystems. They are frequently isolated from surface water, freshwater fish, healthy and diseased fish and has the potential to be a foodborne pathogen. Therefore, one hundred samples of fresh *Tilapia niloticus* and *Mugil cephalus* fish were collected randomly from local fish markets for detecting the incidence and identification of different *Aeromonas* spp. As well, the bactericidal effects of cinnamon, rosemary and garlic essential oils at a concentration of 0.5 and 1% on the experimentally-infected *Oreochromis niloticus* with *A. hydrophila* were investigated. The obtained results revealed that the incidence of *Aeromonas* spp. in *Tilapia niloticus* and *Mugil cephal* were 34 (68%) and 31 (62%), respectively. The most frequently identified *Aeromonas* species isolated from *Oreochromis niloticus was A. caviae* 18 (36%), *Aeromonas sobria* 14 (28%) and from *Mugil cephalus*. *A. caviae* 17 (34%), *A. sobria* 13 (26%), respectively.Garlic oil at 0.5% ensured a better reduction percent of *A. hydrophila* than cinnamon and rosemary at 0.5 and 1% with a maintaining sensory trait. We concluded that essential oils of cinnamon, rosemary and garlic has different percent of bactericidal activity against Aeromonas hydrophila specially garlic oil so, we recommended addition of garlic oil during preparation of fish fillet for human consumption.

Key words: Fish · *A. hydrophila* · Antibacterial · Garlic · Cinnamon · Rosemary

INTRODUCTION

Aeromonas spp. is ubiquitous in aquatic ecosystems. They are frequently isolated from freshwater fish, surface water, sewage, healthy or diseased fish, which able to cause infection in humans [1].

Aeromonas (Family *Aeromonadaceae*) are gram-negative, straight non spore-forming rods, generally cytochrome oxidase positive and facultative anaerobic. They are characterized by being unable to grow at 6% NaCl [2]. Fishes are highly prone to contamination with *A. hydrophila* and become a means of transmitting pathogenic bacteria [3]. Studies indicate that fish is most often and most severely contaminated with microorganisms of the *Aeromonas* spp. The identification of the *Aeromonas* spp. isolated from fish and fish products indicated *A. hydrophila* as predominant species [4].

One of the emerging bacterial pathogen associated with foodborne diseases is *Aeromonas hydrophila*, frequently found in raw meat and drinking water. A greater risk of infection is reported in young children, elderly people and immunocompromised patients [5].

Aeromonas species are pathogens contaminated food and cause foodborne gastroenteritis in humans with its virulence factors such as extracellular toxins (enterotoxins, hemolysin, protease, phospholipase, hydrolytic enzymes), structural features (pilli, S-layer, lipopolysaccharide), adhesion and invasion [6]. Moreover, isolated A. hydrophila strains from patients with gastroenteritis are haemolytic [7]. Collected information on 16 outbreaks/incidences of Aeromonas-associated gastroenteritis implicated a range of suspect foods including fish, land snails, oysters, prawns, shrimp cocktail, dried fish sauce and egg salad. Adults are the largest age group reported among cases [8].

Corresponding Author: Basma F. El-sobary, Veterinary Medicine Directorate in Kafr El-Sheikh, Egypt.
E-mail: basmafawzy30@gmail.com.

Prevalent motile species of *Aeromonas* associated with fish disease following molecular identification include the following: A. veronii associated with channel catfish and A. sobria associated with *Oreochromis niloticus* [9].

Spices are rich in essential oils recognized for notable antimicrobial activity [10]. Spices antimicrobial effectiveness depend on the kind of spice, its composition and concentration, kind and concentrations of the target microorganism, substrate composition and processing and food storage conditions [11]. Among many spices primarily used for flavoring foods and at the same time have their antimicrobial potential recognized are garlic, onion, rosemary and cinnamon [12].

Essential oils as antimicrobial agents present two principal characteristics: i) their natural origin meaning more safety for consumers and environment; and ii) there is low risk of rising microbial resistance to their action because essential oils are mixtures of several compounds that, apparently, present different antimicrobial action modes becoming more difficult to the microbial adaptability [13] .

Musa [14] observed that garlic was active against *A. hydrophila* as well as other many bacteria isolated from fish and shrimp. More recently, garlic extract showed to be active against three *Aeromonas* species *A. hydrophila*, *A. caviae* and *A. sobria* isolated from seafoods [15].

The present study aimed to detect an incidence of *Aeromonas* species in fresh *Orechromis niloticus* and *Mugil cephalus* and identify different *Aeromonas* species in both types, in addition to study bactericidal effects of essential oils such as cinnamon, rosemary and garlic at a concentration of 0.5 and 1% on *Aeromonas hydrophila* growth.

MATERIALS AND METHODS

Sample Collection: A total of 100 samples of freshly dead *Orechromis niloticus* and *Mugil cephalus* (50 of each) collected from fish markets at Kafr El-Sheikh and El Bihara governorates. The samples collected in an insulated plastic bag and transferred quickly as soon as possible for further examinations.

Detection of *Aeromonas* Species

Preparation of Samples: Fish samples were collected from fish markets in plastic bag then ten grams of the fish sample were thoroughly mixed with 225 ml of alkaline peptone water using a sterile blender for 1–1.5 minutes. The prepared sample was incubated at 37°C for 6 hours for enrichment, 1ml from each pre-enrichment broth was transferred to 9 ml tryptic soya broth (TSB) which incubated at 37°C for 24 hours for enrichment of *Aeromonas* species[16].

Isolation of *Aeromonas* Species: A loop full from broth was streaked aseptically onto *Aeromonas* selective agar plates supplemented with ampicillin (5 mg/L) and then incubated at 37°C for 24 hours according to Ashiru [17]. Purified isolates were used as stocks for further morphological and biochemical identifications [18].

Preparation of *Orechromis niloticus* Fillets: Piece of each of fresh *Orechromis niloticus* fillets (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 the microorganisms attached to its surface.

Preparations of Inoculate [19]: *Aeromonas hydrophila* strain was obtained from Food Analysis Center, Faculty of Veterinary Medicine, Benha University. Bacteria were subcultured on Brain Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. The cells were harvested by centrifugation (3000×g, 15 min), washed twice and resuspended with saline (NaCl, 0.85%, w/v).

Inoculation of *Orechromis niloticus* Fillets with Tested Bacteria: For inoculation of the *Orechromis niloticus* fillets, 1 ml of the dense suspension 5×10^6 /g for *Aeromonas hydrophila* was employed.

Essential Oil Extraction: The extraction of active ingredients from dry cinnamon, rosemary and garlic plants were done according to the technique developed by Tandon and Rane [20] . In brief; the dried plant material was size reduced with milling using hammer mill. Extraction of the plant material was carried out by immersion in absolute methanol for three days with agitation using automatic shaker. Then filtration through a piece of gauze to remove solid plant particles were done, the extract was re-filtered through filter paper to remove fine or colloidal particles from the extract. The enriched extract was concentrated by evaporation of the solvent with heating in water bath at 65°C until solid mass was obtained. Finally, drying the extract by spreading under shaded area till complete dryness, then stored in the refrigerator until using. Each extract was used for preparation of 1% (w/v) solution.

Mixing of *Orechromis niloticus* Fillets with Essential Oils: The inoculated samples were divided into 7 groups; the 1st was untreated control, while the 2nd group was mixed with cinnamon extract (0.5%) for 15 minutes, 3rd group was mixed with cinnamon extract (1%), the 4th was mixed with rosemary extract (0.5%) for 15 min, 5th group was mixed with rosemary extract (1%) for 15 minutes, the 6th was mixed with garlic extract (0.5%) for 15 min and the last 7th group was mixed with garlic extract (1%) for 15 minutes. The control and treated minced meat samples were labeled and packaged as triplicates, then stored at 2±1°C inside the refrigerator. All groups (either control or treated) were subjected to a microbiological assessment at day zero (within 2 hours after treatment) then periodically every 3 days (0, 3rd, 6th and 9th days).

Enumeration of the Tested Bacteria: In order to enumerate A. hydrophila, 100µl of a suitable dilution of the bacteria grown in Brian Heart Infusion (BHI) broth were surface plated on Aeromonas selective agar plates. Enumerations were carried out after incubating of the plates at 37°C for 24 hours.

Sensory Evaluation: Sensory evaluation was performed using the scoring test developed by Klinic and Cakli [21]. Accurately, 5 panelists evaluated the sensory attributes of *Oreochromis niloticus* fillets samples. The fillets samples were blind-coded by special codes; the panelists were not informed about the experimental approach. They were asked to give a score for each of color, odor and consistency while the fillets were raw. The panelists were asked to wash their mouths with warm water between samples.

Statistical Analysis: The obtained results were statistically evaluated by application of one-way ANOVA test using SPSS (version 16; SPSS Inc., Chicago, USA).

RESULTS

The data illustrated in table (1) showed that the incidence of Aeromonas species in fresh Oreochromis niloticus and Mugil cephalus was 34 (68%) and 31(62) %, respectively.

Regarding to Table (2) showed that the most prevalent *Aeromonas* species isolated from fresh *Oreochromis niloticus* were A. caviae 18 (36%), *Aeromonas sobria* 14 (28%), *Aeromonas veronii* 8 (16%), *A. hydrophila* 7(14%) and *Aeromonas fluvialis* 3 (6%), respectively.

The presented data in Table (3) revealed that most prevalent *Aeromonas* species isolated from fresh *Mugil cephalus* were *A. caviae* 17 (34%), followed by *Aeromonas sobria* 13 (26 %), then *Aeromonas veronii* 9 (18 %), *A. hydrophila* 6 (12%) and *Aeromonas schubertii* 5 (10%), respectively.

Regarding to Table (4) illustrated that treatment of *Oreochromis niloticus* fillets, experimentally infected with *A. hydrophila* by intensity 5×10^6 /g, with essential oils (0.5%) of different plant extracts such as cinnamon, rosemary and garlic showed different a reduction percent at different storage times.; Treatment with cinnamon 0.5% showed a reduction percent of 85.8, 94.6 and 98.6 % after 3, 6 ad 9 days of treatment; rosemary 0.5 % showed reduction percent of 93.6, 98.9 and 99.9 % after 3, 6 and 9 days of treatment, finally garlic 0.5% showed a higher reduction percent reach to 98.7 % after 3 days of treatment and 100 % at 9 days of treatment.

The obtained results in Table (5) revealed that treatment of Oreochromis niloticus fillets, experimentally infected with A. hydrophila by intensity 5×10^6 /g, with essential oils (1%) which extracted from different plant such as cinnamon, rosemary and garlic showed a different reduction percent at different storage times; cinnamon 1 % showed reduction percent 95.1, 98.1 and 99.6 % after 3, 6 ad 9 days of treatment; rosemary 1 % showed reduction percent 98.4, 99.9 after 3 and 6 days of treatment and reach to 100 % after 9 days of treatment, finally garlic 1 % showed a higher reduction percent reach to 99.8 % after 3 days of treatment and 100 % at 6 and 9 days of treatment.

Table (6) illustrated that sensory criteria such as appearance, odor, texture and flavor of *Oreochromis niloticus* changed during different storage times and may be spoiled after 9 days of storage, treatment with different essential oil 0.5 % of different plant such as cinnamon, rosemary and garlic help in maintain sensory criteria during different storage times. Garlic essential oils 0.5 % is better than cinnamon and rosemary 0.5% in maintain sensory traits till the end of storage times without major changes in the sensory criteria.

Table (7) revealed that sensory criteria of *Oreochromis niloticus* changed during different storage times and may be spoiled after 9 days of storage, treatment with different essential oils 1 % of different plant such as cinnamon, rosemary and garlic help in maintain sensory criteria during different storage times. Garlic essential oil 1 % gave the better result in conserving sensory criteria of the examined experimental *Oreochromis niloticus* fillets with *A. hydrophila* till the end of storage time without major changes in the sensory criteria.

Table 1: Incidence of *Aeromonas* species in fresh *Oreochromis niloticus* and *Mugil cephalus* (50 of each)

		Aeromonas species	
Type of fish	No. of examined samples	No	Percent
Oreochromis niloticus	50	34	68
Mugil cephalus	50	31	62

Table 2: Frequency distribution of *Aeromonas* species in fresh *Oreochromis niloticus* (n=50).

Aeromonas species	No	Percent
Aeromonas hydrophila	7	14
Aeromonas caviae	18	36
Aeromonas sobria	14	28
Aeromonas veronii	8	16
Aeromonas fluvialis	3	6
Total	50	100

Table 3: Frequency distribution of *Aeromonas* species in fresh Mugil cephalus (n=50).

Aeromonas species	No	Percent
Aeromonas hydrophila	6	12
Aeromonas caviae	17	34
Aeromonas sobria	13	26
Aeromonas veronii	9	18
Aeromonas schubertii	5	10
Total	50	100

Table 4: Bactericidal effect of different essential oils (0.5%) on *A. hydrophila* inoculated *Oreochromis niloticus* fillets by intensity of 5×10^6/g (n=5).

	Treatment							
	Control		Cinnamon		Rosemary		Garlic	
Storage time	Count	R %	Count	R %	Count	R %	Count	R %
Zero time	$5.0\times10^6\pm 0.3\times10^6$	0	$5.0\times10^6\pm 0.3\times10^6$	0	$5.0\times10^6\pm 0.3\times10^6$	0	$5.0\times10^6\pm 0.3\times10^6$	0
3 days	$4.8\times10^6\pm 0.9\times10^6$	4.0	$7.1\times10^5\pm 1.8\times10^5$	85.8	$3.2\times10^5\pm 0.7\times10^5$	93.6	$6.2\times10^4\pm 1.0\times10^4$	98.7
6 days	$4.4\times10^6\pm 0.6\times10^6$	12.0	$2.7\times10^5\pm 0.5\times10^5$	94.6	$5.3\times10^4\pm 0.9\times10^4$	98.9	$1.2\times10^3\pm 0.1\times10^3$	99.9
9 days	$4.3\times10^6\pm 0.5\times10^6$	14.0	$6.8\times10^4\pm 1.1\times10^4$	98.6	$1.4\times10^3\pm 0.3\times10^3$	99.9	0	100

R %= Reduction % ND= Not detected

Table 5: Bactericidal effect of different essential oils (1%) on *A. hydrophila* inoculated *Oreochromis niloticus* fillets by intensity of 5×10^6/g (n=5).

	Treatment							
	Control		Cinnamon		Rosemary		Garlic	
Storage time	Count	R %	Count	R %	Count	R %	Count	R %
Zero time	$5.0\times10^6\pm 0.3\times10^6$	0	$5.0\times10^6\pm 0.3\times10^6$	0	$5.0\times10^6\pm 0.3\times10^6$	0	$5.0\times10^6\pm 0.3\times10^6$	0
3 days	$4.8\times10^6\pm 0.9\times10^6$	4.0	$2.3\times10^5\pm 0.4\times10^5$	95.4	$8.0\times10^4\pm 1.7\times10^4$	98.4	$9.1\times10^3\pm 2.2\times10^4$	99.8
6 days	$4.4\times10^6\pm 0.6\times10^6$	12.0	$9.6\times10^4\pm 2.0\times10^4$	98.1	$6.6\times10^3\pm 1.3\times10^3$	99.9	0	100
9 days	$4.3\times10^6\pm 0.5\times10^6$	14.0	$1.6\times10^4\pm 0.3\times10^4$	99.6	0	100	0	100

R %= Reduction % ND= Not detected

Table 6: Changes in sensory traits of control and essential oils (0.5%) treated *Oreochromis niloticus* fillets (n=5)

	Trait					
Storage time	Appearance (5)	odor (5)	Texture (5)	Flavor (5)	Overall (20)	Grade
1. Control:						
Zero time	5	5	5	5	20	Excellent
3 days	3.4	3.0	3.6	3.2	13.2	Middle
6 days	2.0	1.6	2.0	1.4	7.0	Poor
9 days	1.2	1.0	1.6	1.0	4.8	Spoiled
2. Cinnamon oil:						
Zero time	5	5	5	5	20	Excellent
3 days	4.2	4.0	4.0	4.4	16.6	Good
6 days	3.2	3.2	3.4	3.6	13.4	Middle
9 days	2.6	2.4	2.6	2.8	10.4	Poor
3. Rosemary oil:						
Zero time	5	5	5	5	20	Excellent
3 days	4.6	4.4	4.4	4.6	18.0	Good
6 days	4.0	3.6	3.8	3.8	15.2	Good
9 days	3.2	3.0	3.2	3.4	12.8	Middle
4. Garlic oil:						
Zero time	5	5	5	5	20	Excellent
3 days	4.8	4.6	4.2	4.6	18.2	Very good
6 days	4.4	3.6	3.8	3.8	15.6	Good
9 days	3.4	3.2	3.2	3.6	13.4	Middle

Table 7: Changes in sensory traits of control and essential oils (1%) treated *Oreochromis niloticus* fillets (n=5)

	Trait					
Storage time	Appearance (5)	odor (5)	Texture (5)	Flavor (5)	Overall (20)	Grade
1. Control:						
Zero time	5	5	5	5	20	Excellent
3 days	3.4	3.0	3.6	3.2	13.2	Middle
6 days	2.0	1.6	2.0	1.4	7.0	Poor
9 days	1.2	1.0	1.6	1.0	4.8	Spoiled
2. Cinnamon oil:						
Zero time	5	5	5	5	20	Excellent
3 days	4.2	3.4	3.6	3.8	15.0	Middle
6 days	3.2	2.8	3.0	3.2	12.2	Middle
9 days	2.4	1.6	2.2	2.6	8.8	Poor
3. Rosemary oil:						
Zero time	5	5	5	5	20	Excellent
3 days	4.4	3.8	4.2	4.4	16.8	Good
6 days	3.8	3.0	3.2	3.6	13.6	Middle
9 days	3.0	2.4	2.6	3.4	11.4	Middle
4. Garlic oil:						
Zero time	5	5	5	5	20	Excellent
3 days	4.6	3.8	4.0	4.6	17.0	Good
6 days	4.2	3.4	3.6	4.0	15.2	Good
9 days	3.4	3.0	3.0	3.6	13.0	Middle

DISCUSSION

Although motile Aeromonas appropriately receive much notoriety as pathogens of fish, it is important to note that these bacteria also compose a part of the normal intestinal microflora of healthy fish. Therefore, the presence of these bacteria, by itself, is not indicative of disease and, consequently, stress is often considered to be a contributing factor in outbreaks of disease caused by these bacteria [22].

The obtained results in Table 1 agrees with Abd El-Malek [23] who revealed that *Aeromonas* spp. could be isolated from wild and cultured Nile tilapia samples with the percentage of 40% and 36%, respectively.

Lower incidence of *Aeromonas* obtained by Eissa *et al*. [24] who reported that the prevalence of motile aeromonad septicemia in cultured and wild Nile Tilapia (*Oreochromis niloticus*) was 10 % and 2.5% respectively. On the contrary, higher results (100%) of *Aeromonas* spp. in Tilapia reported by Manna *et al*. [25] in a related study in India.

Castro-Escarpulli *et al*. [26] indicated that all β-haemolytic strains of A. hydrophila isolated from frozen fish were carriers of the virulence factor (aerolysin gene).

Our results nearly similar to Yecel *et al*. [27] who affirmed that among freshwater fish species, *Aeromonas caviae* was the most prevalent species (66%), followed by *Aeromonas hydrophila* (22.6%) and *Aeromonas veronii* biovar sobria (11.6%) while in sea fish *Aeromonas veronii* biovar sobria was the most prevalent *Aeromonas* species (41.5%) followed by *Aeromonas hydrophila* (30.1%) and *Aeromonas caviae* (28.3%).

Erdem *et al*. [28] isolated 78 strains from the skin and the intestinal tract of catfish and Tilapia and identified as followed: 36 *Aeromonas hydrophila*, 22 *Aeromonas caviae* and 20 *Aeromonas veronii* biovar sobria [17] recovered *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas sobria* from catfish and Tilapia and found that *Aeromonas hydrophila* and *Aeromonas sobria* were the predominant species in catfish, while *Aeromonas caviae* in tilapia.

Abd El-Malek [23] reported that *A. hydrophila* strains could be isolated from wild and cultured Nile Tilapia samples with the percentage of 16 % and 12 %, respectively. Regarding A. sobria, it could be isolated only from wild and cultured Nile Tilapia in an incidence of 20 % and 24%, respectively. Meanwhile, only wild Nile Tilapia contaminated with one isolate of A. caviae with the percentage of 4%.

Daskalov [29] has voiced an opinion that the consumption of *A. hydrophila*-contaminated fish and fish products has a major signi?cance for the occurrence of gastroenteritis. Most cases of illness are related to products from aquacultures or refrigerated foods ready for direct consumption.

Previous studies have indicated that A. hydrophila has been isolated from the different fish species [29, 30]. Ye *et al*. [31] isolated 20 strains of *A. hydrophila* from sixty diseased fish samples with hemorrhagic diseases showing 33.3% infection by A. hydrophila.

The safety of fish is affected by numerous factors, such as fish origins, products properties, processing method and preparation before consumption. The risk from fresh fish is low after proper heat processing, yet it increases if the fish is consumed raw, insufficiently thermally processed or lightly treated. Fish contaminated with A. hydrophila could be hazardous, especially for sensitive populations, such as children, elderly persons and immunocompromised people [32].

Garlic has been used for centuries in many societies against parasitic, fungal, bacterial and viral infections. The recent chemical characterization of their sulphur compounds has promoted claims that such compounds are the main active antimicrobial agents [33].

Previous research suggested that those functions are mainly attributed to the bioactive components of garlic, including sulphur containing compounds, such as allin, diallylsulphides and allicin [34]. Many beneficial health properties of garlic are attributed to organosulphur compounds, particularly to thiosulfinates [35].

The present study provides information about the incidence of *Aeromonas* species in fresh fish in Egypt, A. *caviae* then A. *Sobria* are the most frequent identified *Aeromonas* species isolated from *Oreochromis niloticus* and *Mugil cephalus*. Garlic essential oil 0.5 and 1% have a great bactericidal effect against *A. hydrophila* with maintaining sensory criteria than cinnamon and rosemary essential oil 0.5 and 1%. So we recommended addition of garlic oil during preparation or serving of fish fillet for Human consumption.

REFERENCES

1. Hidalgo, R.B. and M.J. Figueras, 2013. *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. Journal of Fish Diseases, 36: 371-388.
2. Martin-Carnahan, A. and S.W. Joseph, 2005. Family I. *Aeromonadaceae* Colwell, MacDonell and DeLey 1986. In: Bergey's Manual of Systematic Bacteriology, 2nd edn, Vol. 2 (ed. by D.J. Brennan, N.R. Krieg, J.T. Staley & G.N. Garrity), pp: 556-578. Springer-Verlag, New York
3. Seethalakshmi, I., S.K. Jayaraman, M.S. Manoharan and S. Valsalam, 2010. Virulence and cytotoxicity of seafood borne *Aeromonas hydrophila*. Brazilian Journal of Microbiology, 41: 978-983.
4. Sharma, I. and A. Kumar, 2011. Occurrence of enterotoxigenic *Aeromonas* species in foods of animal origin in North East India. European Review for Medical and Pharmacological Sci., 15: 883-887.

5. Handfield, M., P. Simard, M. Couillard and R. Letarte, 1996. *Aeromonas hydrophila* Isolated from Food and Drinking Water: Hemagglutination, Hemolysis and Cytotoxicity for a Human Intestinal Cell Line (HT-29). J. Appl. Environ. Microbiol., 62(9): 3459-3461.
6. Igbinosa, I.H., E.U. Igumbor, F. Aghdasi, M. Tom and A.I. Okoh, 2012. Emerging *Aeromonas* Species Infections and Their Significance in Public Health. Scientific World J., article ID 625023, pp: 1-13.
7. Wejdan, R.T.A., A.F. Al-Rubaiae, K.H.A. Oruba and S.N. Noor, 2014. PCR Detection of putative hemolysin and aerolysin genes in an *Aeromonas hydrophila* isolates from diarrhea in Babylon Province. Journal of Natural Sciences Research, 4: 41-47.
8. Kirov, S.M., 2003. Foodborne Microorganisms of Public Health Significance. Eds Alisa D Hocking. Australian Institute of Food Science and Technology, 6th edition. New South Wales, Australia.
9. Beaz-Hidalgo R. and M.J. Figueras 2012. Molecular detection and characterization of furunculosis and other *Aeromonas* fish infections. In: Health and Environment in Aquaculture (ed.by E. Carvalho), pp: 97-132. In Tech, Brazil.
10. Isman, B.M., 2000. Plant essential oils for pest and disease management. Crop Protection, 19: 603-608.
11. Marino, M., C. Bersani and G. Comi, 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. International Journal of Food Microbiology, 67: 187-195.
12. Baydar, H., O. Sagdi and G. Ozkan, 2004. Antibacterial activity and composition of essential oils from *Origanum, Thymbra* and *Sartureja* species with commercial importance in Turkey. Food Control, 15: 169-172.
13. Daferera, D.J., B.N. Ziogas and M.G. Polissiou, 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. michiganensis. Crop Protection, 22: 39-44.
14. Musa, Wei, L.S., C.T. Seng, W. Wee and L.K. Leong, 2008. Potential of Edible Plants as Remedies of Systemic Bacterial Disease Infection in Cultured Fish. Global Journal of Pharmacology, 2(2): 31-36.
15. Durairaj, S., S. Srinivasan and P. Lakshmanaperumalsamy, 2009. *In vitro* Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. Electronic Journal of Biology, 5(1): 5-10.
16. Buller, N.B., 2004. Bacteria from fish and other aquatic animals. A practical Identification Manual, CABI Publishing Co., Cambridge, London, UK.
17. Ashiru, A.W., P.O. Uaboi-Egbeni, J.E. Oguntowo and C.N. Idika 2011. Isolation and antibiotic profile of Aeromonas species from Tilapia fish (*Tilapia nilotica*) and Catfish (*Clarias betrachus*). Pak. J. Nutr., 10: 982-986.
18. MacFaddin, J.F., 2000. Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.1.
19. Tassou, C., E. Drosinos and G. Nychas, 1995. Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4°C and 10°C. J. Appl. Bacteriol., 78: 593-600.
20. Tandon, S. and S. Rane, 2008. Decoction and hot continuous extraction techniques. In: Handa, *et al*., editors. Extraction technologies for medicinal and aromatic plants. Trieste, Italy: ICS-UNIDO; pp: 93-106.
21. Kilinc, B. and S. Cakli, 2004. Chemical, microbiological and sensory changes in thawed frozen fillets of Sardine (*Sardina pilchardus*) during marination. Food Chem., 88: 275-280.
22. Khalil, A.H. and E.H. Mansour, 1997. Toxicity of crude extracellular products of *Aeromonas hydrophila* in *Tilapia Nilotica*. Lett. Appl. Microbiol., 25: 269-273.
23. Abd-El-Malek , A.M., 2017. Incidence and virulence characteristics of Aeromonas spp. in fish, Veterinary World, 10(1): 34-37.
24. Eissa, I.A.M., A.F. Badran, M. Moustafa and H. Fetaih, 1994. Contribution to hareketlie *Aeromonas septicemia* in some cultured and wild freshwater fish. Vet. Med. J. Giza, 42: 6369.
25. Manna, S.K., P. Maurye, C. Dutta and G. Samanta, 2013. Occurrence and virulence characteristics of *Aeromonas* species in meat, milk and fish in India. J. Food Saf., 33: 461-469.
26. Castro-Escarpulli, G., M.J. Figueras, G. Aguilera-Arreola, L. Soler, E. Fernandez-Rendon, G.O. Aparicio, J. Guarro and M.R. Chacon, 2003. Characterization of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. International Journal of Food Microbiology, 84: 41-49.

27. Yucel, N., B. Aslim and Y. Beyatli, 2005. Prevalence and resistance to antibiotics for Aeromonas species isolated from retail fish in Turkey. J. Food Quality, 28: 313-324.
28. Erdem, B., E. Kariptas and T. Kaya, 2010. Siderophore, hemolytic, protease and pyrazinamidase activities and antibiotic resistance in motile *Aeromonas* isolated from fish. Turk. J. Biol., 34: 453-462.
29. Daskalov, H. , 2006. The importance of *Aeromonas hydrophila* in food safety. Food Control, 17(6): 474- 483.
30. Deng, G.C., X.Y. Jiang, X. Ye, M.Z. Liu, S.Y. Xu, L.H. Liu, Y.Q. Bai and X. Luo, 2009. Isolation, Identification and Characterization of *Aeromonas hydrophila* from Hemorrhagic Grass carp. Microbiology (Chinese), 36(8): 1170-1177.
31. Ye, Y.W., T.F. Fan, H. Li, J.F. Lu, H. Jiang, W. Hu and Q.H. Jiang, 2013. Characterization of *Aeromonas hydrophila* from hemorrhagic diseased freshwater fishes in Anhui Province, China. International Food Research Journal, 20(3): 1449-1452.
32. Herrera, F.C., J.A. Santos, A. Otero and M.L. Garcia-Lopez, 2006. Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. Journal of Applied Microbiology, 100: 527-536.
33. Rose, P., M. Whiteman, P.K. Moore and Y.Z. Zhu, 2005. Bioactive Salk (en) yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. Natural Product Reports, 22: 351-368.
34. Amagase H. and J.A. Milner, 1993. Impact of various sources of garlic and their constituents on 7, 12-dimethylbenz[a]anthracene binding to mammary cell DNA. Carcinogenesis, 14: 1627-1631.
35. Block, E., 1992. The organ sulfur chemistry of the genus Allim implications for the organic chemistry of sulfur. Angew. Chem. Int. Ed., 31: 1135-1178.