



Effect of Different Organic Acids on Survival of Larvae and Control of Water Microflora in Milk fish (*Chanos chanos*) Hatchery System

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Abstract

This study reports the use of organic acids (acetic, malic, formic and citric) in fish larval rearing systems for survival and controlling the microflora of rearing water. The milk fish (*Chanos chanos*) larvae was treated for 10, 15, 30 and 60 min with seven concentrations (%) viz., 1, 0.2, 0.1, 0.075, 0.05, 0.025 and 0.02 of organic acids respectively. The percentage survival was zero (1, 0.2, and 0.1% conc) for all the organic acid at various time periods. The effective concentrations for treating fish larvae are 0.05, 0.05, 0.025 and 0.075% concentrations of acetic, malic, formic and citric acid, respectively where 100% survival was observed. Similarly, when the larval rearing water was treated with different concentrations of same organic acids and time period, complete inhibition of microbial flora was observed (1, 0.2 and 0.1% conc) for all organic acids at various time periods. However, at concentrations less than 0.1%, total bacterial count (TBC) and presumptive Vibrio count (PVC) were found in the range of 3.19 to 5.75 and 2.22 to 3.58 log 10 cfu ml⁻¹. In contrast, TBC and PVC in control group were found in the range of 6.37 to 6.90 and 4.35 to 4.74 log 10 cfu ml⁻¹. The acid treatment with concentrations of 0.075, 0.05 and 0.025 except formic acid at 0.025, 0.020 significantly ($p < 0.05$) improved the survival rates of fish larvae and reduced both TBC and PVC in larval rearing water. The present findings strongly recommend the use of organic

acids for treating fish larvae for improved survival and also for reducing pathogenic bacterial load from larval rearing waters.

Keywords: Milk fish, organic acids, survival, bath treatment, bacterial counts

Introduction

Aquaculture contribution to the food resources, income and livelihood is growing and its share having increased from 7 to 39% due to demand of cultured fish (FAO, 2016). However, the major problem faced by the aquaculture industry worldwide are diseases caused due to various biological and non-biological agents (Moriarty, 1996). For effective production in aquaculture, continuous supply of healthy larvae is essential. The current practice of high stocking densities leads to development of stress thus providing an opportunity to the aquatic pathogens to cause disease (Conte, 2004). The common response to disease is the application of antimicrobial agents which are responsible for the development and spread of antibiotic resistance. After the complete ban on the use of antibiotics in aquaculture, alternate strategies viz., vaccination, probiotics, dietary organic acids and immunostimulants have been applied successfully in aquaculture in place of antibiotics (Ng & Koh, 2016).

Organic acids especially short and medium chain acids or their salts have been used as dietary components in feeds aquaculture (Ng & Koh, 2016) as they are recognized as "generally regarded as safe" (GRAS) by USFDA (Bogaert & Naidu, 2000). The concept of organic acids as growth promoters,

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gut microflora manipulation, effects on tissue and enzymes and histological changes, were well established in aquaculture (Castillo et al., 2014; Elala & Ragaa, 2015; Ringo et al., 2016; Ng & Koh, 2016). Several studies have been conducted on the effect of the organic acids in crustaceans (Chuchird et al., 2015; Ng et al., 2015; Romano et al., 2015) and in fishes (Castillo et al., 2014; Elala & Ragaa, 2015). Sodium salts of organic acids in the shrimp diets showed significant inhibition of pathogenic bacteria in the digestive tract of *Litopenaeus vannamei* (Silva et al., 2013). Adams & Boopathy (2013) have suggested the use of formic acid in diets of shrimp to control *Vibrio* disease. Romano et al. (2015) have reported the protective effects of dietary organic acid in shrimp and resistance against the vibriosis. The presumptive *Vibrio* spp. counts from the grow out pond culture was significantly lowered for the shrimp fed with commercial feed added with microencapsulated organic blend (Ng et al., 2015). In olive flounder and red hybrid tilapia, the antimicrobial effect of dietary organic acids on the gut microbiota was reported by Park et al., 2011; Koh et al., 2016 respectively. However earlier studies were restricted to the use of organic acids through diet fed to the animals.

Among various pathogens affecting fish, *Vibrio* spp. is a very important one in marine waters (Ceccarelli & Colwell, 2014) and is responsible for Vibriosis in several fish species (Wang & Leung, 2000). The composition of the *Vibrio* spp. present in the culture and their relation to the disease outbreak in *Penaeus monodon* culture was well studied (Sung et al. 2001). Otta et al. (2001) enumerated total bacterial counts (TBC) of raw sea water (untreated) and the water from shrimp larval rearing tanks and observed that 70% of bacterial population were *Vibrio* spp. and the dominance of *Vibriosis* was observed in the larval culture tanks of *M. rosenbergii* (Prasad et al., 2008). But, there is paucity of information on the use of organic acids as a bath treatment for fish larval rearing and their effect on the water microflora. Therefore, the present study was conducted to determine the effective concentration of organic acids needed for treating the fish larvae for improved survival rates and also reduce the *Vibrio* load from the larval rearing water.

Materials and Methods

Healthy milk fish larvae (0.30 ± 0.03 g) were procured from fish seed hatchery, ICAR-Central

Institute of Brackish Water Aquaculture (CIBA), Chennai and were acclimatized in the indoor laboratory tanks for one week prior to experiment. The larvae were fed daily @ 3% body weight with the feed pellets procured from Nutrition Division of ICAR-CIBA, Chennai. The completely randomized design was followed with three replicates of four acid treated groups (acetic, malic, formic and citric) at seven concentrations (%) (1, 0.2, 0.1, 0.075, 0.05, 0.025 and 0.02) and one control (0.0%) group. For each organic acid, the survival studies were conducted separately. The experiment was carried out in 12 l plastic tubs filled with 5 l of sea water. Ten fish larvae were stocked in each tub and acclimatised for 30 min. The stock solution (2%) organic acids *i.e.*, acetic, formic, malic and citric (Merck, Mumbai, India) were prepared, and larvae were treated for 10, 15, 30 and 60 min with different concentrations of four organic acid. The larvae in the control group were treated with same amount of sterile distilled water. After the acid treatment, complete water was changed, and fresh sea water was added.

The survival rate (%) was noted at the end of each acid treatment in stipulated time intervals of 10, 15, 30 and 60 min and after 24 h for each acid respectively and the survival was (%) calculated as follows:

$$\text{Survival (\%)} = \frac{\text{Total number of animals survived}}{\text{Total number of animals treated}} \times 100$$

To demonstrate the reduction in microflora in organic acids treatment, treated and control water samples (100 ml) were collected from the milk fish larval culture tubs. TBC and PVC were enumerated by serial dilution and plating on Marine agar (Himedia, Mumbai, India) and on Thiosulfate Citrate Bile Salts Sucrose agar (Himedia, Mumbai, India) respectively. The water sample was serially diluted in phosphate buffer saline and 0.1 ml was spread plated onto marine agar and TCBS and incubated at 30°C for 24-48 h in BOD incubator. Bacterial counts were expressed as $\log_{10}$ cfu ml⁻¹ for further statistical analysis. For comparison, water microflora from control group was also enumerated for TBC and PVC.

Feed (1 g) was taken and blended with nine ml of phosphate buffer saline aseptically and spread on the respective plates for enumeration of TBC and PVC.

All the data were analysed in one-way ANOVA using SPSS V. 16 software (SPSS Inc., Chicago,

Illinois, USA). The means were compared using Duncan multiple range test to find the difference at 5% ($p < 0.05$) level.

Results and Discussion

The survival percentage of larvae after ten minutes of different acid treatments is shown in Fig. 1. Complete mortality was observed at 1 and 0.2%, whereas 100% survival was recorded at 0.025 and 0.02% for all the acids. At 0.1%, the survival was 23, 40, 36% for acetic, malic and citric acids respectively. Similarly, at 0.075%, the survival rate was 83, 100, 100% for acetic, malic and citric acids respectively and 100% survival in 0.05% of acetic, malic and citric acids. However, in case of formic acid, the survival at 0.1, 0.075, 0.05 and 0.025 (%) of organic acid were 0, 0, 26, 100 respectively. Significantly, 100% survival was observed in control group (treated with sterile distilled water). In all acid treated groups significant differences were seen ($p < 0.05$) between the concentrations compared to control.

The percentage of survival of larvae recorded for different acid treatments after 15 min is given in Fig. 2. There was significant difference ($p < 0.05$) in percentage of survival at different concentration (for all acids) compared to control group. Complete mortality was observed in treatments with 1 and 0.2% concentration for all organic acids, whereas 100% survival was recorded at 0.025 and 0.02% for all the acids. At 0.1%, the survival was 13, 36, 33% for acetic, malic and citric acids respectively. Similarly, at 0.075%, it was 73, 93, 100% for acetic, malic and citric acids respectively and at 0.05%, the survival rate (%) was 100, 100, 100 for acetic, malic and citric acids respectively. However, in case of formic acid, the survival rate (%) at 0.1, 0.075, 0.05 and 0.025 (%) of acid concentration was 0, 0, 13, 83.

The survival of larvae after thirty minutes of different acids treatment is shown in Fig. 3. At 0.1%, the survival rate (%) was 0, 26, 13 for acetic, malic and citric acids. Similarly, at 0.075%, the survival rate (%) was 73, 86, 100 for acetic, malic and citric

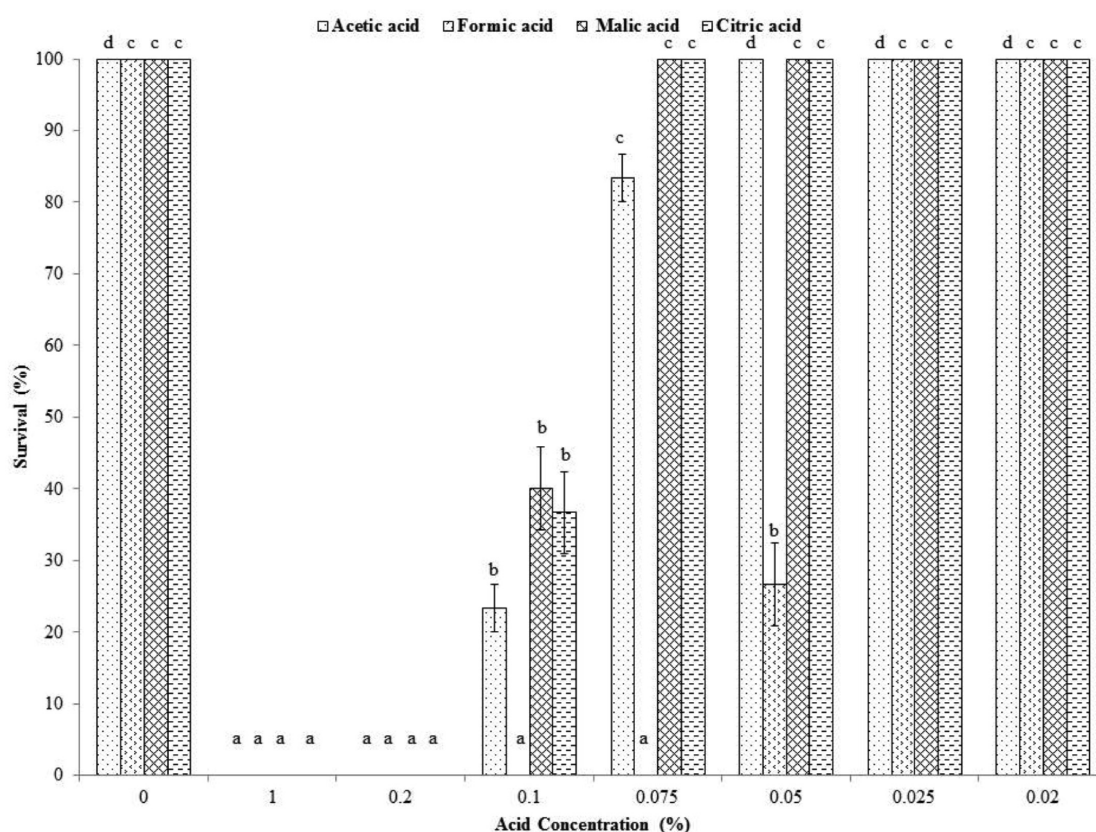


Fig. 1. Survival percentage of larvae recorded at the end of acids treatment after 10 min. Values represents the three replicates used for acids treatments (Mean $\pm$ SE, $n = 3$). Values with different upper case superscripts denote significant difference ($p < 0.05$) among the replicates.

acids and at 0.05%, the survival rate (%) was 100, 100, 100 for acetic, malic and citric acids respectively. However, in case of formic acid, the survival rate (%) at 0.1, 0.075, 0.05 and 0.025 (%) of acid concentrations was 0, 0, 0, 76. Significantly, 100% survival was observed in control group (treated with sterile distilled water). In all acid treated groups significant difference was seen ($p < 0.05$) between the concentrations compared to control.

The survival rate of larvae (%) after 60 min of different acid treatments was nil at 1 and 0.2% (Fig. 4), whereas 100% survival were recorded at 0.02% for all the acids. At 0.1%, the survival rate (%) was 0, 23, 3 for acetic, malic and citric acids. Similarly, at 0.075%, the survival rate (%) was 66, 73, 100 for acetic, malic and citric acids respectively and at 0.05%, the survival rate (%) was 100, 100, 100 for acetic, malic and citric acids respectively. However, in case of formic acid, the survival rate (%) at 0.1, 0.075, 0.05 and 0.025 (%) of acid concentration was 0, 0, 0, 73. There was a significant difference ($p < 0.05$) in percentage of survival at different concentration (for all acids) compared to control group.

The survival rate (%) of larvae obtained after 24 h of different acids treatment is shown in Fig. 5. There was a significant difference ($p < 0.05$) in all acid treatments compared to control. The survival rate was found nil in treatments with 1 and 0.2% concentration for all organic acids, whereas 100% survival was recorded at 0.02% for all the acids. At 0.1%, the survival rate (%) was 0, 23, 0 for acetic, malic and citric acids. Similarly, at 0.075%, the survival rate (%) was 66, 66, 100 for acetic, malic and citric acids respectively and at 0.05%, the survival was 100% for all organic acid treated group. However, in case of formic acid, the survival rate (%) at 0.1, 0.075, 0.05 and 0.025 (%) of acid concentration was 0, 0, 0, 66.

The bacterial counts obtained after treatment of water sample with acetic acid for different time intervals is shown in Table 1. There was a significant decrease in bacterial counts with increase in acid concentrations. Complete inhibition of bacteria was found in water sample treated with 1, 0.2 and 0.1% of acetic acid for all the time periods. TBC significantly ($p < 0.05$) decreased from 5.21 to 4.55;

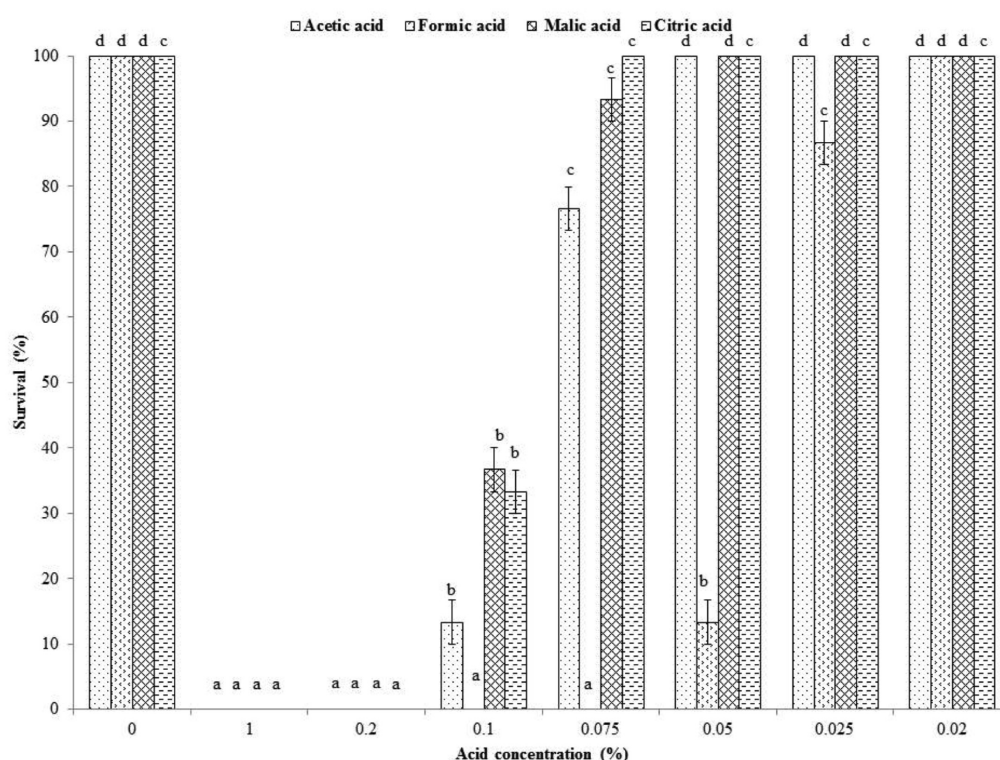


Fig. 2. Survival percentage of larvae recorded at the end of acids treatment after 15 min. Values represents the three replicates used for acids treatments (Mean $\pm$ SE, $n = 3$). Values with different upper case superscripts denote significant difference ($p < 0.05$) among the replicates.

4.82 to 4.27; 4.82 to 4.24 and 4.82 to 4.19 $\log_{10}$ cfu ml^{-1} and PVC also showed significant ($p < 0.05$) decreasing trend from 4.26 to 3.38; 4.08 to 3.32; 4.03 to 3.22 and 3.97 to 3.22 for 0.025, 0.05 and 0.075 % for 10, 15, 30 and 60 min respectively. The microbial counts of TBC and PVC were found to be higher (6.47 to 6.84 $\log_{10}$ cfu ml^{-1} and 4.45 to 4.61 $\log_{10}$ cfu ml^{-1}) in control group.

The bacterial counts recorded after formic acid treatment at different time intervals is given in Table 2. There was inverse relation between bacterial count and acid concentration *i.e.* decrease in bacterial counts with increase in acid concentration. There was complete inhibition of bacteria for 1, 0.2 and 0.1% of acid at 10, 15, 30 and 60 min. TBC significantly ($p < 0.05$) decreased from 4.21 to 3.55; 3.82 to 3.27; 3.82 to 3.24 and 3.62 to 3.19 $\log_{10}$ cfu ml^{-1} and PVC also showed significant ($p < 0.05$) decreasing trend from 3.26 to 2.38; 3.18 to 2.22; 3.13 to 2.22 and 3.08 to 2.22 for 0.025, 0.05 and 0.075 % for 10, 15, 30 and 60 min respectively. The microbial counts of TBC and PVC were found in higher (6.37 to 6.74 $\log_{10}$ cfu ml^{-1} and 4.35 to 4.51 $\log_{10}$ cfu ml^{-1}) in control group.

The bacterial counts obtained after treatment of water sample with malic acid for different time intervals is shown in Table 3. Decrease in bacterial counts was observed as the concentration of acid increases. Complete inhibition of bacteria in water sample treated with 1, 0.2 and 0.1% of malic acid for all the time periods was observed. TBC significantly ($p < 0.05$) decreased from 6.41 to 5.75; 5.88 to 5.47; 5.88 to 5.44 and 5.82 to 5.32 $\log_{10}$ cfu ml^{-1} and PVC also showed significant ($p < 0.05$) reduction from 4.60 to 3.58; 4.51 to 3.55; 4.43 to 3.52 and 4.07 to 3.52 for 0.025, 0.05 and 0.075 % respectively for 10, 15, 30 and 60 min of malic acid treatment. In contrast, in control group, microbial counts of TBC and PVC were found to be in higher *i.e.*, 6.56 to 6.78 $\log_{10}$ cfu ml^{-1} and 4.56 to 4.71 $\log_{10}$ cfu ml^{-1} respectively.

The bacterial counts obtained after treatment of water sample with citric acid for different time intervals is given in Table 4. The complete inhibition of bacteria was observed in water sample treated with 1, 0.2 and 0.1% of citric acid for all the time periods. TBC significantly ($p < 0.05$) decreased from

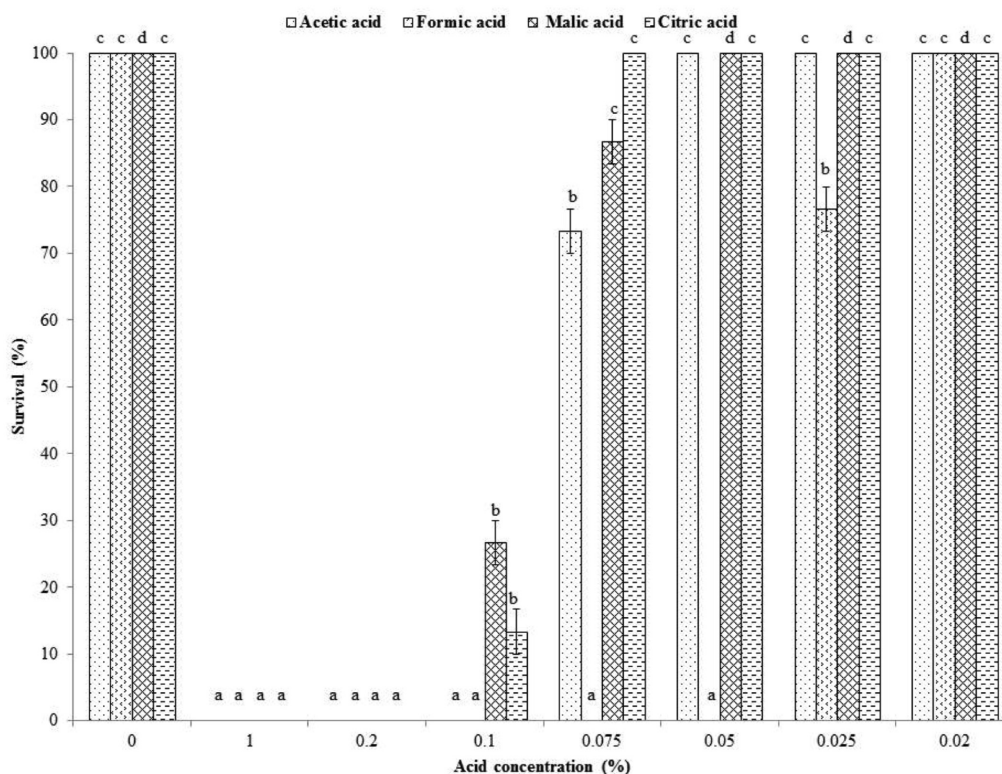


Fig. 3. Survival percentage of larvae recorded at the end of acids treatment after 30 min. Values represents the three replicates used for acids treatments (Mean $\pm$ SE, $n = 3$). Values with different upper case superscripts denote significant difference ($p < 0.05$) among the replicates.

5.41 to 4.75; 4.88 to 4.47; 4.88 to 4.44 and 4.82 to 4.32 $\log_{10}$ cfu ml^{-1} and PVC also showed significant ($p < 0.05$) decreasing trend from 4.46 to 3.58; 4.31 to 3.45; 4.23 to 3.32 and 4.18 to 4.32 for 0.025, 0.05 and 0.075% respectively for 10, 15, 30 and 60 min of citric acid treatment. In contrast, in control group microbial counts of TBC and PVC were 6.67 to 6.90 $\log_{10}$ cfu ml^{-1} and 4.55 to 4.74 $\log_{10}$ cfu ml^{-1} respectively.

The microbial enumeration from the raw sea water (untreated) and feed was carried out to establish the point of entry of *Vibrios* to the larval rearing water. In untreated sea water, the total aerobic bacterial counts were $\log_{10}$ 2.45 cfu ml^{-1} and no *Vibrio* was found. In inert feed pellets, the total aerobic bacterial counts were about $\log_{10}$ 2.1 cfu g^{-1} and no *Vibrio* was found.

The use of organic acids to larval rearing systems results in the development of stress which will modulate the immune system of the fish larvae for fighting with the external pathogens effectively (Elala & Ragaa, 2015). The percent survival was

above 50% for concentrations of 0.075, 0.05, 0.025, 0.02 for three acids tested in the present study except for formic acid where above 50% survival was found for concentrations of 0.025 and 0.02. The results revealed that the effective concentrations at which no mortality observed are 0.05, 0.05, 0.025 and 0.075% for acetic acid, malic acid, formic acid, and citric acid respectively. The effect of citric acid on the white shrimp, *L. vannamei* survival was significantly higher at 1- 4 g kg^{-1} of diet (Su et al., 2014). Formic acid also showed improved survival rates of Pacific white shrimp, *L. vannamei* post larvae after challenge with *Vibrio parahaemolyticus* (Chuchird et al., 2015). The organic acids if used in higher concentration may affect the survival of the animals resulting in mortalities of larvae as evidenced in case of 1, 0.2, 0.1 concentrations for all acids where the survival rates are nil. Therefore, the organic acids should be used at an effective concentration to improve the survival rates of fish larvae.

The primary mode of action of organic acids is its antimicrobial nature *i.e.* limiting growth of bacteria in the gut of the animals when administered as

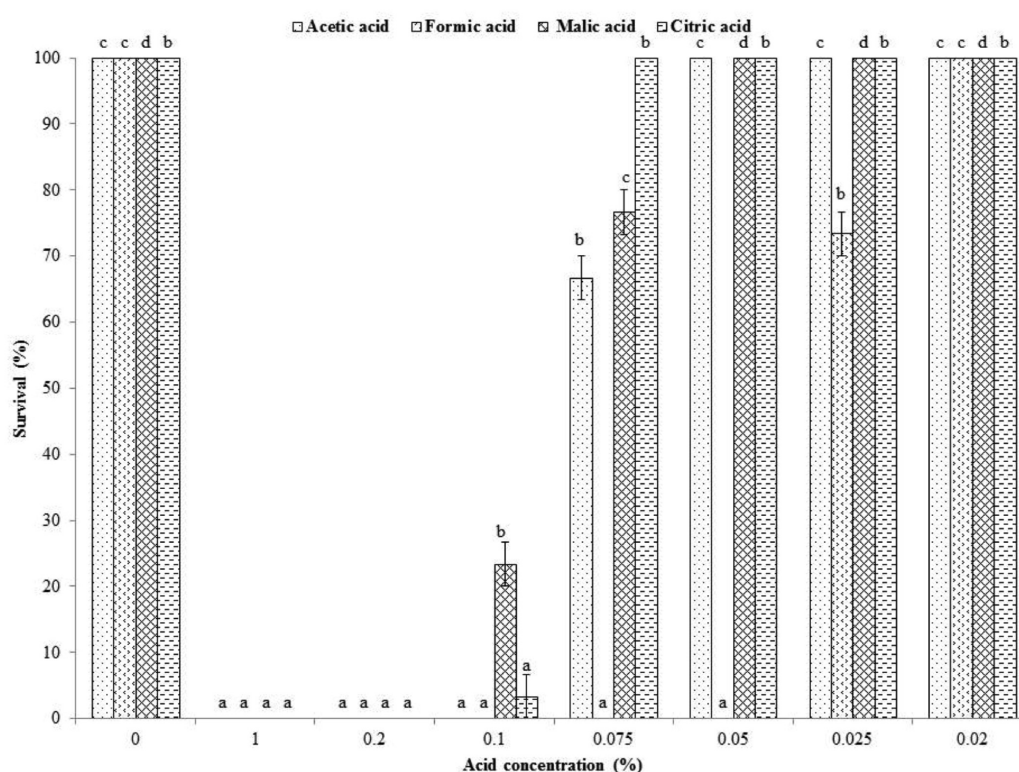


Fig. 4. Survival percentage of larvae recorded at the end of acids treatment after 60 min. Values represents the three replicates used for acids treatments (Mean $\pm$ SE, $n = 3$). Values with different upper case superscripts denote significant difference ($p < 0.05$) among the replicates.

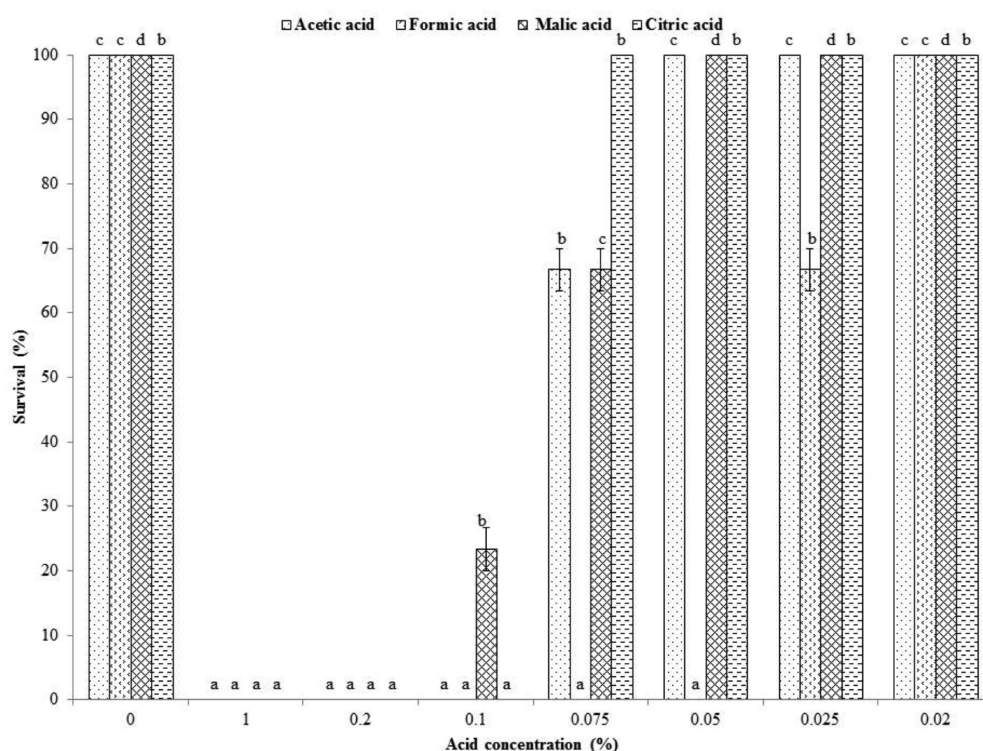


Fig. 5. Survival percentage of larvae recorded at the end of acids treatment after 24 h. Values represents the three replicates used for acids treatments (Mean $\pm$ SE, n = 3). Values with different upper case superscripts denote significant difference ($p < 0.05$) among the replicates.

Table 1. Water microflora (log 10 cfu ml⁻¹) recorded after treatment with different concentrations of acetic acid for respective time period

Sl. No.	Acid concentration (%)		10 min	15 min	30 min	60 min
1.	C	TBC	6.52 ^d $\pm$ 0.08	6.47 ^c $\pm$ 0.13	6.84 ^d $\pm$ 0.08	6.66 ^c $\pm$ 0.06
		PVC	4.45 ^c $\pm$ 0.11	4.50 ^c $\pm$ 0.11	4.61 ^d $\pm$ 0.17	4.54 ^c $\pm$ 0.14
2.	1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
3.	0.2	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
4.	0.1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
5.	0.075	TBC	4.55 ^a $\pm$ 0.07	4.27 ^a $\pm$ 0.07	4.24 ^a $\pm$ 0.06	4.19 ^a $\pm$ 0.11
		PVC	3.38 ^a $\pm$ 0.08	3.32 ^a $\pm$ 0.10	3.22 ^a $\pm$ 0.14	3.22 ^a $\pm$ 0.09
6.	0.05	TBC	4.98 ^b $\pm$ 0.06	4.58 ^b $\pm$ 0.11	4.51 ^b $\pm$ 0.08	4.40 ^{ab} $\pm$ 0.12
		PVC	4.06 ^b $\pm$ 0.04	3.72 ^b $\pm$ 0.08	3.69 ^b $\pm$ 0.14	3.64 ^b $\pm$ 0.14
7.	0.025	TBC	5.21 ^c $\pm$ 0.12	4.82 ^b $\pm$ 0.10	4.82 ^c $\pm$ 0.09	4.82 ^c $\pm$ 0.09
		PVC	4.26 ^c $\pm$ 0.06	4.08 ^c $\pm$ 0.09	4.03 ^c $\pm$ 0.06	3.97 ^b $\pm$ 0.05

Values (Mean $\pm$ SE, n = 3) with different upper case superscripts denote significant difference ($p < 0.05$) among the replicates. ND-not detected. TBC-total aerobic bacterial counts. PVC-Presumptive Vibrio count.

Table 2. Water microflora (log₁₀ cfu ml⁻¹) recorded after treatment with different concentrations of formic acid for respective time period

Sl. No	Acid concentration (%)		10 min	15 min	30 min	60 min
1.	C	TBC	6.42 ^d ±0.08	6.37 ^c ±0.13	6.74 ^d ±0.08	6.56 ^c ±0.06
		PVC	4.35 ^d ±0.11	4.40 ^d ±0.11	4.51 ^d ±0.17	4.44 ^d ±0.14
2.	1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
3.	0.2	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
4	0.1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
5	0.075	TBC	3.55 ^a ±0.07	3.27 ^a ±0.07	3.24 ^a ±0.06	3.19 ^a ±0.11
		PVC	2.38 ^a ±0.08	2.22 ^a ±0.10	2.22 ^a ±0.14	2.22 ^a ±0.09
6	0.05	TBC	3.98 ^b ±0.06	3.58 ^b ±0.11	3.61 ^b ±0.08	3.40 ^{ab} ±0.12
		PVC	3.06 ^b ±0.04	2.72 ^b ±0.08	2.69 ^b ±0.14	2.64 ^b ±0.13
7	0.025	TBC	4.21 ^c ±0.12	3.82 ^b ±0.10	3.82 ^c ±0.09	3.62 ^b ±0.09
		PVC	3.26 ^c ±0.06	3.18 ^c ±0.09	3.13 ^c ±0.06	3.08 ^c ±0.00

Values (Mean±SE, n=3) with different upper case superscripts denote significant difference (p<0.05) among the replicates. ND-not detected. TBC-total aerobic bacterial counts. PVC-Presumptive Vibrio count.

dietary component or in the water when applied as bath treatment. The effect of the organic acids on the gut microbiota have been reported in olive flounder (Park et al., 2011), tilapia (Koh et al., 2016) and white shrimp (Da Silva et al., 2013). At higher concentrations of all tested organic acids (1, 0.2, 0.1 %), there was complete inhibition of bacterial growth. Also, in acid treated water, there was significant (p<0.05) reduction in both the total aerobic bacterial counts as well as presumptive Vibrio count when compared to the untreated rearing water (control). The decrease in microbial counts may be due to the action of the organic acids on the cell membrane of the bacteria lowering the cytoplasmic pH resulting in death of bacteria (Ng & Koh, 2016). The decrease in Vibrio count have also been reported by Da Silva et al. (2013) in Pacific white shrimp gut when sodium propionate is administered through diet. Similar decrease in Vibrio counts was also noticed by Romano et al. (2015) in hepatopancreas of *L. vannamei* when shrimp larvae were fed with organic acid blend. In larval rearing waters, *Vibrio* spp. may dominate from 50 to 70% of the total aerobic microbial flora (Otta et al., 2001) as evidenced in the enumeration of the water microflora for the larval rearing water. In the present study, the water

microflora in the untreated acid water (control) was in the range of 6.37 to 6.90 log₁₀ cfu ml⁻¹ which is similar to the microbial counts reported from the larval rearing waters of shrimp (*P. monodon*) hatchery (Otta et al., 2001).

To determine the entry of pathogenic bacteria i.e. *Vibrio* into the rearing systems, the microbial enumerations were also done for the raw sea water sample (untreated) and feed pellets for total aerobic bacterial counts and presumptive Vibrio count. No Vibrio counts were noticed in the water and feed suggested that the water and feed was free from pathogenic Vibrios and the probable source for the entry of pathogenic Vibrios may be through the larvae. Different microflora may enter into the rearing water through larvae, live feed or through bloodstock (Otta et al., 2001). The higher organic matter content in the larval rearing water is also responsible for the higher bacterial load. Therefore, treating the water periodically will reduce the bacterial microflora especially pathogenic *Vibrio* spp.

In conclusion, the treatment of organic acids improved the survival rates of fish larvae and also the treatment of the larval water with different organic acids reduced the pathogenic *Vibrio* spp.

Table 3. Water microflora (log 10 cfu ml⁻¹) recorded after treatment with different concentrations of malic acid for respective time period

Sl. No	Acid concentration (%)		10 min	15 min	30 min	60 min
1.	C	TBC	6.56 ^c ±0.05	6.63 ^c ±0.11	6.66 ^c ±0.05	6.78 ^c ±0.10
		PVC	4.56 ^c ±0.11	4.65 ^c ±0.07	4.64 ^d ±0.06	4.71 ^c ±0.15
2.	1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
3.	0.2	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
4	0.1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
5	0.075	TBC	5.75 ^a ±0.07	5.47 ^a ±0.07	5.44 ^a ±0.05	5.32 ^a ±0.17
		PVC	3.58 ^a ±0.08	3.55 ^a ±0.14	3.52 ^a ±0.14	3.52 ^a ±0.09
6	0.05	TBC	5.83 ^a ±0.11	5.72 ^b ±0.05	5.81 ^b ±0.08	5.53 ^{ab} ±0.16
		PVC	4.23 ^b ±0.17	3.89 ^b ±0.03	3.85 ^b ±0.06	3.79 ^{ab} ±0.07
7	0.025	TBC	6.41 ^b ±0.12	5.88 ^b ±0.05	5.88 ^b ±0.03	5.82 ^b ±0.09
		PVC	4.60 ^c ±0.07	4.51 ^c ±0.15	4.43 ^c ±0.06	4.07 ^b ±0.14

Values (Mean ± SE, n = 3) with different upper case superscripts denote significant difference (p<0.05) among the replicates. ND-not detected. TBC-total aerobic bacterial counts. PVC-Presumptive Vibrio count.

Table 4. Water microflora (log 10 cfu ml⁻¹) recorded after treatment with different concentrations of citric acid for respective time period

Sl. No.	Acid concentration (%)		10 min	15 min	30 min	60 min
1.	C	TBC	6.72 ^d ±0.08	6.67 ^c ±0.13	6.90 ^c ±0.03	6.86 ^c ±0.06
		PVC	4.55 ^c ±0.11	4.70 ^d ±0.11	4.71 ^d ±0.17	4.74 ^c ±0.12
2.	1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
3.	0.2	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
4	0.1	TBC	ND	ND	ND	ND
		PVC	ND	ND	ND	ND
5	0.075	TBC	4.75 ^a ±0.07	4.47 ^a ±0.07	4.44 ^a ±0.05	4.32 ^a ±0.17
		PVC	3.58 ^a ±0.08	3.45 ^a ±0.14	3.32 ^a ±0.14	3.28 ^a ±0.14
6	0.05	TBC	5.05 ^b ±0.12	4.72 ^b ±0.05	4.71 ^b ±0.08	4.63 ^a ±0.09
		PVC	4.20 ^b ±0.10	3.85 ^b ±0.05	3.72 ^b ±0.09	3.62 ^b ±0.09
7	0.025	TBC	5.41 ^c ±0.12	4.88 ^b ±0.05	4.88 ^b ±0.03	4.82 ^b ±0.09
		PVC	4.46 ^c ±0.06	4.31 ^c ±0.15	4.23 ^c ±0.06	4.18 ^b ±0.06

Values (Mean ± SE, n = 3) with different upper case superscripts denote significant difference (p<0.05) among the replicates. ND-not detected. TBC-total aerobic bacterial counts. PVC-Presumptive Vibrio count.

load from the larval rearing water. The effective concentration for treating the fish larvae was observed at 0.05, 0.05, 0.025 and 0.075% for acetic acid, malic acid, formic acid, and citric acid respectively after 15 min treatment.

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