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Effect of Salinity and pH Changes on the Toxicity of Abamectin in *Artemia franciscana* using Response Surface Methodology

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Abstract

The excessive increase in pesticides consumption has made concerns about aquatic ecosystems. Hence, the present study focused on the toxicity level of Abamectin as a widely used pesticide in *Artemia franciscana*. The lethality of Abamectin at different pHs and salinity was investigated to simulate the real natural status of *A. franciscana.* The salinity ranges of 10-255 and 10-130 $g.l^{-1}$ with the pH range of 4-11 for water with a constant Lethal concentration 50 (LC_{50}) (0.145 $\mu g.l^{-1}$ from the toxicity test) were considered and evaluated using response surface methodology (RSM). A significant variation in *Artemia* mortality was observed at 0.145 $\mu g.l^{-1}$ of Abamectin in the salinity ranges of 10-255 $g.l^{-1}$, irrespective of the effect of pH. In the second phase of the experiment, a significant variation of mortality was observed in the level of LC_{50} in the salinity range of 10-130 $g.l^{-1}$, which was associated with pH shifts (p<0.05). In addition, R^2 adjusted, and predicted R^2 of the model were equal to 0.985, 0.976, and 0.96, respectively. The reduction in the pH from 7.5 and the salinity from 30 $g.l^{-1}$ intensified the lethal effects of Abamectin. The simultaneous increase in pH and salinity decreased the mortality level. Also, the increase in salinity raised the mortality rate. These findings may reflect the stressor effects induced by the change of physicochemical parameters on the tolerance of *A. ftanciscana*'s in confronting pollution exposure. Hence, it can be concluded that a severe decrement in salinity and pH can intensify the LC_{50}, which exerts an adverse impact on osmoregulation and high energy demand.

1. Introduction

The release of pesticides into surface water has increased due to agricultural modernization and industrialization through surface runoff, drainage, and accidental leaks [1,2]. Their harmful impacts on living organisms are associated with their mobility and stability [3,4]. Abamectin classified as Class 2 of pesticides, has a wide application in agriculture and livestock. It is derived from the fermentation of Actinomyces called *Streptomyces avermitilis* and utilized as an insecticide due to its gastrointestinal and contact effects and acaricide. Abamectin is a mixture of Avermectin compounds from 16-membered macrocyclic lactones. Avermectins contain four main components: A_1, A_2, B_1, and B_2, with compounds consisting of A_{1a}, A_{2a}, B_{1a}, and B_{2a}, as well as four sub-compounds of A_{1b}, A_{2b}, B_{1b}, and B_{2b}. Moreover, Abamectin (B_1) contains 80% B_{1a} and 20% B_{1b} in its structure [5]. This pesticide is highly popular due to its ease of use and strong performance. It affects the nervous system of insects by acting on GABAergic neurons [5], which inhibits synapses connecting glutamate-sensitive chloride channels [6]. This pesticide causes diarrhea, vomiting, drowsiness, and hypotension in humans. Furthermore, it can spark respiratory and heart failure in severe poisoning [7].

According to the Abamectin's insolubility in water and lipophilic property, it can be transmitted through the food chain and disrupt the reproduction, biological function, and survival of non-target aquatic organisms. This pesticide, for instance, induces oxidative stress in the liver of *Oreochromis mossambicus* by producing free radicals, altering antioxidants, and reducing the activities of antioxidant enzymes such as glutathione peroxidase, glutathione s-transferase, and superoxide dismutase. Furthermore, lipid peroxidation, enzyme inhibition, and liver necrosis are other adverse impacts of Abamectin [8]. Numerous investigations have been carried out on Abamectin toxicity concerning the lethal concentration of 50% (LC_{50}) due to its high molecular weight and immobility. In addition, *Daphnia magna* was recognized as the most sensitive aquatic species to Abamectin with an LC_{50} value of 0.34 μgl^{-1} [9,10]. However, according to the aforementioned detrimental impacts of Abamectin and a lack of relevant literature considering *Artemia* sp., it is essential to investigate the toxicity level of Abamectin.

Artemia sp., as the main link of the food chain, can accumulate and magnify pollutants in contaminated ecosystems. Due to their wide distribution, they can be a good bio-indicator for ecosystem health [11]. *Artemia* sp. is recognized as the most suitable in vitro test organism to assess toxicity because of available information about its biology and ecology, ease of accessibility, small size, rapid hatching, and sensitivity to various contaminants. It is also low cost with a short testing time compared to other laboratory organisms. It should be mentioned that the sensitive endpoint of mortality is commonly considered as a response to toxic substances [12]. *Artemia* sp. typically inhabits saline and shallow-water ecosystems and may be influenced by pesticides like Abamectin in the discharge of agricultural waste [13]. Therefore, the investigation of Abamectin toxicity in *Artemia* sp. can provide beneficial data concerning its function and response.

Conventional laboratory methods for toxicity tests are not only costly and time-consuming but also unable to demonstrate the mutual effect of the test variables, while response surface methodology (RSM) can reduce test costs and produce better results without undermining the statistical validity. RSM is a statistical method that draws on experimental data to determine regression models and optimize laboratory conditions [14]. The two most common methods used in RSM are central composite design (CCD) and Box-Behnman design (BBD). CCD is a widely accepted approach with the capability to predict the responses as well as optimize the operation condition. Hence, an accurate estimation of the regression model and the mutual effect of the variables can be achieved by the application of this technique. Moreover, this method is employed in a set of mathematical and statistical

techniques to perform a minimum number of experiments, saving time compared to other common experimental methods [15]. Although the BBD approach gives optimized conditions in a minimum number of experimental runs as compared to CCD, little collinearity, rotatable or nearly rotatable, and insensitive to outliers and missing data are limited its application in environmental studies. Furthermore, the BBD doesn't predict well at the concerns of the design space and doesn't contain any point at the vertices of the cubic region created by the upper and lower limits for each variable. Therefore, the CCD was chosen as the best option for the experimental design.

Since the toxicity of Abamectin in *Artemia* has not been studied yet, its toxicity test was performed using *A. franciscana* under laboratory conditions. For this purpose, the influence of environmental factors, including temperature, pH, and salinity on the lethal effect of pollutants on aquatic organisms was investigated [16]. Therefore, the present study focused on the modulation of salinity and pH shifts on the variation of LC_{50} levels in *A. franciscana* using the RSM methodology.

2. Materials and Methods

2.1 *Preparation of A. franciscana*

To obtain the nauplii of *A. franciscana,* 1 g of the pure cyst was placed in plastic cones for hatching. The encapsulation conditions were based on the standard method: 24 h at a light intensity of 2000 Lux, salinity of 35 $g.l^{-1}$, pH= 7.8, the water temperature of 29 °C, and aeration in 1 L of water. After the encapsulation process, Napliuses removed from the cysts were collected by the photolysis or positive phototropism and accumulated on the surface of the beaker by Pipette Pasteur [17]. *Artemia* Nauplii were fed by *Dunaliella teriolecta* until maturity after passing the instar I stage [18]. The Abamectin pesticide (Vertimec brand), available on the market as an emulsion of 1.8% EC, was used to determine the lethal range in the adult group of *A. franciscana* following the *Artemia* maturation. The statistical range of lethal concentrations of Abamectin was determined according to the standard O.E.C.D method. Accordingly, a series of finding experiments was used to specify the alteration range of Abamectin concentration. The minimum lethal and maximum nonlethal concentrations of Abamectin were chosen to kill 100% of *Artemia* and survive any *Artemia* at 24 h, respectively. The water temperature was maintained constant during the experiment at 25 °C with pH= 7.5 and salinity of 35 $g.l^{-1}$. After preparing the main solution, each concentration was pipetted into Petri dishes and 20 adults of *Artemia* were added. A toxicity test was performed without a water change. After 24 h, the number of dead *Artemia* in each petri dish was recorded by a loop. Acceptance mortality was determined based on immobility after 10 seconds of continuous observation [19]. After preliminary tests, the toxicity range was chosen between concentrations of 0.05 to 1 $\mu g.l^{-1}$ (including 0.05, 0.125, 0.25, 0.5, 0.75 and 1 $\mu g.l^{-1}$ concentrations). At the end of the experiment, the results were analyzed using Probit Program Analysis ver. 1.5 with a 95% confidence interval (CI) and the values of $LC_{10,}$ $LC_{15,}$ $LC_{50,}$ $LC_{85,}$ $LC_{90,}$ $LC_{95,}$ and LC_{99} were calculated.

2.2 *Experimental design and data analysis*

In this study, the Design-Expert software 7.0 (DOE, Stat-Ease Inc., Minneapolis, MN, USA) and the standard central composite design (CCD) method were used. This method includes three parts (1) a complete or a fractional factorial, (2) an extra-axial plan where the points are at a certain distance from the center, and (3) a central point. Axial points ensure the model prediction's variance is constant at all points relative to the center point. The 2n factorial runs or similar variables with 2n axial runs and a central run (nc) (with four replications) were used to

investigate the experimental error. Axial points were also selected for the probability of reproducibility. Each factor was evaluated at 5 levels, including -1.41, -1, 0, +1 and +1.41 (α= 1.4). The process optimization was carried out in 3 steps of performing statistical tests to estimate the significance level of experimental runs and predict the response of the model and its accuracy. Therefore, the impact of variables on mortality was investigated based on the following quadratic mathematical equation.

$$Y = b_0' + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{j>1}^{n} b_{ij} X_i X_j$$

Where Y is the observed response, and b_0, b_i, b_{ij}, and b_{ii} are the intercept, linear, interaction, and squared coefficient, respectively. Also, X_i and X_j are the coded values of pH and salinity factors, X is the independent variable, and n indicates the number of variables.

The RSM was performed to evaluate the significant relationship between pH and salinity as independent variables and their effect on the mortality of *A. franciscana* at a constant concentration of 0.145 µg.l^{-1} (LC_{50}). Moreover, the mortality rate was selected as the response. According to the salinity range of 10-255 g.l^{-1} and pH= 4-11, 20 experimental runs were made at a constant concentration of LC_{50} (0.145 µg.l^{-1}) as shown in table 1 [20].

Table 1. Probability value calculation

Concentration µg/l	Number of test organism	Mortality%	Lethal concentration	Obtained concentration µg/l	Confidence level	Slope±SE
control	20	0	LC_{10}	0.043	0.020-0.067	
0.05	20	20	LC_{15}	0.054	0.028-0.080	
0.125	20	40	LC_{50}	0.145	0.102-0.191	
0.25	20	60	LC_{85}	0.385	0.287-0.590	0.37±2.43
0.5	20	75	LC_{90}	0.486	0.352-0.802	
0.75	20	100	LC_{95}	0.686	0.471-1.279	
1	20	100	LC_{99}	1.307	0.794-3.137	

The primary analysis of the results revealed the insignificance impact of the variables due to the extensive range of salinity. Hence, the salinity range was limited to 10-130 g.l^{-1}. Then, the adult Artemia for the evaluation of treatments was utilized at a constant concentration of LC_{50}. The range and values of each variable in both actual and coded states are illustrated in Tables 2 and 3. Additionally, the linear, 2D, and 3D interactions of variables were evaluated. Based on the percentage of observed mortality, the fit quality of the polynomial model was defined by the R^2 coefficient, and the analysis of variance (ANOVA) test was used to determine the significant level of each variable's effect. For statistical analysis, the coefficient of determination (R^2), adjusted R^2, and prediction R^2 as well as the predicted residual error sum of the square were evaluated at 95% CI level.

Table 2. The CCD experimental design considering pH (X_1: 4-11) and salinity (X_2:10-255) impact on the mortality

Run	Coded setting levels		Actual levels of variables		Mortality %
	X_1[a]	X_2[b]	X_1[a]	X_2[b]	
1	0	-1	7.5	10	20
2	-1	1	4	250	100
3	1	0	11	130	20
4	0	1	7.5	250	100
5	0	1	7.5	250	100
6	-1	0	4	130	100
7	0	0	7.5	130	100
8	0	0	7.5	130	100
9	-1	1	4	250	100
10	0	-1	7.5	10	20
11	1	0	11	130	20
12	1	-1	11	10	100
13	1	1	11	250	100
14	0	0	7.5	130	100
15	0	0	7.5	130	100
16	1	1	11	250	100
18	-1	-1	4	10	100
18	-1	0	4	130	100
19	-1	-1	11	10	100
20	-1	-1	4	10	100

Table 3. The CCD experimental design considering pH (X_1: 4-11) and salinity (X_2:10-130) impact on the mortality

Run	Coded setting levels		Actual levels of variables		Mortality %
	X_1[a]	X_2[b]	X_1[a]	X_2[b]	
1	-1	-1	5	27	90
2	1	-1	10	27	70
3	1	1	10	113	40
4	1.4	0	11	70	40
5	0	1.4	7.5	130	50
6	0	0	7.5	70	60
7	-1	-1	5	27	90
8	1	-1	10	27	70
9	0	-1.4	7.5	10	90
10	1.4	0	11	70	40
11	0	-1.4	7.5	10	90
12	0	1.4	7.5	130	50
13	-1	1	5	113	80
14	0	0	7.5	70	60
15	1	1	10	113	40
16	-1.4	0	4	70	90
18	0	0	7.5	70	60
18	-1	1	5	113	80
19	-1.4	0	4	70	90
20	0	0	7.5	70	60

3. Results and Discussion

The mortality of *A. franciscana* when exposed to Abamectin and the LC_{50} results under the standard conditions at different concentrations are listed in Table 1. Results suggested a surge in *Artemia* losses by increasing the toxin concentration. According to probit analysis, the concentration of LC_{50} Abamectin was estimated at 0.145 $\mu g.l^{-1}$.

3.1 *The effect of pH and salinity interaction on the rate of LC_{50}*

The CCD design was used to measure the interaction effect of pH and salinity shifts on the lethal changes of Abamectin LC_{50} (0.145 $\mu g.l^{-1}$) in *A. franciscana.* According to the fitting values of both experimental categories in the residual plot, the residual variance was constant. Also, no sinusoidal changes were observed in the residual distribution diagram of the data (Figures 1 and 2). Thus, independent test data was accepted. Tables 4 and 5 demonstrate the results of the ANOVA analysis used to confirm the model's validity.

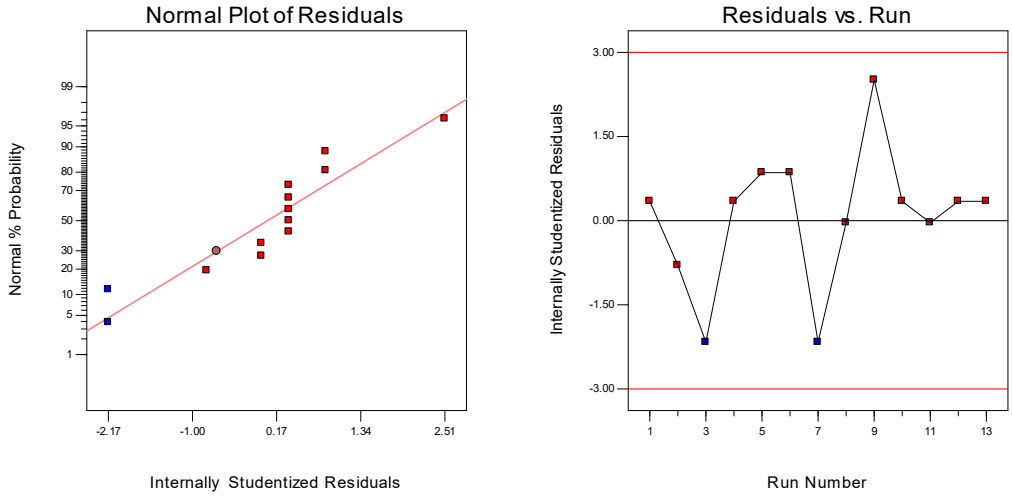

Figure 1. Normal probability plot of residuals and residuals vs. predicted response for the salinity of 10-255 $g.l^{-1}$ and pH 4-11

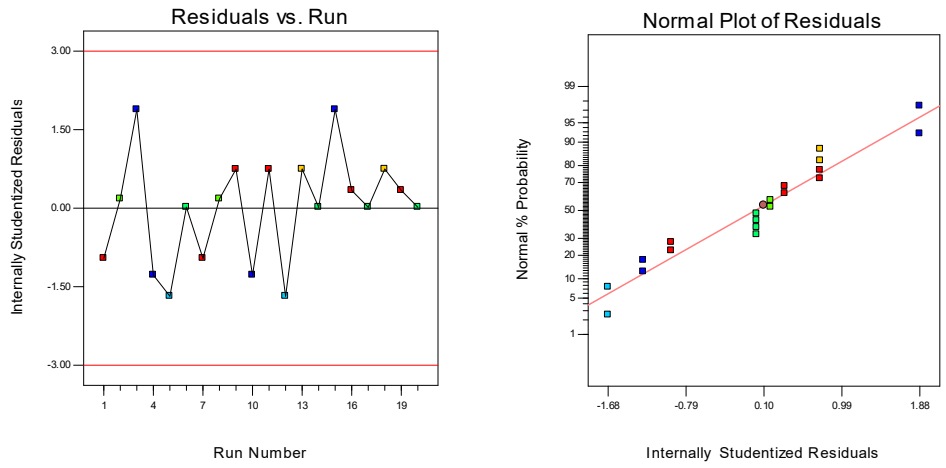

Figure 2. Normal probability plot of residuals and residuals vs. predicted response for the salinity of 10-130 $g.l^{-1}$ and pH 4-11

Table 4. Results of ANOVA for response surface quadratic model for the effect of LC_{50} in *A. franciscana* at the salinity of 10-255 $g.l^{-1}$

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob>F	Remarks
model	21.50	5	4.30	0.35	0.8692	Not significant
X1	10.67	1	10.67	0.86	0.3846	
X2	10.67	1	10.67	0.86	0.3846	
X_1X_2	0.00	1	0	0.00	1.00	
X_1^2	0.053	1	0.053	4.237E-003	0.9499	
X_2^2	0.053	1	0.053	4.237E-003	0.9499	
residual	86.80	7	12.40			
Lack of fit	86.80	3	28.93			
Pure error	0	4	0.00			
Correlation total	108.31	12				

Table 5. Results of ANOVA for response surface quadratic model for the effect of LC_{50} in *A. franciscana* at the salinity of 10-130 $g.l^{-1}$

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob>F	Remarks
model	7093.37	5	1418.67	156.85	<0.0001	Significant
X1	4267.68	1	4267.68	471.83	<0.0001	
X2	2327.27	1	2327.27	257.30	<0.0001	
X_1X_2	200.00	1	200	22.11	0.0003	
X_1^2	89.74	1	89.74	9.92	0.0071	
X_2^2	292.02	1	292.02	32.29	<0.0001	
residual	126.63	14	9.04			
Lack of fit	126.63	3	42.21			
Pure error	0	11	0.00			
Correlation total	7220.00	19				

According to the results, pH has no significant effect on the lethality of LC_{50} concentration in *A. franciscana* at a salinity range of 10-255 $g.l^{-1}$ and a pH range of 4-11. The values of R^2= 0.19, Adj R^2= -0.37, pred R^2= -0.77 as well as the values PRESS= 735.25 were computed. However, limiting the salinity range of 10-130 $g.l^{-1}$ caused significant changes in the results. The highest R^2 and the lowest coefficient of variance (CV) were obtained with an insignificant lack of fit ($p>0.05$). The values of R^2, Adj R^2, pred R^2, and PRESS were 0.98, 0.96, 0.77, and 267.91, respectively. The F-value (156.85) and *p*-value<0.05 indicated that the model was significant. The response of pH and salinity changes to the mortality of *A. franciscana*, when exposed to the Abamectin, was investigated using the empirical second-order polynomial model. Hence, the final equation in the actual factors was derived from the equations 1 and 2. In these equations, a positive sign indicated the synergistic effects while a negative sign indicated the diminishing effects of independent factors.

Equation 1(salinity 10-255 $g.l^{-1}$ in pH 4-11):

$$\text{Mortality} = +9.51 - 0.212*\text{pH} + 0.0136*\text{salinity} + 0.0000*\text{pH}*\text{salinity} - 0.01126*\text{pH}^2 - 9.578*\text{salinity}^2$$

Equation 2 (salinity 10-130 $g.l^{-1}$ in pH 4-11):

$$\text{Mortality} = +148.32 - 10.91*\text{pH} - 0.369*\text{salinity} - 0.0466*\text{pH}*\text{salinity} + 0.507*\text{pH}^2 + 3.1167*\text{salinity}^2$$

Figure 3 shows a contour plot and a 3D plot in the salinity range of 10-255 g.l^{-1}, where salinity alone can perform a significant effect on mortality changes. This could be due to the wide range of salinity chosen in this part of the experiment, which has a significant effect on mortality, irrespective of the pH effect on severe mortality, especially in the presence of Abamectin. However, in the second category of the experiment, the mortality rate increased with a pH reduction from 7.5, in the salinity of less than 30 g.l^{-1} in comparison with LC_{50}. Then, with the salinity increasing from 30 g.l^{-1} to 100 g.l^{-1} in the pH range of 7.5 to 10, the mortality rate dropped to the LC_{50} level. However, at higher levels, the mortality rate spiked significantly (Figure 4). According to the actual data and model responses, the optimum condition was obtained at a pH of 8.3 and salinity of 79 g.l^{-1}, resulting in a mortality of 52.57%.

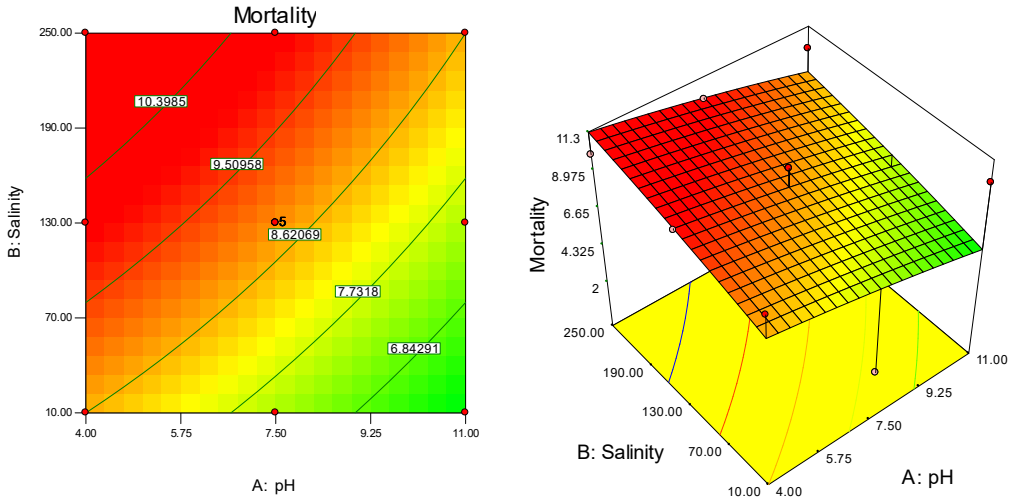

Figure 3. The effect of pH (4-11) and salinity alteration (10-255 g.l^{-1}) on the mortality

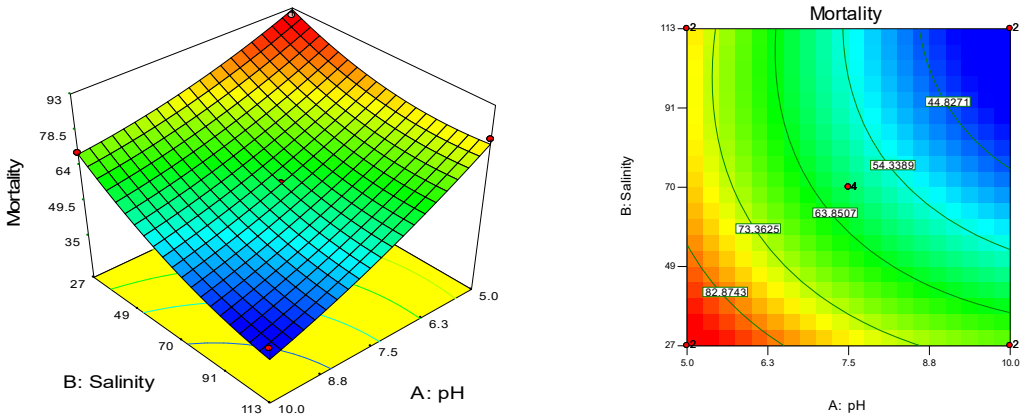

Figure 4. The effect of pH (4-11) and salinity alteration (10-130 g.l^{-1}) on the mortality

The toxicity experiment in aquatic organisms can be utilized to estimate the potentially harmful effects of contaminants in the aquatic environment [14]. Pesticides generate complicated conditions in aquatic ecosystems in connection with non-target organisms [21]. Accordingly, due to the widespread application of Abamectin as a pesticide and its destructive impacts on aquatic organisms, the LC_{50} level of this pesticide was investigated in *A. franciscana* as a model. Also, since the effect of operational variables on the toxicity of this pesticide on *Artemia*

sp. has not been investigated yet, the present study was conducted to shed light on the function and sensitivity of *A. franciscana*. The results of the toxicity test of Abamectin on *A. franciscana* indicated that the increment of pesticide level raised mortality. The LC_{50} level of Abamectin was estimated at 0.145 $\mu g.l^{-1}$. The results demonstrated that *A. franciscana* is highly sensitive to Abamectin and the toxicity of this pesticide is considerably different from the other studied substances. Thus, Abamectin with an estimated LC_{50} of 0.145 $\mu g.l^{-1}$ must be classified as toxic for aquatic organisms. In the study of Varo et al. [22], the LC_{50} levels of dichlorvos and chlorpyrifos organophosphate toxins in *A. salina* were 9.3 and 3.19 $mg.l^{-1}$, respectively. Rahnama et al. [23] also calculated LC_{50} levels of paraquat toxins (2.701 $mg.l^{-1}$), dichlorophenoxy-acetic acid (14.475 $mg.l^{-1}$), trifluralin (0.446 $mg.l^{-1}$), and glyphosate (17.431 $mg.l^{-1}$) in *A. franciscana*. Moreover, in the study of Shaala et al. [24], the LC_{50} level of Diuron in Napoli *A. salina* was estimated as 23.27 $mg.l^{-1}$ after 24 h. The results of the recent study were also comparable in terms of the toxicity of heavy metals. According to Damasceno et al. [25], the LC_{50} of zinc and nickel in *Artemia* sp. was 224 and 534 $mg.l^{-1}$, respectively. Thus, the high sensitivity of *Artemia* to Abamectin in the present study, despite its resistance, could explain the grave risk of this contaminant entering other aquatic organisms. Some physicochemical factors, such as salinity, pH, hardness, and temperature, can directly affect the toxicity potential of contaminants by influencing degradation, bioavailability, and mobilization processes [16, 26]. Therefore, in addition to measuring the LC_{50} of Abamectin, the effect of salinity and pH on LC_{50} was considered. The results elucidated an increase in mortality in response to the salinity concentrations of less than 30 $g.l^{-1}$ and more than 100 $g.l^{-1}$. Similarly, other studies have also depicted *Artemia* sensitivity to low salinity in the presence of nickel and zinc enhancement, which could be related to the saline ion's impact on metal bioavailability [25]. Furthermore, the reduction of ions, especially chloride, not only decreases the interaction between water ions and metals but also magnifies the effect of free metal ions. The osmoregulation process is one of the main physiological factors affecting the behavior of toxins. Although the isosmotic point in *Artemia* is 9.5 $g.l^{-1}$, an increase in mortality at less than 30 $g.l^{-1}$ could be attributed to the effect of the toxin on the osmoregulation function in *A. franciscana*. Among different types of organisms, *Artemia* sp. is able to tolerate severe environmental conditions and maintains its balance in a broad range of salinities through specialized cells (sodium-potassium pumps from isoenzymatic hemolymph) that discard the excess salt from the body [27]. These crustaceans can tolerate and survive in the moderate salinity of 60 to 100 $g.l^{-1}$ [28]. In addition, they regulate osmotic conditions by means of a hyper-hypoosmotic mechanism when the salinity is changed. This process is carried out through protein consumption by the enzyme Na^+/K^+ ATPase, which triggers the secretion of Na^+ and Cl^- from the plasma membrane during the osmoregulation process. For higher concentrations of ambient salt, these cells need more activities and energy to stabilize the osmotic balance. As a result, their durability and survival are debilitated due to higher energy consumption [29]. *Artemia* can also maintain osmosis in the presence of hypo-hyper-osmotic conditions by altering intracellular compounds. Under hypo-osmotic conditions, this crustacean moderates the condition by reducing intracellular ions, amino acids, and protein catabolism while augmenting the oxidation of amino acids and their secretion into the cytosol [30]. It is worth noting that the body can maintain its osmotic balance by increasing the concentration of intracellular substances in hyperosmotic conditions. This process is carried out by upgrading the protein catabolism and amino acids, which release leucine and glutamic acid into the cell, and hydrolyzing cellular proteins to increase amino acids [31]. Therefore, although *Artemia* sp. is capable of implementing the process of osmotic homeostasis at low and high salinities. The excessive energy consumption in different salinities to maintain the osmotic condition of the body as well as the use of intracellular

compounds, may have an adverse effect on other existing biological mechanisms [32]. Hence, severe changes in salinity not only impose direct stress on the organism but also exacerbate the effect of the toxin on the organism. The results of the first phase of the experiment (in the range of 10 to 255 $g.l^{-1}$ salinity) in the present study suggest that the increased *Artemia* mortality in the LC_{50} can be due to the intensity of stress-induced in the osmoregulation process. However, this trend took a downturn in the second range of salinity (i.e., 10 to 130 $g.l^{-1}$). In this regard, Damasceno et al. [25] reported the combined effect of nickel and zinc toxicity on *Artemia* sp. at low salinity. They linked toxicity surge to variations in normal physiological characteristics. Considering the intermittent pH alteration in aquatic ecosystems, its impact on toxicity was also considered [33]. pH can affect the hydrolysis of toxins and subsequently the extent of toxic which may be received by organisms. Addressing literature, water pH has an adverse effect on toxin stability. It means that the hydrolysis of toxin enhances the acidic pH and in this way, reduces its effectiveness. Several studies have also reported a reduction in the toxicity of various pesticides with the pH decline [16, 34].

In addition, as reported in some studies, the effect of pH limit on organisms is greater than the effect of the toxin. Berge et al., [35] suggested that the acidification of the ocean may reduce phytoplankton growth rates due to direct pH effects. Moreover, a severe pH reduction in aquatic ecosystems not only decreases and disrupts reproductive efficiency, proper respiration, locomotor function, and nutrition but also deteriorates vital cellular mechanisms [36]. Therefore, toxicity enhancement in the present study induced by pH reduction from 7.5 could be due to the direct effect of pH (in acidic conditions) on the cellular structure of the gill membrane, which allows more toxin absorption. pH directly affects the tolerance of living organisms by creating stress that intensifies the effect of toxins. Zhang et al. [37] studied the effect of pH on the toxicity of polystyrene nanoplastic on *Daphnia magna* and they found the same results. At acidic pH, the presence of NH_4^+ molecules significantly contributes to toxicity by transferring Na^+ ions in the osmoregulation process, which has a negative impact on survival. Tellez et al. [38] also reported that pH changes could negatively affect aquatic organisms, especially first-level organisms, due to the solubility, bioavailability and mobility of heavy metals. Khatikarn et al. [39] and Fu and Bae [40] have reported that low pH levels increased the toxicity of TGS in *Aliivibrio fischeri* and *Dario rerio* (Zebrafish) embryos because of the enhancement of ionized TGS proportion at low pH conditions.

Another possible factor associated with the ineffectiveness of low pH in reducing mortality is the Abamectin chemical properties. According to Awasthi et al. [41], Abamectin is resistant to pH changes and remains stable even at a pH of about 4. Therefore, the toxin stability against pH reduction can also be a crucial factor in augmenting the effect of the toxin on lowering pH and the simultaneous impact of the salinity factor. Several studies evaluated the effect of each factor on toxicity. However, the impact of several stressors, such as the simultaneous effect of salinity and pH on toxin stress, has rarely been investigated. This could provide more accurate results about the interaction of different factors. Chen et al. [34] found that the highest effect of food deficiency in the presence of toxins was observed at pH<6. They suggested that in order to gather more information on each factor, studies should explore a combination of factors simultaneously. Soares et al. [16] in their study about the effect of pH and nitrite on the rate of LC_{50} changes in *Macrobranchium pantanalense* and *M. amazonicum* demonstrated that pH had an opposite effect on cypermethrin toxicity in two shrimp species. They attributed this difference to the disparity of two shrimp species in terms of susceptibility to pollutants.

4. Conclusions

In the present study, the best toxin tolerance range in *Artemia* was LC_{50} 0.145 µg.l^{-1} which was obtained at the salinity of 35 to 100 g.l^{-1} and pH range of 7.5 to 10. Also, the optimum condition was obtained at pH of 8.3 and salinity of 79, resulting in mortality of 52.57%. The results showed that the Abamectin toxin decreases the durability of *A. franciscana* by intensifying the stress caused through osmoregulation, despite the survival and durability of *A. franciscana* in various salinity ranges from 9 to 350 g.l^{-1}. Therefore, the alteration of effective parameters in toxicity research can reflect the real impact of the toxin. This study also revealed that employing several factors and exploring the impact of stress on toxicity will enhance the adaptability of results to real-life conditions. According to the obtained results in the present investigation and the high sensitivity of *A. franciscana* to this pesticide, it can be concluded that Abamectin has a higher toxicity than others. Hence, the entry of this toxin into the ecosystem underlines the need for meticulous management and appropriate strategies to reduce its consumption.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding this article.

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