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Toxicity Test on Different Brands of Food Seasonings Using Brine Shrimp (*Artemia salina*) Lethality Test

Kenneth Dave B. Borja, Vhebs Terez L. Buhion and Everlita E. Canalita
Integrated Developmental School – College of Education, Mindanao State University-Iligan Institute of Technology, Andres Bonifacio Avenue,Tibanga,9200, Iligan City, Philippines.

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Abstract:

Toxicity testing is performed to assess the safety or hazards presented by substances such as consumer products, pharmaceuticals, natural products and industrial chemicals. Many of the current toxicity test methods include the use of laboratory animals such as mice, rats or rabbits, however in this study lower species of test organisms were used – the *Artemia salina*, which are commonly called brine shrimp.

This study was conducted to test for the toxicity potential of three different brands of commercially sold food seasonings using brine shrimp lethality test. This is an assay that will determine the toxicity potential of a substance using the mortality rate of brine shrimp nauplii and identify the median lethal concentration (LC_{50}) using regression line analysis .Ten (10) brine shrimp nauplii were exposed to the three different brands of commercially sold food seasonings in the Philippines with three different concentrations (10, 100, and 1000 ppm) of the test solutions. Each concentration was replicated thrice. The mortality rate of brine shrimps exposed to each brand was determined and recorded within a 24-hour period.

Statistical analysis using two-way ANOVA showed that there is no significant interaction between the brands of artificial food seasonings and their respective concentrations. It showed further that there is no significant difference in the mortality rate of brine shrimps among the three different brands of food seasonings; however, significant difference existed among the three concentrations. The highest mortality rate was observed at 1000 ppm while the lowest mortality rate was observed at 10 ppm for all brands. As the concentration increases, mortality rate of brine shrimp increases. In addition, regression line analysis showed that Brand A, Brand B, and Brand C have an LC_{50} of 535 ppm, 542 ppm, and 438 ppm, respectively. This implies that the substance has an ingredient that could affect the activity of brine shrimp cells.

Key words: Toxicity, Median Lethal Concentration (LC_{50}), Brine Shrimp Lethality Test (BSLT)

1. Introduction

Food seasonings whether natural or artificial are very in-demand and instantly accepted due to its accessibility and flavoring effects. It adds and enhances the flavor to ordinary dishes making the food taste more appealing. Commercially sold food seasonings occur in different varieties. Despite their differences, they have one thing in common; all of these products are made in the laboratory by trained professionals, a "*flavorist"* who blends appropriate chemicals together in the right proportion [11].

Although the Food and Drug Administration (FDA) has classified food seasonings as a food ingredients that are "generally recognized as safe," however many rumors about its harmful effects, remained controversial. For this reason, the researchers had come to an idea of investigating the toxicity potential of artificial food seasonings using brine shrimp lethality test (BSLT). This brine shrimp assay was proposed by Michael et al [3], and latter developed by Vanhaecke et al. [4], Sleet and Brendel [5]. The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extracts, heavy metals, pesticides and toxicity testing of substances [6-10]. The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs makes Brine Shrimp Lethality Test (BSLT) a very useful bench top method [7] .

Brine shrimps, *Artemia* are small marine crustaceans that can live in a wide range of salinities (10ppt to 100ppt) and have a long life span in the wild (exceeding to six months). These organisms are found favor as a model organism use in toxicological assays, despite the recognition that it is too robust an organism to be a sensitive indicator species. In pollution research *Artemia*, the brine shrimp, has had extensive use as a test organism and in some circumstances is an acceptable alternative to the toxicity testing of mammals in the laboratory [1]. The fact that millions of brine shrimp are so easily reared has been an important help in assessing the effects of a large number of environmental pollutants on the shrimps under well controlled experimental conditions.

It has been said that using too much artificial food seasonings can harm people's health and even trigger cancer and hair fall incidences. This study could provide baseline information regarding the toxicity effects of commonly used food seasonings in the Philippines.

2. Materials and Methods

The toxicity of the food seasonings was evaluated using the tiny crustacean, brine shrimp (*Artemia salina*) nauplii as test organisms. The test consisted of three major steps: hatching of shrimps, preparation of test solution, and Brine Shrimp Lethality Test (BSLT).

A. Brine Shrimp Hatching

The brine shrimp eggs were obtained at the Center for Aquaculture Research, Enterprise and Services of MSU Naawan, Misamis Oriental. Seawater was used as the hatching medium in this experiment. The seawater used in this experiment was taken at least a hundred meters away from the seashore. It was then sterilized and filtered to make sure that it's free from solid materials that may be mistaken as eggs of brine shrimps during the experiment. A pinch of these brine shrimp eggs were placed in the hatching aquarium. The researchers waited for 24 hours for the nauplii to be harvested.

B. Preparation of the Test Solution

Test solutions using the three different brands of food seasonings were prepared using serial dilution method. A 10,000 ppm stock solution was first prepared by dissolving 20mg of each food seasoning in 2 mL distilled water. Using a pipette, test solutions were transferred to 10mL test tubes corresponding to 1 000, 100, and 10 ppm, respectively. Three replicates were prepared for each dose. The standard procedure of serial dilution was shown below:

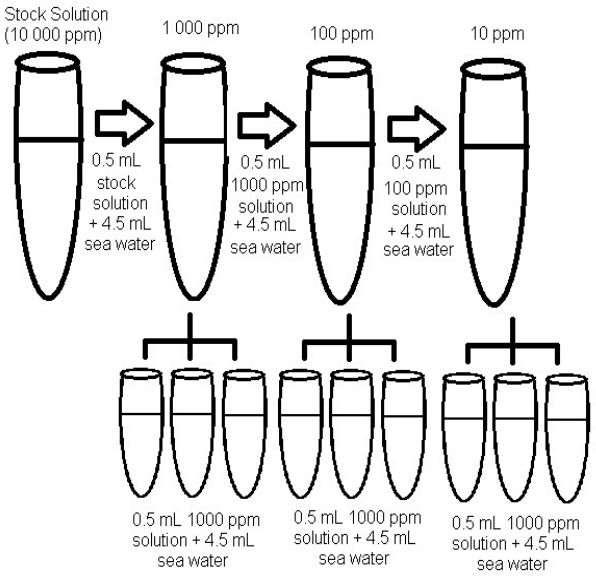

Figure 1. Serial Dilution Method

C. Brine Shrimp Lethality Test (BSLT)

Brine shrimp lethality bioassay was carried out to investigate the toxicity effect of the three different brands of food seasonings in the Philippines. Brine shrimps were hatched using brine shrimp eggs in a small glass aquarium filled with seawater (pH=8.5) under constant aeration for 48 hours. After hatching, active nauplii were collected from the brighter portion of the hatching chamber and use for the assay. Ten active

nauplii were drawn using a glass pipette and place in each test tube containing the different concentrations of test solutions. Experiments were conducted along with the negative control set up (using seawater only).

Ten active brine shrimp nauplii were placed on each test tube using Pasteur's pipette. The number of dead brine shrimps was then recorded 24 hours after subjecting each brine shrimp in each of the solutions.

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values were obtained from the best-fit line plotted concentration versus percentage lethality using the open stat software. Furthermore, two-way ANOVA were also used in determining the significant difference among the lethality levels of the three different brands of food seasonings and among concentrations.

3. Results and Discussion

The brine shrimp nauplii were exposed to three different brands of food seasonings with three different concentrations. Mortality rates of brine shrimps were determined and recorded within 24 hours of exposure. Sterilized sea water was used as a negative control in this experimentation.

Table 1. Mortality Rate of Brine Shrimps Subjected to the three Different Brands and Concentrations of Food Seasonings

Concentrations (ppm)	% Mortality		
	Brand A	Brand B	Brand C
1 000	76.67%	83.33%	100%
100	53.33%	33.33%	36.67%
10	10%	0%	6.67%
Negative Control	0%	0%	0%

Table 1 showed that the degree of lethality was found directly proportional to the concentration of food seasonings in all brands. Maximum mortalities of brine shrimps were manifested in 1000 ppm whereas least mortalities were at 10 ppm concentration in all brands of food seasonings.

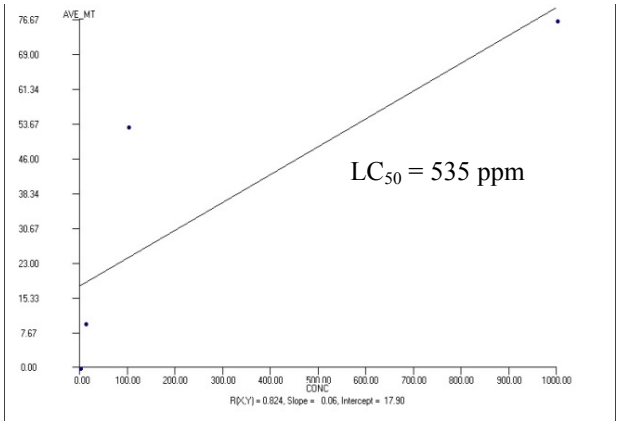

Figure 2. Best Fit Line for Brand A

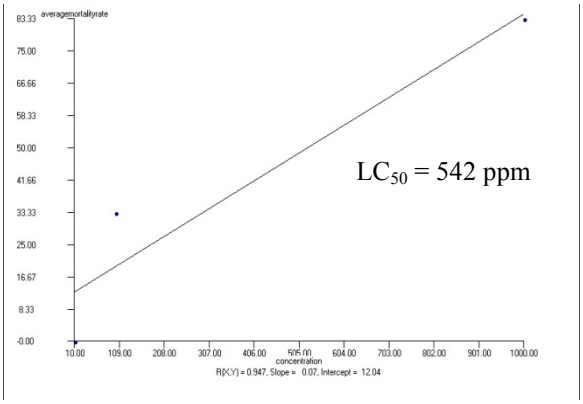

Figure 3. Best Fit Line for Brand B

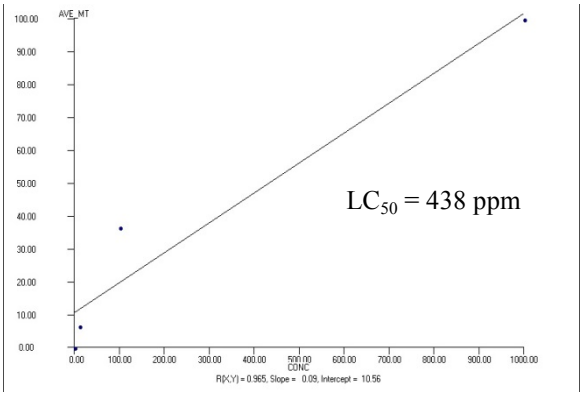

Figure 4. Best Fit Line for Brand C

LC_{50} values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii mortalities against the concentrations of the food seasonings and the best fit line was obtained by means of regression analysis. As shown in the figure 2, 3 and 4, there was a strong, positive linear relationship between mortalities of brine shrimps and concentrations of food seasonings. The calculated coefficient of determination of brands A, B, and C were 67.9%, 89.7 % and 93.2 %, respectively. These coefficients can explain that the total variations of the mortality were due to the variations of concentrations, an indicative of the presence potent toxic components that warrants further investigation.

Table 2. Two-way ANOVA of the Average Mortality Rates

Source	*SS*	*df*	*MS*	*F*	*p-value*
Brand	7.39	2	3.69	0.7	0.5
Concentration	437	3	146	27.7	5.72×10^{-8}
Interaction	14.4	6	2.4	0.46	0.83
Error	126	24	5.25		
Total	584	35			

The results of the two-way ANOVA showed that there was no significant difference in the average mortality among the three brands of food seasonings (at $\alpha = 0.05$ level of significance). However, significant difference existed in the average mortality of brine shrimp at different concentrations. Hence, the result suggested that mortality of brine shrimps were greatly affected by increasing concentration of food seasonings regardless of the brand.

4. Conclusion

Based on the results, data showed that Brand C affects lethality of brine shrimps the most, with an LC_{50} of 438 ppm concentraton. It was then followed by brand A and B that killed 50% of the brine shrimp at 535 ppm and 542 ppm, respectively. Furthermore, the brine shrimp lethality exposed to the three different brands of food seasonings were found to be concentration-dependent regardless of the brand of food seasoning. From the results obtained using two-way ANOVA, it can be concluded that there is no significant interaction between the brand and concentration of food seasonings. Hence, the two factors were analyzed independently. It showed no significant difference in the mortality rates of brine shrimps among the three brands of food seasonings, however significant difference in the mortality rates existed among the three different concentrations.

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