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ENRICHMENT OF *Artemia franciscana* AND TOXICITY ANALYSIS OF CHLORPYRIFOS ON ENRICHED ARTEMIA

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AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The present study highlights the importance of Artemia as a common fish feed worldwide The Artemia cyst were collected, decapsulated, incubated for harvesting, and washed and separated. The Artemia was fed with two different formulations of feed and the growth of Artemia were observed. The feed supplemented with groundnut oil cake and wheat flour established a faster growth rate than the feed supplemented with spirulina powder and rice bran. Aminoacid analysis is carried out by HPLC in following two types of Artemia samples. The cytotoxicity test was performed using chlorpyrifos pesticide. Enriched Artemia is tested for chlorpyrifos toxicity by giving different concentrations (25, 50,100, 200, 300) in µl for 5 hours. LC50 value of the chlorpyrifos from the given concentration on enriched Artemia is = 187.6 µg/ml. Adult enriched Artemia cannot tolerate the toxicity for even a few hours. The observations proved that adult enriched Artemia was easily susceptible to various toxic agents such as pesticides, parasites, and other microbes. Enriched Artemia nauplii are very much less sensitive to the pesticide when compared with adult Artemia. It can tolerate the toxic over the adult Artemia. As a result, enriched nauplii are the best food source for freshwater and marine fishes and other crustaceans.

Keywords: Artemia; livefeed; enrichment; crustacean and toxicity.

1. INTRODUCTION

In the presence of live feed for fish larviculture, Brine shrimp (Artemia) is the most commonly used live feed worldwide Artemia belongs under the phylum Arthropoda. The tiny crustacean survives only in the natural salt lake areas [1]. They consist of both zygogenetic and parthenogenetic characteristics of reproduction. While culturing Brine shrimp, the temperature (30ºC), Ph(8-10), Salinity(28-30PPT) should be in the optimum level. For hatching nauplii from the cyst, vigorous aeration and illumination (light) are needed at the beginning. Later on, sufficient aeration is enough for them to survive.

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The main focus of giving nutrition to Artemia is the fatty acids and proteins. Proteins are an essential nutrition component for aquatic animals, especially brine shrimp. Lipids play a vital role in energy production and the fatty acids required for the development and growth of brine shrimps [2]. Some foods for Artemia that are commercially available include yeast, soya powder, spirulina, wheat flour, etc. These food types also supply nutrition and promote the growth of Artemia. Enriched Artemia has a high energy content, and also it has all essential fatty acids and amino acids. If the larvae take enriched Artemia as a food, it will be reached faster growth and get more nutrition. Artemia is the most successful live feed for fishes and larva, containing a high amount of protein and amino acids [3]. The amino acid content of enriched Artemia is detectable by HPLC (High-Performance Liquid Chromatography).

Chlorpyrifos is one of the insecticides (pesticides), which is an organophosphate (OP). It is highly toxic to aquatic animals and some animals like rats, insects, etc. [4]. The mode of action of chlorpyrifos in brine shrimp is the blockage of acetylcholine (Ach). Effect of chlorpyrifos can change the normal function of acetylcholine. Chlorpyrifos is non-toxic if it does not bind with enzymes. The toxic form is created after the body tries to destroy the chemical. The toxic form is called "Chlorpyrifos oxon" is persistently binds to the enzyzmes. After binding with the enzymes, the nerves, muscles and enzymes fail to function normally. An acute toxicity test is used to identify the toxicity effect of chlorpyrifos on Artemia [5].

The toxicity results are shown statistically by the probit analysis. Probit Analysis is a regression type that is used to detect responsible variables. The method of regression is described as fitting a line to the data. The relationship of the response is compared variable or dependent variable (Y) to the independent variable (X) [6].

In toxicology, probit analysis is a commonly used method to analyze the toxicity of chemicals to living organisms. It is established by testing the response of living animals under different concentrations. The LC50/LD50 is accomplished through the concentration (LC50) or dose (LD50) at which 50% of the population responds.

2. MATERIALS AND METHODS

2.1 Artemia Culture

Artemia cysts was hatched out under the optimum level of Ph, temperature, and salinity. Before hatching, Decapsulation process was carried out to prevent bacterial contamination and promoted high hatching rate [7].

After hatching, total Artemia was separated from decapsulation vessel.

2.2 Artemia Enrichment

Artemia was enriched by fed with two different commercial food combination. Combine feeding technique for Artemia, obtained high nutrition [8].

Artemia nauplii was introduced into both A and B cultures. Groundnut oil cake and wheat flour were selected for sample A, and spirulina powder and rice bran were selected for sample B.

Ongrowing Artemia was measured in the course of feeding till they reaches the adult. The measurement was analysed and compared one sample with another sample.

2.3 Amino Acid Analysis

The amino acid profile of enriched Artemia was identified and analysed the amino acid content by using HPLC [9].

2.4 Toxicity Test of Chlorpyrifos

The toxicity of chlorpyrifos was identified in the enriched Artemia. The toxicity test was accomplished by giving a different concentration of chemical on enriched Artemia and the LC50 value was calculated. [10]

3. RESULT AND DISCUSSION

3.1 Decapsulation

Fig. 1. The decapsulation of the Artemia cyst. The shells of the Artemia cyst was chemically removed by addition of NaOCl and NaOH. The colour of the cyst turned from brown to orange. The decapsulated cyst yielded the high hatching rate.

Table 1. Growth comparision

DAYS	SAMPLE A	SAMPLE B
7	500µm	450µm
12	5mm	2.5mm
15	7mm	4.5mm
20	10.5mm	7mm

3.2 Measurement

While Artemia enrichment, Artemia was observed under the microscope and measurement was taken in two culture samples. In case of the small tiny size of Artemia, it was measured by using oculometer.

Artemia was enriched and fed with two different combination of food. Sample A containing Artemia fed with combination of Wheatflour and Groundnutoil cake which attained higher growth rate than the Sample B.

3.3 Aminoacid Analysis

In both samples, four amino acids have been identified. From sample A (**Isoleucine, Proline, Threonine and Glutamine**) has indentified where from Sample B as same as sample A but Glutamine was replaced by Methionine. In these detection of amino acids Sample A has higher percentage of amino acids than the sample B.

3.4 Cytotoxicity Test

The five different concentration of chlorpyrifos were given to Enriched Artemia. The number of alive artemia and dead Artemia values were taken for mortality rate calculations.

The mortality rate was calculated and compared between Adult enriched Artemia and Enriched Nauplii. From the result Enriched nauplii is resistant to toxicity compared with Enriched Adult Artemia.

Fig 1. Decapsulation

Fig. 2. Decapsulated egg (40x magnification)

SAMPLE A

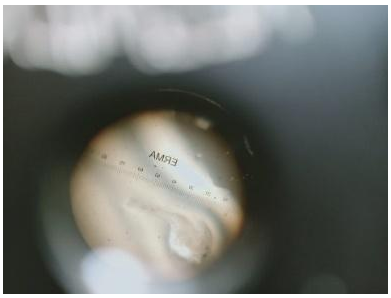


Fig. 3. 7th day (500µm)

SAMPLE B

Fig. 4. 7th day (450µm)

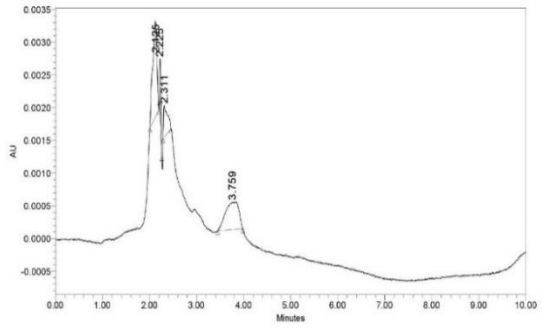
SAMPLE A

Fig 5. 20th day (40x magnification) (10.5mm)

SAMPLE B

Fig 6. 20th day (40x magnification) (7mm)

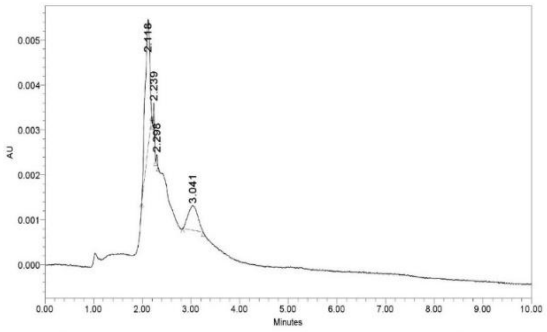
SAMPLE A



	Amino acids	RT	Area	% Area	Height
1	ISOLEUCINE	2.125	9187	42.11	1465
2	PROLINE	2.225	1612	7.39	912
3	THREONINE	2.311	2772	12.71	486
4	GLUTAMINE	3.759	8246	37.79	419

Fig. 7

SAMPLE B



	Amino acids	RT	Area	% Area	Height
1	ISOLEUCINE	2.118	19233	68.66	2859
2	PROLINE	2.239	930	3.32	625
3	THREONINE	2.298	339	1.21	200
4	METHIONINE	3.041	7511	26.81	548

Fig. 8

Table 3. LC50 value of chlorpyrifos

Conc	Log C	No.of Artemia taken	No.of dead Artemia	No.of alive Artemia	% of the Mortality	LC 50 µg/ml
20	1.60206	8	0	8	0	
50	1.712667	8	2	6	25	
100	2.014162	8	3	5	37.5	
200	2.31245	8	4	4	50	187.6
300	2.488045	8	6	2	75	

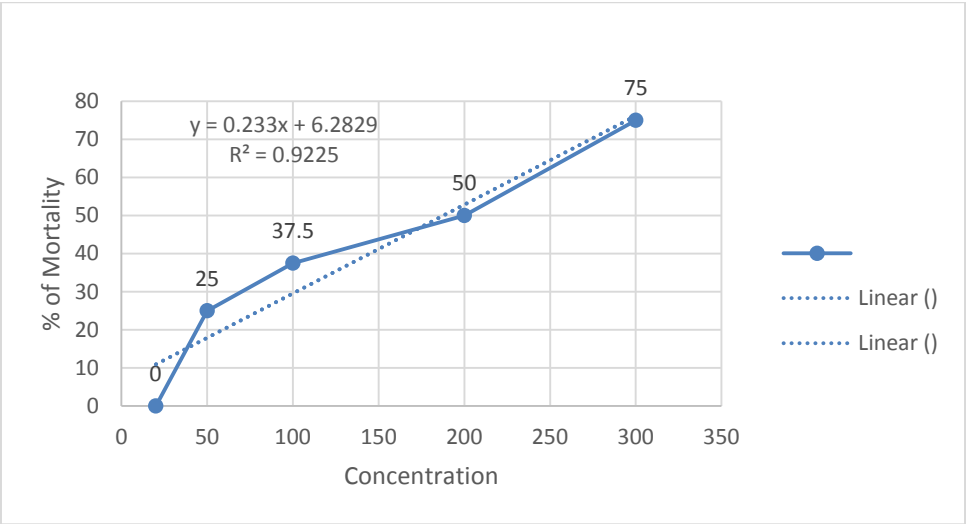


Fig. 9. The mortality rate and the LC value was calculated by the regression analysis. It was expressed in graphical interpretation. The LC50 value of chlorpyrifos on enriched Artemia was 187 µg/ml

The mortality rate of adult and nauplii enriched Artemia was showed in graphical presentation. The mortality rate of Enriched adult artemia was increased more than Enriched nauplii.

In an enrichment process, sample A containing Artemia established a faster growth than sample B. Four important amino acids were identified in both samples. Amino acids in sample A has a higher value than sample B. So the feed combination given to

Table 4. Mortality rate comparison between Adult and Nauplii *Artemia*

CONC (PPM)	Total no. of Artemia taken	No.of. Adult Artemia taken	No. of adult Artemia alive	No. of Nauplii taken	No. of Nauplii alive	Mortality rate of Adult Artemia	Mortality rate of nauplii
20	8	4	4	4	4	0	0
50	8	4	2	4	4	50	0
100	8	4	2	4	3	50	25
200	8	4	1	4	2	75	50
300	8	4	0	4	2	100	50

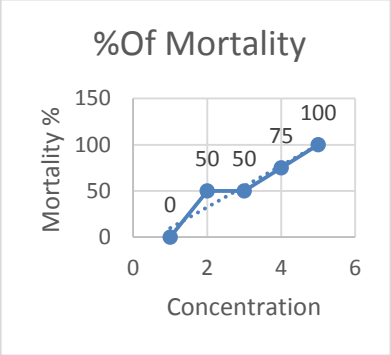


Fig. 10. Mortality rate of Adult *Artemia*

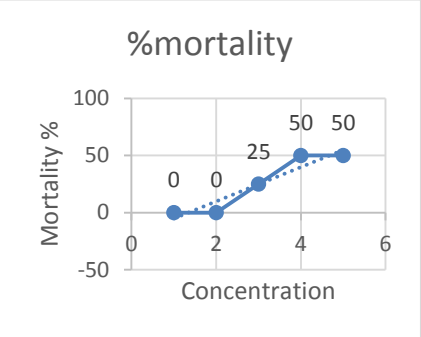


Fig. 11. Mortality rate of Nauplii

sample A is denoted as the best nutritive food for Artemia.

The toxicity test of chlorpyrifos was accomplished on enriched Artemia by giving different concentrations. After the exposure of chlorpyrifos on enriched adults and nauplii, it was blocked the neurotransmitter (acetylcholine) and affected the nervous system. The acetylcholine blockage of enriched Artemia nauplii is lesser than the enriched Adult Artemia. LC50 value of the chlorpyrifos from the given concentration on enriched Artemia is 187.6 µg/ml**.**

According to the mortality rate of enriched adult Artemia and nauplii, enriched adult Artemia cannot tolerate the toxicity for even a few hours. As verified, enriched adult Artemia is easily affected to toxic by either bacteria or other parasites. Enriched nauplii are less sensitive to the toxic in contrast to enriched adult Artemia, and they can tolerate the toxic over the enriched adult Artemia. As a result, enriched Artemia nauplii is the best source of live feed for ornamental fishes and other crustaceans.

4. CONCLUSION

In course of Artemia culture the main source of feed was normally given to Artemia is microalgae and some algae. Algae culture consumes more amount time so the feeding process may delay. Rather than algae , commercial food like wheat flour, groundnut oilcake, Spirulina powder, Rice bran are readily available and promotes high nutrition. The combination of wheat flour and growndnut oilcake established highgrowth of Artemia.

After enrichment, the toxicity of chlorpyrifos was analysed to both enriched adult Artemia and Artemia nauplii. After findings, enriched Artemia nauplii was less sensitive to toxic when compared with Adult Artemia. So I suggest, enriched nauplii is the best feed source for ornamental and crustaceans rather than enriched adult Artemia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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