

Effects of Commercial Herbal Extracts Enriched *Artemia nauplii* on Enzymatic and Nonenzymatic Antioxidants Activities of *Oreochromis mossambicus*



Zoology

KEYWORDS : *Oreochromis mossambicus*, *Artemia salina*, Herbs, Enzymes, Antioxidants

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ABSTRACT

In the present study the enzymatic and nonenzymatic activities of *Oreochromis mossambicus* larvae fed with commercial herbal enriched *Artemia nauplii* was analysed. Unenriched *Artemia* (Control), *Coriandrum sativum* (Experiment-I), *Centella asiatica* (II), *Triconella foenum* (III), and *Amaranthus polygonoides* (IV) enriched with *Artemia salina* for 50 days. The effect of herbs on the level of antioxidant (The Phenol Tannins Ascorbic acid, α -Tocopherol, Ferric Free Antioxidant, Invitro Lipid Peroxidation and Measurement of Thiobarbituric Acid Reactive Substances) and enzyme activity (Alkaline Phosphatase (ALP) Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT), were analyzed after the experimental period. It was statistically significant for both control and Experimental groups at ($p < 0.05$) level.

Introduction

Aquaculture is currently the fastest growing segment of food production in the world and by 2002 was contributing approximately 30% by weight of fish and shellfish consumed worldwide (FAO, 2004). Tilapia is the third most cultured fish, after carp and salmonids. In the last decade, the production of farmed tilapia has shown a tremendous increase jumping from 383,654 metric tons in 1990 to 1,505,804 metric tons in 2002.

Artificial feeds are developed either to supplement or to replace natural foods in the culture systems. The extensive aquaculture system mainly depends on natural foods. Food, especially the first formulated food of larvae is vital for achieving good survival rates (Gosh et al., 2007). Dey and Chandra, (1995) produced disease resistance fry of *Catla catla* through immersion herbal treatment (Neem, Garlic and Turmeric). Vitamin C as an important water soluble antioxidant in biological fluids and as an essential micro nutrient for normal metabolic functions (Jaffe 1984).

Tannins are able to react with proteins, digestive enzymes, polysaccharides and other molecules, reducing digestibility of the amino acids of the diet. Condensed tannins, it has been reported as responsible for the inhibition of the enzymatic activity of the cellulose, peptidase, amylase, lipase, proteolytic enzymes and the alpha-galactosidase (Chung et al., 1998).

Lipid peroxidation is the oxidative deterioration of poly unsaturated fatty acids to form free radicals intermediates and peroxides which damage cellular constituents (Wanger et al., 1994). Non-enzymatic antioxidant also acts as a free radical scavenger such as Glutathione, Vitamin A, Vitamin C and Vitamin E and other compounds like phenol and caffeic acids (Van et al., 1993). The aim of the present study was to determine the liver marker enzymes and antioxidants activities in *Artemia salina* enriched with *Centella asiatica*, *Triconella foenum*, *Coriandrum sativum*, *Amaranthus polygonoides* and unenriched *Artemia salina* (Control) fed to *O. mossambicus* for the experimental period of 50 days.

Materials and Methods

Experimental Animal

Oreochromis mossambicus fries were collected from Saravana fish farm in Kaveripatti, Namakkal District, Tamil Nadu, India. After acclimatization of 10 days, fifty seedlings of *O. mossambicus* were maintained in Plastic containers.

Herbal Enrichments

The experimental period was restricted to 50 days. Experimental fish fries were fed with enriched *Artemia Nauplii* and Control with Unenriched *Artemia Nauplii*, *Coriandrum sativum* (I), *Centella asiatica* (II), *Triconella foenum* (III) and *Amaranthus polygonoides* (IV) enriched *Artemia Nauplii*.

Antioxidant Studies

The Phenol content were estimated using the method of Malik and Singh, 1980, Estimations of Tannins (Robert, 1971), Estimation of Ascorbic acid (Roe and Keuther, 1953), Estimations of α -Tocopherol (Emmerie-Engel, 1938, as described by Rosenberg, 1992), Ferric Free Antioxidant (Yildirim et al., 2000), Induction of Invitro Lipid Peroxidation and Measurement of Thiobarbituric Acid Reactive Substances by (Ohkawa et al., 1979).

Enzymatic Studies

Estimation of AP by Method of (King and Armstrong, 1934), estimation of GOT and estimation of GPT by (Reitman and Frankel, 1957).

Statistical analysis

Statistical analysis of experimental data was performed by One-Way ANOVA with Duncan test at the level of 95% using SPSS 16 (Statistical significance was set at the level of $p < 0.05$ with \pm SD).

RESULTS

Enzymological Studies

The activity of liver marker enzymes like AP, GOT and GPT were estimated. The AP Enzyme was found to be high *O. mossambicus* larvae fed with *C. sativum* supplementations enriched *Artemia Nauplii* (114.44 IU/L) compared with other group and Control was gradually decreased. The *O. mossambicus* larvae tissue GOT enzyme activity was observed in control (22.01 IU/L). In *O.*

mossambicus larvae fed with *C. sativum* extract enriched Artemia nauplii, the GOT activity 45.73 IU/L better compared with the other three supplementations (graph 1).

The amount of GPT alterations in fishes fed with different herbals like *C. sativum* supplementations enriched Artemia nauplii showed the level of 41.64 IU/L. The GPT activity was low in Control group as 25.65 IU/L and fishes fed with *A. polygonoides* supplementations enriched Artemia activity 29.30 IU/L in lower level compared with other groups. All the enzymatical statistical values significantly ($p < 0.05$). (Graph 1).

Antioxidant Studies

The aerobic organisms are protected from oxidative stress induced by free radicals and active oxygen species by an array of defense system. The present studies proved that the herbal antioxidants played a major role for aquatic larviculture.

Vitamin C (Ascorbic Acid) level

O.mossambicus larvae fed with Artemia nauplii enriched with *C.asiatica* supplementations showed the increased ascorbic level of 19.46 mg/g compared with other group and Control. The estimated the amount of Vitamin E α - Tocopherols in *O. mossambicus* larvae fed with unenriched Artemia nauplii normally 1.080 μ g/g. *C.sativum* supplementations fed fishes was found 2.439 μ g/g compared with *C. asiatica*, *T. foenum* and *A. polygonoides* (Table.1). The level of Tannin was observed in control as 43.65mg/g. Larvae fed with *C. asiatica* supplementations was very high relatively (88.16 mg/g), *C.sativum*, *T. foenum* and *A. polygonoides* supplementations was significantly found to be decreased as 77.65 mg/g 66.75 mg/g and 55.74 mg/g (Table.1). The Total Phenol content from *O. mossambicus* fed with different herbal supplementations like supplementations fed *C.sativum* fishes was very high 73.12 mg/g. *C. asiatica*, *T. foenum* and *A. polygonoides* supplementations gradually shown decreased values such as 67.57 mg/g, 51.86 mg/g and 45.30 mg/g (Table.2). The Ferric free antioxidant was found to be more or less same in fishes fed with *C.asiatica* supplementations and Control as 88.91% 87.06% respectively. In *C. sativum* supplementations, comparatively very highest percentage of antioxidant was noted 117.59%. In *A. polygonoides* moderate amount was recorded in 95.45 and in *T. foenum* supplementations lowest percentage was recorded (Table.2). The lipid peroxidation on *O. mossambicus* fed with different supplementations *C. sativum* enriched Artemia nauplii free radical scavenging activity was very high (9.28%) compared with other feed and control (Table.2). Based on the above experiment results, biochemical composition and antioxidant level the herbal feed *C. sativum* is the suitable feed for larval rearing of *O. mossambicus*. All the statistical values significantly ($p < 0.05$).

Table 1. Showing Vitamin C, E and Tannin level of *O. mossambicus* fed with different Herbals enriched Artemia Nauplii

Commercial Herbals	Feeding period	Total Phenol (mg/g)	Ferric Free Antioxidant (%)	Invitro Lipid Peroxidation (%)
Control	50 Days	28.73±2.76 ^c	87.06±1.96 ^{bc}	5.07±0.65 ^c
Ex1	50 Days	73.12±2.87 ^{a*}	117.59±2.99 ^{a*}	9.28±1.87 ^{a*}
Ex2	50 Days	67.57±1.88 ^b	88.91±3.88 ^b	4.75±0.87 ^{cd}
Ex3	50 Days	51.86±2.09 ^c	71.06±1.09 ^d	7.46±0.32 ^c
Ex4	50 Days	45.30±1.98 ^d	95.45±2.00 ^b	8.45±0.43 ^b

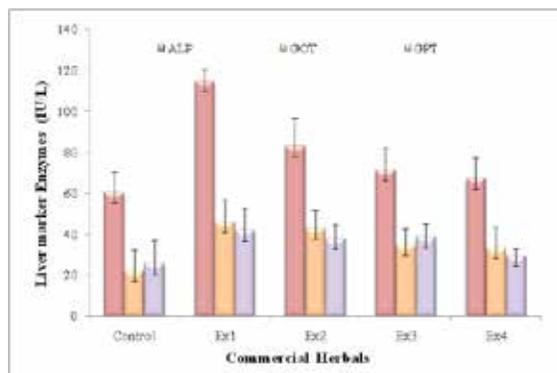
Mean ± SD (n=5) Mean values within the same row sharing the same superscript are Significant different (P >0.05).

Table 2. Showing the Total Phenol Ferric free Antioxidant and Lipid peroxidation level of *O. mossambicus* fed with different Herbals enriched Artemia Nauplii

Commercial Herbals	Feeding period	Ascorbic acid (mg/g)	α -Tocopherol (μ g/g)	Tannin (mg/g)
Control	50 Days	14.16±1.00 ^c	1.080±1.65 ^c	43.65±3.00 ^c
Ex1	50 Days	18.92±2.32 ^{ab}	2.439±1.22 ^a	77.65±0.98 ^b
Ex2	50 Days	19.46±1.98 ^a	1.646±1.98 ^b	88.16±1.23 ^{a*}
Ex3	50 Days	16.17±2.02 ^b	1.584±1.23 ^c	66.75±2.09 ^c
Ex4	50 Days	15.17±1.09 ^{bc}	1.979±1.98 ^b	55.74±2.98 ^d

Mean ± SD (n=5) Mean values within the same row sharing the same superscript are Significant different (P >0.05).

Graph 1. Enzyme activity of *O. mossambicus* fed with different Herbals enriched Artemia Nauplii



Discussion

In recent years, there is an increasing interest in the use of herbals as dietary and therapeutic supplements indicate that growth and modulating immune function in fish and shellfish (Harikrishnan et al.,2011). One of the main strategies in developing a larval rearing system is the establishment of a feeding regime that will result in optimal growth, survival and health of the fish larvae (Citarasu et al.,1998). The present study proved that the herbal antioxidant played a major role for aquaculture larviculture. Vitamin E deficiency can lead to immunological malfunctions and reduced disease resistance in salmonid fish (Waagbo,1994).

The growth rate promotion by Livol (IHF-100), a new growth promoter from Indian Herbs, was studied by Jayaprakas and Euphrasia (1996). *Allium sativum* was used as a growth promoter in *O.niloticus* (Diab et.al.,2002), and also *Clitoria Ternatea* Plant Parts on Growth Performance, Biochemical and Enzymatic Activities of Pearl Spot (*Eetroplus Suratensis*). Uthayakumar et al., 2013. This finding is in agriment with some of the earlier studies with plant derived immunostimulants. A. significant reduction in mortality was observed in tilapia fed with diets containing an ethyl acetate extract of *R.officinallis* leaves (Abutbul et al., 2004) or an aquas extract of *E.alba* leaves Christyapapita et al., 2007) The present study proved that, *O.mossambicus* was a successful candidate to be considered for commercial fish culture. All the herbals proved to be enhancing the growth of the fishes and it should be further analysed in other fishes also.

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