Medicinal Plant as Immuno stimulant for Health Management in *Cyprinus carpio*

S. Agneswari[1], M. Jansi M[2] and M.M. Malini[2]

[1]Department of Zoology, Vivekananda College, Agasteeswaram – 629701, Affiliated to Manonmaniam Sundaranar University, Tirunelveli - 627012
[2]Research Department of Zoology, S.T. Hindu College, Nagercoil, Affiliated to Manonmaniam Sundaranar University, Tirunelveli - 627012

Abstract

Aquaculture is the main source to increase fish supply. Fast development of aquaculture and increasing fish demand lead to intensification of fish culture, and risk of diseases. Until now, chemotherapy has been widely used to prevent and treat disease out breaks, although use of chemical drugs has multiple negative impacts on environment, fish and human health e.g. resistant bacterial strains and residual accumulation in tissue. Hence, disease management in aquaculture should concentrate on environmental friendly methods. Recently, increasing attention is being paid to the use of plant products for disease control in aquaculture as an alternative to chemical treatments. Treatment of bacterial disease with different herbs has been safely used in organic aquaculture. Plant products have been reported to stimulate appetite and promote weight gain, to act as immune-stimulant and to have antibacterial properties in fish due to active molecules such as alkaloids, terpenoids, saponins and flavonoids. The present study deals with the treatment of Aeromonas veronii and Enterobacter ludwigii infected fish Cyprinus carpio with medicinal plant Amaranthus dubius. Based on the results it is appropriate to conclude that the ethanol plant extract of A.dubius may act as a potent Immuno-stimulant in preventing and controlling bacterial disease in C.carpio than aqueous plant extract.

Key words: *Aquaculture, medicinal plants, bacteria.*

1. Introduction

In fish aquaculture, culturing different species of freshwater fish continues to increase each year, also the aquaculture industry is recently facing a serious setback due to infectious diseases [1]. Factors such as overcrowding, periodic handling, high or sudden changes in temperature, poor water quality and poor nutritional status contribute to physiological changes in fish such as immunosuppression and susceptibility to infection [2].

Enhancement of the immune system seems to be the most promising method for preventing fish diseases. The immune system of fish has two integral components. First component is the innate, natural or nonspecific defense system formed by a series of cellular and humoral components, and the second is the adaptive, acquired or specific immune system characterized by the humoral immune response through the production of antibodies and the cellular immune response is mediated by T. lymphocytes capable of reacting specifically with antigens [3]. Immuno- stimulant substances include synthetic compounds such as antibiotics [4], bacterial [5] and plant compounds [6] which increase the immunity of fishes.

Antibiotics used in medicines have been tried experimentally to treat bacterial infections of fish problems especially in ornamental fish culture [7]. The prevention and treatment of fish diseases by the extensive use of antimicrobial agents have undoubtedly contributed to an increase in the frequency of resistant strains [8]. Commercial vaccines are

expensive for fish farming practices and are specific against particular pathogens [9]. The use of medicinal plant is an alternative to antibiotics in fish health management [10]. These herbs are not only safe for consumers but also widely available throughout Asia and they also have a significant role in aquaculture [11]. Many studies have been proved that herbal additives enhanced the growth of fishes and also protected them from the diseases. Further medicinal plant extracts have minimal side effects, are easily biodegradable, inexpensive, locally available, and are easily prepared [12].

Plant extracts have been reported to favour various activities like antistress, growth promotion, appetite stimulation, enhancement of tonicity and immune-stimulation, maturation of culture species, aphrodisiac cand antipathogenic properties in fish and shrimp aquaculture due to active principles such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils [13]. Besides, their use could reduce costs of treatment, and more environmental friendly as they tend to be more biodegradable than synthetic molecules and they are less likely to produce drug resistance in pathogens due to the high diversity of plant extract molecules [14]. Herbs and probiotics are promising alternative tool to supplement antibiotics, chemicals or vaccines for fishes [15]. The phytochemicals have various activities such as antistress, antimicrobial and antistimulants [16]. But limited reports were available on the immuno-stimulant effect of *Amaranthus dubius* on fishes. So this present study is focused on immunostimulant effect of aqueous and ethanol extract of *A.dubius* on *Cyprinus carpio*.

2. Materials and Methods

The common carp, *C.carpio*. L of similar age groups were obtained from J.J. Fish farms, Nagercoil, Kanyakumari district, Tamil Nadu, India. The fishes were belonged to the length group of 12.0 ± 1.0 cm and weight 15.0 ± 1.0 gm. Infected fishes with bacterial diseases were collected to isolate the pathogen. The gut samples were collected from the infected C. *carpio* and the homogenized sample was used for bacterial notation [17]. The homogenized samples were serially diluted from 10^{-1} to $10^{-9}$and 0.1 ml of each sample was spread over an Tryptone Soy Agar medium (TSA) for culture. The plates were incubated at 37^0C for 24 to 47 hours. Distinct colonies appeared on each plate were sub cultured on sterile plates with freshly prepared TSA medium to obtain pure cultures [18]. The bacterial populations were expressed as number of colony forming units (cfu) per ml of the samples analyzed. Single isolate colonies from the streaked plated were transferred to TSA slants for further study. The isolates were identified by cultural, morphological and biochemical characteristics as per [19], [20]. The pure cultures were maintained in agar slants for future use. Two major pathogens were selected *(Aeromonas veronii* and *Enterobacter ludwigii)* to induce *C.carpio*. Crude plant extract of *A. dubius* was prepared by Soxhlet extraction method. The powdered plant materials (25 gram) were extracted with ethanol (99.9%) and aqueous, at 40 - 80˚C [21].

Healthy fishes were separated into 5 groups of ten fishes each. The isolated pathogens *(A. veronii* and *E. ludwigii)* of 0.5 ml suspension was administered to each group [22]. After 2 days, the diseased fishes were treated with aqueous and ethanol leaf extract of *A. dubius* except control. The blood sample was collected after 24 hours and biochemically analyzed. The blood samples were used for determining haemogloblin content [23]. Total and differential count of white blood cells (WBC) were performed using standard methods Houston (1990). Thrombocytes were determined using a Neubauer haemocytometer [23].

Enzymes were measured according to Marousc *et al*., [24], Plasma glucose was determined using glucose kit supplied by Boehring Mannheium kit, [25]. Total protein

content, albumin and globulin were estimated calorimetrically [26]. The above parameters were estimated at 7th day, 14th day, 21stday and 28th day of experiment.

3. Results

A. veronii and *E. ludwigii* induced *C. carpio* were treated with aqueous and ethanol leaf extract of *A. dubius* and the haematological parameters observed were presented in Table 1. The treatment of pathogen induced *C. carpio* with aqueous and ethanol leaf extract exhibited a slight variation in the blood parameters. Hb, WBC, Neutrophil and Lymphocytes were enhanced on 28th day of treatment in A. *veronii* and *E. ludwigii* induced *C. carpio* treated with aqueous and ethanol leaf extract. The platelet count was found to be increased as 6.5 ± 0.31 and 6.84 ± 0.08 Lakhs / cu. mm in *A. veronii* induced *C. carpio* treated with aqueous and ethanolic leaf extract respectively. But the platelet count was reduced on 28th day treatment in *C. carpio induced* with *E. ludwigii* with aqueous and ethanolic leaf extract.

SGOT, SGPT and alkaline phosphatase concentration of pathogen induced fishes treated with both ethanol and aqueous leaf extract was illustrated in Table 2. SGOT, SGPT concentration were observed to be decreasing on 28th day of treatment of *C. carpio with* aqueous and ethanolic leaf extract whereas the concentration of alkaline phosphatase increased on 28th day of treatment.

The level of protein in leaf extract treated fish was higher than the control value throughout the experimental period. The level of protein showed an increase in ascending fashion from day 2 (5.24 ± 0.08mg / dl) to 28th day (10.6 ± 0.41 mg / dl) in *E. ludwigii* infected fish treated with aqueous extract of *A. dubius*. A sudden rise was noticed on 14th (8.9 ± 0.41 mg / dl) and 28th day (10.32 ± 0.109 mg / dl) in *E. ludwigii* induced *C. carpio* with ethanol extract. Globulin and albumin content were increased in both treatments in *C. carpio.*

In all experimental groups glucose level was gradually decreased than the control in *C. carpio* infected with *A. veronii and E. ludwigii* from 2nd day to 28th day and was presented in Table 3.

Table 1. Haematological assessment of pathogens induced fish treated with A. dubius leaf extract

Sample	Days	HB gm/ %		TWBC cells / cu. mm		Neutrophil %		Lymphocyte (%)		Platelet count (Lakhs / cu. mm)	
		A. veronii	*E. ludwigii*	*A. veronii*	*E. ludwigii*	*A. veroni*	*E. ludwigii*	*A. veroni*	*E. ludwigii*	*A. veronii*	*E. ludwigii*
Control	2	13.9±0.27	11.9±0.23	15041.6±245.79	15958.3±102.6	15±0.89	9.5±1.04	79.6±1.03	84±1.26	4.3±0.20	4.8±0.19
	7	15.3±0.33	15.5±0.34	12600±104.88	14558.3±128.12	9.6±1.03	4.2±0.75	86.5±3.61	91.6±2.42	5.2±0.24	4.7±0.2
	14	14.73±0.39	14.7±0.28	11483.3±121.10	14166.6±225.09	11.4±0.80	6.5±0.49	85.3±1.21	89.1±1.47	5.15±0.19	4.1±0.21
	21	15.8±0.25	13.45±0.33	13016.6±172.24	11991.6±111.43	7.7±0.41	4.5±0.37	90±0.89	92.1±2.04	4.5±0.19	4.8±0.24
	28	14.3±0.33	15.9±0.24	13950±104.88	15391.6±326.21	7.9±0.20	6±0.54	88.3±1.03	90±1.26	3.2±0.20	3.5±0.11
Aqueous	2	9.32 ± 0.24	5.44 ± 0.089	10520 ± 327.10	12380 ± 268.3	5.2 ± 0.44	4 ± 1	80.2± 0.44	88.6± 2.1	4.5± 0.08	4.0± 0.1
	7	14.7 ± 0.28	7.62 ± 0.52	11020±44.72	13440± 338.23	6.6 ± 0.54	5.8±0.54	85.8± 1.09	88± 1.41	4.16± 0.1	3.6 ± 0.1
	14	13.9 ± 0.27	5.12 ± 0.10	11400 ± 223.6	13540 ± 89.44	7.4 ± 0.54	6.6±0.54	86.4± 1.94	90.2± 0.4	4.58± 0.1	3.5± 0.08
	21	15.3 ± 0.33	14.7 ± 0.83	12200±273.86	13660 ± 151.67	8.6± 0.89	7.2±1.30	86 ± 1.41	93.2± 1.6	5.4 ± 0.14	3.0± 0.1
	28	15.8 ± 0.25	15.7 ± 0.67	13140 ± 219.08	13820± 204.93	9.8 ± 0.44	9.82± 0.2	89.24± 1.3	93.6± 2.0	6.5±0.31	2.8 ± 0.1
Ethanol	2	10.28 ± 0.22	8.16 ± 0.13	9030 ± 109.54	12140 ± 384.70	6.08 ± 0.08	6.8± 0.44	67.6 ± 2.50	91.4± 2.1	4.18 ± 0.10	4.4± 0.1
	7	8.32 ± 0.20	8.3 ± 0.27	9726 ± 140.99	13120 ± 443084	6.74 ± 0.19	6.8± 0.83	77.2 ± 3.11	87.8± 1.6	4.08 ± 0.04	4.0± 0.08
	14	6.72 ± 0.38	8.52± 0.17	12270 ± 363.31	12880 ± 109.54	7.2 ± 0.2	7.6± 0.54	86.4± 2.30	90.8 ± 0.83	5.6 ± 0.24	3.7± 0.1
	21	14.7 ± 0.83	10.32 ± 0.40	13380 ± 164.31	12630 ± 156.52	7.12 ± 0.21	8.2± 0.83	90.4 ± 0.54	94.2± 1.0	6.14 ± 0.13	3.5± 0.1
	28	15.7 ± 0.067	15.7 ± 0.67	14140 ± 207.36	13940 ± 219.08	9.82 ± 0.4	9.8± 0.44	93 ± 1.22	94.4± 0.8	6.84 ± 0.08	3.0± 0.1

Table 2. Enzyme concentration of pathogens induced fish treated with *A. dubius* leaf extract

Sample	Days	SGOT (µg/ml)		SGPT (µg/ml)		Alkaline Phosphatase (µg/ml)	
		A. veronii	*E. ludwigii*	*A. veronii*	*E. ludwigii*	*A. veronii*	*E. ludwigii*
Control	2	1205±61.23	1331.6±36.009	166.8±2.13	163.8±3.18	155±10.48	156.6±10.32
	7	103.5±3.72	1328.3±22.28	97.1±1.16	163±3.46	245±13.78	160.8±6.64
	14	40±12.64	1241.6±54.55	18.1±1.83	162.6±2.25	113.3±4.08	146.6±5.16
	21	19.16±2.40	1315.3±11.77	12.3±2.25	168±2.44	52.5±4.18	178.3±4.08
	28	56.6±4.67	1061±2.52	42.8±2.31	540.8±7.35	109.1±3.76	364.1±4.91
Aqueous	2	185.8 ± 1.09	161.4 ± 2.19	146.6 ± 1.67	145.4 ± 0.54	135.6 ± 0.54	133.4 ± 2.30
	7	175.8 ± 1.09	149.8 ± 1.48	156.2 ± 1.64	148.4 ± 2.30	137.4 ± 1.34	136.2± 1.64
	14	168.8 ± 1.64	142.4 ± 2.50	159.6 ± 0.89	141.8 ± 2.04	155 ± 10.48	141.6 ± 1.51
	21	165.8 ± 1.30	146.4 ± 1.34	157.2 ± 1.09	141 ± 1.41	131 ± 1.22	146 ± 1
	28	160.8 ± 1.09	140.6 ± 2.60	151.4 ± 1.516	136.4 ± 0.54	200 ± 6.32	209.2 ± 0.83
Ethanol	2	170.6 ± 0.89	168.6 ± 15.64	150.6 ± 0.89	152.6 ± 2.50	135.8 ± 1.09	141.4± 0.89
	7	166.2 ± 1.64	168.6 ± 2.190	147 ± 1.41	147.8 ± 2.58	141 ± 0.70	142.4 ± 2.19
	14	166.2 ± 1.64	164.4 ± 2.60	140.6 ± 0.89	145.4 ± 0.89	148 ± 0.70	142.8 ± 1.78
	21	160.8 ± 1.09	158.6 ± 2.19	136.2 ± 1.64	140.8 ± 1.09	152.8 ± 1.78	148.8 ± 2.16
	28	156.4 ± 1.34	154. 8 ± 2.86	130 ± 0.70	141.6 ± 1.94	194 ± 3.74	200 ± 6.32

Table 3. Biochemical Composition of pathogens induced fish treated with A. dubius leaf extract

Sample	Days	Protein (mg/dl)		Albumin (mg/dl)		Glubulin(mg/dl)		Glucose (mg/dl)	
		A. veronii	*E. ludwigii*	*A. veronii*	*E. ludwigii*	*A. veronii*	*E. ludwigii*	*A. veronii*	*E. ludwigii*
Control	2	9.1 ± 0.25	10.3 ± 0.40	1.5±0.27	1.0±0.13	1.16 ± 0.18	1.3 ± 0.44	92.5 ± 3.61	103.5 ± 4.03
	7	12.3 ± 0.25	9.4 ± 0.33	2.5±0.13	1.4±0.2	1.06 ± 0.054	1.16 ± 0.18	107.1 ± 5.26	94.6 ± 3.50
	14	11.2 ± 0.27	9.6 ± 0.51	7.2±0.2	1.3±0.20	0.78 ± 0.08	0.8 ± 0.07	76.5 ± 2.07	94.1 ± 2.78
	21	10.5 ± 0.31	9.4 ± 0.80	5.1±0.09	1.6±0.16	0.56 ± 0.080	0.84 ± 0.089	64 ± 3.63	103.6 ± 4.27
	28	10.7 ± 0.88	10.1 ± 0.39	6.28±0.19	5.3±0.13	0.54 ± 0.054	0.58 ± 0.10	103.6 ± 7.31	105.8 ± 3.37
Aqueous	2	6.0± 0.044	5.24 ± 0.08	0.58 ± 0.10	0.56 ± 0.080	0.54 ± 0.05	0.54 ± 0.054	123 ± 2.73	125.4± 68.68
	7	5.7 ± 0.2	5.48 ± 0.21	0.58 ± 0.10	0.8 ± 0.1	0.54 ± 0.05	0.66 ± 0.054	123 ± 2.73	118.6 ± 0.89
	14	9.1 ± 0.25	5.38 ± 0.08	0.84 ± 0.089	0.78 ± 0.08	0.68 ± 0.16	0.54 ± 0.089	121.4 ± 1.14	115.6 ± 0.89
	21	10.4 ± 0.37	8.08 ± 0.25	1.06 ± 0.054	1.16 ± 0.18	0.8 ± 0.07	1.3 ± 0.44	126.2 ± 1.09	111.8 ± 1.095
	28	5.22 ± 0.10	10.6 ± 0.41	1.22 ± 0.044	2.4 ± 0.070	1.5 ± 0.27	2.6 ± 0.89	92.5 ± 3.61	92.2 ± 2.68
Ethanol	2	6.08 ± 0.10	5.54 ± 0.35	0.66 ± 0.08	0.58 ± 0.10	0.52 ± 0.044	0.46 ± 0.05	117.2 ± 1.78	119.2 ± 0.83
	7	5.8± 0.134	5.6 ± 0.07	0.72 ± 0.10	0.82 ± 0.08	0.7 ± 0.07	0.6 ± 0.12	115.6 ± 0.89	122.8 ± 2.58
	14	5.56 ± 0.05	8.9 ± 0.41	1.1 ± 0.1	0.54 ± 0.05	0.8 ± 0.07	0.44 ± 0.05	115.6 ± 0.89	116.8 ± 1.78
	21	8.8 ± 0.89	5.26 ± 0.05	1.1 ± 0.1	1.98 ± 0.17	1.08 ± 0.08	0.25 ± 0.08	109.2 ± 1.30	115.8 ± 0.44
	28	10.2 ± 0.44	10.3 ± 0.10	1.24 ± 0.05	2.22 ± 0.21	1.8 ± 0.83	0.2 ± 0.1	97.2 ± 1.30	93.4±2.70

4. Discussion

Medicinal plant extract as immunostimulant has significant effect on pathogens. In the present study the pathogens *A. veronii* and *E. ludwigii* were used to induce to *C. carpio.* Then the fishes were treated with aqueous and ethanol leaf extract of *A. dubius.*

Hematological and biochemical profiles of blood can provide important information about the internal environment of the organisms [27]. HB, WBC, Neutrophil, Lymphocyte, and Platelet count were increased in pathogen induced fishes treated with ethanol leaf extract of *A. dubius*. This was supported by [28] in *Clarias batrachus* treated with *Ocimum sanctum* leaf extract. The results were coinciding with the results obtained by [29] who treated common carp with dietary *Aloe vera* extracts.

In agreement with the present findings, [30] reported that WBC and RBC counts were higher in *Labeo rohita* fingerlings fed with *Magnifera indica* kernel when compared to control. [31] also reported that there was an increase in the WBC count after feeding the common carp with immunostimulants like chitin. Similar results were obtained by [32] who tested the immunostimulatory effects of various medicinal plant extracts, such as mistletoe (*Viscumalbum*), nettle (*Urticadioica*) and ginger (*Zinger officinale*), in rainbow trout, [33]. The significant increase in the total leukocyte counts with ginger extract can be considered as an indicator for improvement in general resistance. [34] reported about the increase in

neutrophils and lymphocyte counts in fishes treated with *Withania somnifera* herbal extracts which can be attributed to the specific immune response.

Phosphatase enzyme is considered as a member of lysosomal enzyme which is a valuable parameter of macrophage activation [35]. In our study SGOT, SGPT and alkaline phosphatase activities of fish *C. carpio* treated with the leaf extract of *A. dubius* is enhanced significantly when compared to the control fish. The enhancement of serum phosphatase activity in fish may be due to the increased production of enzyme by the macrophage cells. Similar observations were reported by Rao *et al*.,[36] who revealed that *Achyranthe saspera* enhanced the serum alkaline phosphatase activity in *L. rohita*. Pratheepa and Sukmaran [37] also reported about enhanced alkaline phosphate activity of A. *hydrophila* infected *C. carpio* treated with *Euphorbia hirta*.

In the present study, Pathogen induced fishes treated with leaf extract of *A. dubius* showed enhanced total protein, albumin, globulin. Similar resuts were reported in rainbow trout fed with garlic, ginger, *Lourus nobilis* and Coggyri *acoggyria* [38]. Thus the increase of total protein, albumin and globulin in the present study was attributed to increased immunostimulatory effect.

The decreased level of glucose in *C. carpio* in the present study indicated that plant extracts reduce the stressors. Similar observations were found in *L. rohita* fingerlings [39], *Clarias batrachus Lin.* [40] and black tiger shrimp, *Penaous monodon* [41] that glucose level was reduced after feeding with herbal immunostimulant diets.

Thus the use of herbals and their active compounds are recognized to improved immunity and disease resistance [42]. The leaf of *A. dubius* has been shown to contain water soluble phenolic compounds such as alkaloid, glycosides, saponin etc. that might act as a potential immunostimulant. The present work revealed that the ethanolic leaf extract of *A. dubius* is highly effective on pathogen induced *C. carpio*. So that it can be recommended to treat the fishes from pathogens.

5. Conclusion

In the ancient age itself medicinal plants are used to cure the ailments. In this study deals with the treatment of Aeromonas veronii and Enterobacter ludwigii infected fish Cyprinus carpio with medicinal plant Amaranthus dubius. Based on the results it is appropriate to conclude that the ethanol plant extract of A.dubius may act as a potent Immuno-stimulant in preventing and controlling bacterial disease in C.carpio than aqueous plant extract.

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