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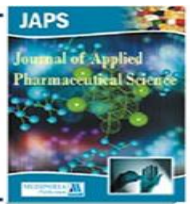

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Studies on the immouostimulant activity of *Coriandrum sativum* and resistance to *Aeromonas hydrophila* in *Catla catla.*

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ABSTRACT

The present study was designed to evaluate the immunostimulant potential of *Coriandrum sativum* is fish *Catla catla*, post challenged with *A.hydrophila*. Fish were divided into 2 groups one received control diet, another received *Coriandrum Sativum* incorporated diet for 14 days. The haematological parameters and serum protein were analyzed between control and experiment. There was a significant increase in TEC ($P<0.01$) and TLC ($P<0.01$) in the immunostimulant (IS) administered diet. Remarkable increase in Hb content from as 6.9.to 7.39% and serum protein level from 0.56 to 0.58g% was noted. The immunostimulant administered post challenged *Catla catla* showed a decrease in TEC at 10^3 cfu/ml and a drastic increase in TLC(<0.01) in both 10^3 and 10^5 cfu/ml Hb content on IS incorporated post challenged *Catla catla* showed an increase from 8.7 – 11.5g%.

Key words: *Catla catla, Coriandrum sativum, Aeromonas hydrophila,* Immunostimulant.

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INTRODUCTION

The use of immunostimulants in fish culture for the prevention of disease is a promising new development. Immunostimulants seem to represent a useful alternative to vaccination and chemotherapy in the control of fish disease, which enhance the non – specific immune response (Sakai 1999, Esteban et al., 2004). In aquaculture, there are many studies reporting a variety of substances including synthetic products(Rao et al., 2006), bacteria (Goetz et al., 2004, Engstad et al., 1992) animal and plant products (Hardie et al., 1991, Ardo et al., 2008, Rao et al., 2006) as immunostimulants to enhance the immune system of cultured fish. The development of plant based vaccines are in advanced stage. Natural plant products have various activities like antistress, appetizer, antimicrobials and immunostimulants (Citarasu et al., 2002, 2003). The advantages of herbal therapeutants over synthetic or formulated ones are: a)90% utilization by the culture organism b) Provide many phytocompounds c) do not cause damage to physical system d) Supply pigments such as chlorophyll, carotenoids, xanthophylls and very complex Vitamins in the most desirable form (Stephan et al., 2006).

Coriandrum sativum is an annual and herbaceous plant belonging to the Apiaceae family. It is a medicinal plant, native of Southern Europe and Western mediterranean region and it is cultivated worldwide. The fruit of coriander is diuretic, antipyretic, stomachic and cures bronchitis. The leaves are hypotonic, analgesic, anti inflammatory useful in jaundice and tuberculosis (Tan et al., 2004). As a medicinal plant it is used as an antimicrobial substance (Delaquis et al., 2002, Singh et al., 2002, Eyayyar et al., 2001). Coriander oil has properties of analgesic, stimulant, antibacterial and antiinfectious (Isao Kubo, 2004).

The regular monitoring of fish blood serves the diagnostic purpose in establishing the health status of fish. By analyzing blood cell characteristics some clues for diagnostic and prognosis of the disease state may be found (Anderson, 2003). The present study is aimed to evaluate the immunostimulant potential of *Coriandrum sativum* in the fish *Catla cata*, post challenged with *Aeromonas. hydrophila* by analyzing the hematological and serological parameters.

MATERIALS AND METHODS

The experimental fish *Catla catla* (125± 30g) were collected from kallidaikuruchi fish farm and allowed to acclimatize to laboratory conditions for one week. During acclimatization they were fed with rice bran and groundnut oil cake and water was renewed daily. During the experimental period the water quality variables such as temperature (28± 1°c) p^H (7.4±0.2) salinity (10±2%) and dissolved oxygen (>5 mg^{-1}) were recorded.

Feed Preparation

The basic diet (control diet) was prepared by mixing rice bran 10g wheat bran 10g, soya flour 23g, dry fish meal 24g, groundnut oil cake 23g and Tapioca flour 10g, made as dough, sterilized in pressure cooker for 30min, cooled and made in the in the form of noodles by adding a little amount of sunflower oil. They were shade dried and broken into small desirable pieces and stored. Immunostimulant diet was prepared using the same proportion by using 2g of *C. sativum* powder, which was collected from the local market.

Experimental design

Experiment I:

Experiment I consisted of two groups, one control and one experimental of 15 fishes each. The control group received normal diet and the experimental group received feed formulated with *C.sativum* powder. (IS diet) the fishes were fed with these diets for 14 days, and haematological parameters and serum protein level were analyzed after 24 hrs, 72hrs, 7th and 14th day respectively.

Experiment 2:

Both control feed and experimental feed fed fishes, after 14 days were infected with the bacteria *Aeromonas hydrophila* previously grown in nutrient broth for 24 hrs. 10^{-3} and 10^{-5} (cfu/ml) bacteria were injected intramuscularly and again haematological and serum proteins were studied after 24hrs, 72 hrs, and on 7th day

Haematological and Serological analysis

The blood was collected from the fishes by puncturing the heart by using 1 ml insulin syringe. For serological analysis the collected blood were centrifuged at 2500 rpm for 14min. Total erythrocyte count (TEC) and total leucocyte count (TLC) were carried out using Haemocytometer with improved Neuberger ruling chamber (Weber and Sons,England). Haemoglobin content was estimated by cyanomethomoglobin method (Hemocor-D,Crest Biosystems). Blood smears stained with May – Grunewald's Giemsa stain was used for differential leucocytes. The data were analyzed statistically and students 't' test was used to test the level of significance. For serum protein estimation Gornall's biuret method was followed.

RESULT AND DISCUSSION

Experiment I.

The values of TEC, TLC, were higher immunostimulant incorporated diets and the increase was highly significant P(<0.01) on the 7th and 14th day when compared with the standard feed pellets (Table 1&2).

Table- 1: TEC (million cells/mm^3) in *C.catla* administered with control and IS diet.

Duration Days	Sample	RBC/ million cells ± SD
1	Control	0.62 ± 0.043
	IS	0.63 ± 0.074*
3	C	0.62 ± 0.043
	IS	0.71 ± 0.024**
7	C	0.67 ± 0.059
	IS	0.76 ± 0.031*
14	C	0.70 ± 0.020
	IS	0.81 ± 0.035**

P; ** more significant * significant.

Table – 2 : TLC (million cells/mm^3) in *C.catla* administered control with and IS diet .

Duration Days	Sample	RBC/ million cells ± SD
1	C	245700 ± 5030.17
	IS	322300 ± 29941.6*
3	C	250200 ± 23757.4
	IS	314244 ± 35938.9
7	C	211100 ± 30822.8
	IS	327900 ± 28659.9**
14	C	223950 ± 35442.9
	IS	331900 ± 199864

P; ** more significant * significant.

Table 3: DLC (%) in *C.catla* administered with control and IS diet.

Duration Days	Sample	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
1	C	43	30	12	9	6
	IS	44	31	17	4	4
3	C	40	23	18	10	9
	IS	41	22	16	11	10
7	C	40	31	17	8	4
	IS	36	27	14	13	10
14	C	39	23	19	9	10
	IS	43	32	15	7	3

DLC could not envisage marked differences however here and there fluctuations where found among the types of cells (Table 3) Higher percentage of heamoglobin were estimated in immunostimulatant administered fishes 6.9to 7.3%, whereas in control it decreased from 6.1to 4.7% (fig 1) Serum protein exhibited an increase from 0.56 to 0.58g% in experimental fish. (fig 2).

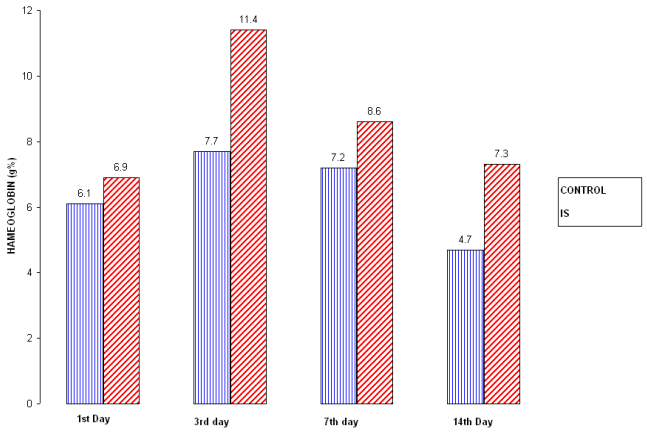

Fig 1: Hb content (g%) *C.catla* in relation to control and IS diet.

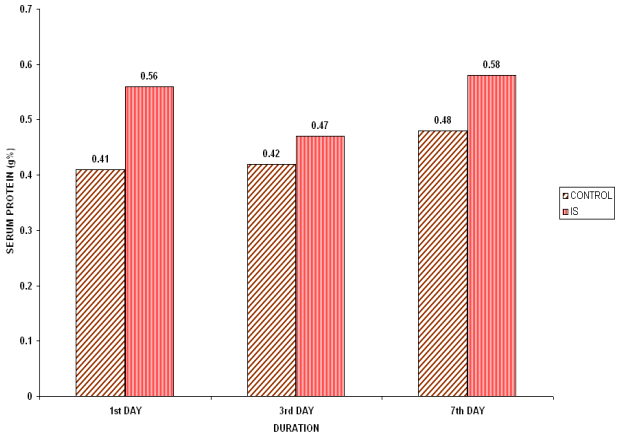

Fig 2: Antiprotease activity of Serum protein (g%) in *C.catla* administered with control and IS diet.

Experiment II

A significant decrease in TEC was noticed in IS fed fishes when infected with *Aeromonas hydrophila* at a dosage of 10^{-3} cfu/ml ($P<0.01$) in 7th day. However an increase of TEC was observed in IS fed fishes infected with *Aeromonas hydrophila* at a dosage of 10^{-5} cfu/ml (Table 4). A significant increase of TLC is noted on 3rd day ($P<0.01$) and ($P<0.05$) on both 10^3 and 10^5 (cfu/ml) in the Is administered infected fishes (Table-5). Hb content highly increased in 10^5 cfu/ml concentration on experimentally immunostimulated infected fishes, with a range of 8.7to 11.5 g% (fig-3) Agglutination tests also revealed better results in IS stimulated infected fishes.

Immunostimulants when administered through diet have been perceived as potentially playing a vital role in aquaculture (Galindo villegas and Hosokowa 2004). *Corianderum sativum* is considered as a potent immunostimulant. Reports available show that both leaves and seeds contain antioxidants, but leaves were found to have a stronger effect (Helle, Anna and kanl, 2004).

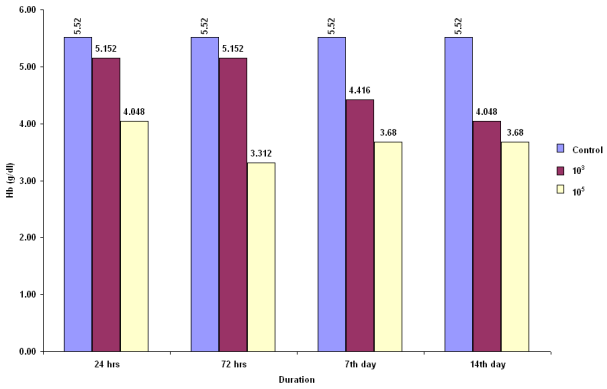

Fig 3: Hb content (g%) in *C.catla* preadministered with IS diet and post challenged with *Aeromonas hydrophila.*

Table – 4: TEC (million cells/mm^3) in *C.catla* pre administered with IS diet and post challenged with *Aeromonas hydrophila.*

Duration Days	Dosage (cfu/ml)	RBC/ million cells SD
1	C	0.74 ± 0.0305
	10^3	0.64 ± 0.070*
	10^5	0.82 ± 0.016**
3	C	0.78 ± 0.019
	10^3	0.63 ± 0.08**
	10^3	0.75 ± 0.025*
7	C	0.74 ± 0.035
	10^3	0.53 ± 0.046**
	10^5	0.66 ± 0.036**

P; ** more significant * significant

Table -5 : TLC (million cells/mm^3) in C.*catla* pre administered with IS diet and post challenged with *Aeromonas hydrophila.*

Duration Days	Dosage (cfu/ml)	RBC/ million cells SD
1	C	244350 ± 49575.8
	Is 10^3	333500 ± 28982.7***
	10^5	433300 ± 3685.2**
3	C	275400 ± 13523.8
	10^3	329600 ± 28043.2**
	10^5	471300 ± 1371165.8*
7	C	288550 ± 25070.1
	10^3	438050 ± 139965.8*
	10^5	328900 ± 41820.1*

P; *** highly significant ** more significant * significant

Table 6: DLC (%)in *C.catla* preadministered with IS diet and post challenged with *Aeromonas hydrophila.*

Duration Days	Dosage (CFU/ml)	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
1	C	45	23	19	9	4
	10^3	43	30	12	6	9
	10^5	42	33	14	4	7
3	C	44	23	20	8	5
	10^3	38	31	21	6	4
	10^5	37	29	23	8	3
7	C	43	22	21	9	5
	10^3	43	32	15	7	3
	10^5	42	30	16	9	3

experimentally infected elicited an alteration in TEC, TLC, DLC and Hb content. There is an increase in TEC in immunostimulant administered diet which agrees with the findings of Gopalakannan and Arul 2006, who reported this in common carp fed with IS chitin. Immune enhancement increase white blood cell phagocytosis, inhibits HIV – 1 replication and improves D_{4+} and T-lymphocyte counts (Mishra et al., 2009). Immunostimulatory activity of *Coriandrum sativum* is evidenced by increased proliferation of lymphocytes and production of interlukin (John Britto et al., 2004).

Disease challenge studies showed an increase in TEC, TLC, DLC, Hb content and serum protein content. TEC in immunostimulant administered catla when post challenged with *A.hydrophila* exhibited a significant decrease on prolonged exposure at a severe dosage (10^3 cfu/ml) But there is a Significant increase at 10^5 cfu/ml indicating the immunostimulatory activity of *C.sativum*, there by enhancing the nonspecific immunity(Gopala Krishnan and Arul 2006).

In IS administered *catla* monocytes showed an increase, lymphocytes level decreased, while neutrophils showed a slight change and eosinophils remained unchanged (Rao et al., 2006). *Coriandrum sativum* enhanced the antibody production in fishes to make antibody response against salmonella species (Stephen et al., 2006). which was similar to our results.

CONCLUSION

From the above results it is clear that *Coriandum sativum* acts as a potent immunostimulant which induces the blood parameters in the experimental fish *catla.* It can be used as a dietary additive or as an adjuvant to heighten the immune response. The efficacy of *Coriandrum sativum* is clearly proved as it causes an increase in TEC, TLC, DLC and Hb content which may enhance the defence mechanism. It is found to be a good choice as a diet supplement to induce some level of disease resistance and enhancement of non specific immunity and act as a potent immunostimulant in fishes.

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