Effects on growth performance and survival rate of *Cyprinus carpio* (Koi carp) fingerlings fed diets augmented with *Coriandrum sativum* (Coriander) against *Aeromonas hydrophila* infection

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Abstract

In most aquaculture systems, more than 50% of total cost is being expended for feed. Hence, developing a nutritionally sound, low-cost feed to minimize the aquaculture risks is a timely need. Occurrence of bacterial diseases to fish fingerlings is caused due to various pathogenic bacteria and majorities were identified as *Aeromonas sp*. To overcome the loss due to pathogenic diseases, enhancement of the immunity of culture fish can be practiced. The use of preparations and infusions of herbal plants to treat diseases and improve their immunological response against many pathogens has been practiced worldwide for centuries. *Coriandrum sativum* (Coriander- E, *Kothtamalli*- S) is a medicinal plant that has antibacterial property. Accordingly, an experiment was designed to determine the growth performances and survival to pathogenic *A. hydrophyla* on koi carp (*Cyprinus carpio*) fingerlings, fed with Coriander augmented prepared ration. Koi carp fingerlings of average weight (3.8942±0.27 g) were used to determine the performance of the four feed types. Complete Randomized Block Design (CRBD) was used for the eight weeks feeding trial with four treatments containing three replicates. Fishes were stocked in similar numbers in glass tanks and fed twice a day with commercial feed (Diet 1), prepared ration enriched with 5 g infusion of *C. sativum* (Diet 2), prepared ration enriched with 10 g infusion (Diet 3), and un-enriched prepared ration (Diet 4). Body weight was measured bi-weekly. At the end of the feeding trial, *A. Hydrophyla* bacteria were introduced to each fish through intraperitoneal injection of 0.8 ppm cell concentration and mortalities of each experiment were recorded. Highest Specific Growth Rate (SGR) level (0.500±0.023) was in Diet 1 followed by Diet 2 (0.479±0.013). The lowest SGR was in Diet 4 (0.423±0.016). There was no significant difference ($p>0.05$) among SGR and weight gain from four different diets. Challenge test results indicated that the lowest mortality percentage (32.73%) which is relevant to highest immunity enhancement was from Diet 3. Diet 1 and Diet 4 fed fishes showed the highest mortality (99.53%).

Keywords: immunity, enrichment, *Aeromonas hydrophila*, *Coriandrum sativum*

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Introduction

Ornamental fish industry is one of the fastest growing sectors around the world. Due to intensive farming practices, infectious diseases pose a major threat to the aquaculture industry, causing heavy losses to farmers (Gomez-Flores *et al.*, 2011). In order to achieve optimal fish production, better prophylactic, diagnostic and therapeutic measures are acceptable during fish farming operations. The recent expansions of aquaculture have led to a growing interest in understanding fish disease, so that they can be treated or prevented at least with partial success (Stoskopf, 1993). Consequently, cultivated fish can become more susceptible not only to pathogenic but also to opportunistic bacteria. Bacterial diseases are one of the major threats to aquaculture industry worldwide. Many bacteria have been reported to have caused disease outbreaks in farmed fish culture resulting in serious economic losses to the industry. The use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases (Subasinghe, 1997).

Aeromonas hydrophila infection is a plague in fresh and warm water fish farming worldwide. It is a ubiquitous organism present in the aquatic environment causing diseases in fish under stress. The bacterium causes hemorrhagic septicemia, which is characterized by the presence of small superficial lesions, focal hemorrhages particularly in the gills and opercula, ulcers, abscesses, exophthalmia and abdominal distension (Austin and Austin, 1987).

As application of antibiotics and chemicals in culture is often expensive and undesirable, it leads to antibiotic and chemical resistance and consumer reluctance. Some medicinal plant extracts or products have been used to control fish and shellfish diseases, which act as immune-stimulants, modulating the immune response to prevent and control fish and shellfish diseases. These immune-stimulants mainly facilitate the function of phagocytic cells, increase their bactericidal activities, stimulate the natural killer cells, complements lysozyme activity, and antibody responses in fish and shellfish which confer enhanced protection from infectious diseases.

Plants or their byproducts are preferred since they contain several phenolic, polyphenolic, alkaloid, quinone, terpenoid, lectine, and polypeptide compounds many of which have been shown to be very effective alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds (Gomez - Flores *et al.*, 2011). Additionally, in aquaculture, herbal medicines are also known to exhibit anti-microbial activity, facilitate growth and maturation of cultured species; besides under intensive farming the anti-stress characteristics of herbs will be of immense use without posing any environmental hazard. Administration of herbal extracts or their products at various concentrations through oral (diet) or injection route enhance the innate and adaptive immune response of different freshwater and marine fish and shellfish against bacterial, viral, and parasitic diseases.

The use of preparations and infusions of herbal plants to treat diseases and improve their immunological response against many pathogens has been practiced worldwide for centuries, but their effectiveness must be scientifically validated to increase the credibility of their use (Mainardi *et al*., 2009; Gomez-Flores *et al*., 2011 and García-Alvarado *et al*., 2001).

C. sativum is a medicinal plant, native of Southern Europe and Western Mediterranean region and cultivated worldwide. *C. sativum* is being used as a medicine for thousands of years and is still used in folk medicine. It has been used to manage diabetes (Gray and Flatt, 1999), used as an anti-fungal (Basilico & Basilico, 1999), antioxidant (Chithra & Leelamma, 1999), hypolipidemic (Chithra & Leelamma, 1997), antimicrobial (Delaquis *et al*., 2002; Singh *et al*., 2002; Elgayyar *et al*., 2001), hypocholesterolemic (Chithara & Leelamma, 1997) and anticonvulsant (Hossein & Mohammed, 2000) substance. Coriander oil has properties of analgesic, stimulant, antibacterial and anti infectious (Isao Kubo, 2004).

The present study is aimed to evaluate the immune-stimulant potential and growth performance of *C. sativum* in the fish koi carp fingerlings, post challenged with *A. hydrophila*.

Materials and Methods

Experimental diets, diet preparation and design of the research

Four types of dietary treatments were prepared as Diet 1 (Commercial feed), Diet 2 (5 g of coriander seed powder infusion enriched feed), Diet 3 (10 g of coriander seed powder infusion enriched feed) and Diet 4 (un-enriched feed). Experimental feed was prepared using commercially available, comparatively low-cost feed ingredients, except imported fish meal. The composition of the laboratory prepared feed used in the present study is given in Table 1. All the dry ingredients i.e., fish meal, soya bean meal, wheat flour, krill meal, rice bran, were weighed according to the formula and mixed thoroughly to prepare the dough. Vitamin and mineral mixture were mixed with the oil and then with the dough. The dough was first steamed, rolled in to strings and finally dried at 60 ºC overnight and crushed into small particles and sieved manually using a plastic sieve to get the required particle size. It was then divided in to three portions (Diet 2, 3 and 4) and stored under room temperature. All dietary ingredients were obtained commercially.

Table 1. Composition of the formulated feed used in this study

Ingredients	Amount %
Fish meal	25
Prawn meal	10
Soya bean meal	20
Rice bran	20
Sunflower oil	12
Wheat flour	10
Vitamin-mineral mixture	3

Enrichment of the Diet 2 and 3 was done by incorporating 5 g and 10 g of coriander infusion into the enrichment medium separately and mixing thoroughly with formulated feed. Ingredients of enrichment medium were given in Table 2. A cost analysis was performed to estimate the price for producing 1 kg of formulated feed in the laboratory**.**

Table 2. Composition of enrichment media

Composition	Diet 3	Diet 4
Coriandrum sativum	5 g/50 mL	10 g/50 mL
Water	40 mL	40 mL
Egg yolk	30 mL	30 mL
Gelatin	5 g	5 g

Source of fingerlings, maintenance and feeding trial

Koi carp fingerlings were purchased from a commercial aquarium, brought to the indoor experimental aquarium at NARA and acclimatized to experimental conditions for 7 days before starting the experiment. Twelve rectangular glass tanks (22” × 10” × 10”), each filled with aerated fresh water were used for stocking acclimatized fingerlings. The fingerlings (initial weight: 3.89 ± 0.27) were kept at a density of 10 individuals in each tank. Feed trial was conducted as four treatments; T1, T2, T3 and T4, each treatment conducted in triplicates. Fingerlings in one group of tanks were fed with commercial feed (Diet 1) - T1. The T2, T3 and T4 groups of tanks were fed with 5 g of *C. sativum* infusion enriched feed (Diet 2), 10 g of *C. sativum* infusion enriched feed (Diet 3) and un-enriched feed (Diet 4) respectively. The commercial feed was used in the study to compare the results of the treatments with formulated/ enriched feed. The tanks in each group were arranged to follow a completely randomized design. Fingerlings were fed for eight weeks twice a day offered at 0900 h and 1600 h up to satiation throughout the experimental period. All tanks were siphoned out daily and kept clean while carrying out two thirds of water volume exchange and providing continuous aeration throughout the day. Determination of pH, temperature, salinity and dissolved oxygen levels in the experimental tanks were checked once a week (APHA, 1998).

Proximate compositions of the experimental feeds were analyzed using the following procedures: Moisture content was quantified after drying the sample in an oven at 105 ºC until a constant weight was gained. Ash by incineration in 550 ºC for 5 hours; protein (N × 6.2) by the Kjeldahl method after acid digestion; lipids by diethyl ether extraction in a soxtec system according to AOAC standards (2009) (Table 3). Mortality levels and abnormal fish behavioral patterns if any were also recorded daily.

Growth performance and survival of fingerlings in the experimental tanks

The growth parameters, Food Conversion Ratio (FCR), Specific Growth Rate (SGR), weight gain and survival rate were calculated after eight weeks using the following formulae.

Weight gain (%) = (FBW-IBW/IBW) x 100

Daily Growth Coefficient, DGC (% d^{-1}) = $\{(FBW^{1/3} – IBW^{1/3})/D\}$ x 100

Specific Growth Rate, SGR (d^{-1}%) = {[ln FBW-ln IBW]/D} x 100

Food Conversion Ratio, FCR = (Feed intake/weight gain) x 100

Food Efficiency Ratio, FER = 1/ FCR

Survival Rate, SR (%) = (N_2/N_1) x100

Where, IBW= Initial Body Weight (g); FBW= Final Body Weight (g); and D= Duration of the experimental period in days. N_1= Number of fish at the start of the experiment; N_2= Number of fish on termination of the experiment.

Preparation of bacteria to intraperitoneal ingestion to the fish (challenge test)

Pure culture, obtained from pre-tested pathogenic *A. hydrophilla* stored in laboratory was taken to test the immunity of the treated fish. The culture was inoculated on Tryptic Soy Agar (TSA) and incubated at 30 ^{0}C for 24 hours for getting sub cultures. Bacteria grown on TSA was inoculated to nutrient broth and incubated at 37 ^{0}C for 24 hours in shaking water bath (YCW-012S, GEMMYCO). After incubation the culture was transferred to sterilized 15 mL centrifuge tubes aseptically and centrifuged at 5000 RPM for five minutes in a cooling centrifuge (Sigma 1-15 PK). The supernatant was discarded while the pellet containing bacteria at the bottom of centrifuge tube was re-suspended in same volume of sterilized distilled water using a vortex mixer. This was repeated three times and after that, 2-3 mL of saline was added.

Before starting the experiment, LD_{50} value of bacterial culture was quantified by injecting the culture into fifteen carp fingerlings to get a general idea on minimum amount that has to be injected to the fish for observing the bacterial disease symptoms.

The suspension was quantified as 0.8 ppm for LD_{50} value using UV-VIS Spectrophotometer (UV-1700 Pharma Spec) and diluted using saline water till required concentration obtained. Finally pour plate technique was used for bacterial enumeration (5.85×10^7 CFU/mL).

Artificial ingestion for treated fish

Three fish from each tank in each treatment were taken for immunity test and were anesthetized using 50 ppm of Tricaine Methane Sulfonate (MS-222) bath. After sedation, fish were artificially infected by intraperitoneally injecting pre-tested pathogenic bacteria of *A. hydrophilla* suspension of 0.1 mL of pathogenic bacteria (0.8 ppm of cell concentration under 540 nm in Spectrophotometer) and those challenged fish were again kept in the same relevant tank and given the aeration continuously to reduce the extra stress. The fish were observed very closely for clinical symptoms for five days and behavioral changes, disease symptoms and survival rate of the injected fishes were recorded daily.

Statistical analysis

The Minitab 16 software was used in the statistical analysis and Microsoft Excel for graphical illustration. Results were presented as means ± Standard Error of Means (SEM). Differences among the control and treatment means were analyzed using one way ANOVA at 95% confidence interval.

Results and Discussion

Results of the proximate composition of four diets indicate that the highest protein percentage is with Diet 1 (30.51%) and experimental feed protein percentage is more or less similar to 27% for all other three feeds (Table 3). Fat percentage level also high in the commercial feed as it is 9.53 and the experimental feed has been adjusted to 6.5 crude fat level considering whether the low fat and low protein percentage can be used for the better growth of Koi carp fingerlings. Results of the study indicated that the similar growth performances can be obtained with experimental feed (27% protein) and commercial feed (30% protein) without significant differences. However, it is evident that there is no effect of the enrichment with *C. sativum* on protein or fat content of the feed (Table 3). Slight increment of ash content was observed in the two enriched feeds (Diet 2 and 3).

Table 3: Proximate composition (%) of the feeds (dry basis)

	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)	Diet 4 (%)
Crude protein	30.51	26.81	26.93	27.04
Crude fat	9.53	6.52	6.50	6.54
Moisture	10.01	17.38	17.19	17.61
Ash	12.84	18.89	18.46	14.98

Growth

The initial average weight of Koi carp fingerling increased from 3.77 to 4.99 g, 3.77 to 4.88 g, 4.05 to 5.22 g and 4.02 to 5.10 g in the fish fed with Diet 1, Diet 2, Diet 3 and Diet 4 respectively during the experimental period (Figure 1). The numerically highest average final weight was found in the Diet 3 and the numerically lowest weight was in Diet 2 fed fish. However, there were no significant difference in the final mean body weight of four different diets fed fish ($p > 0.05$) ensuring that the protein percentage is sufficient to the Koi carp stages to achieve the normal growth rate. Some deviation was observed in the growth pattern as it showed a weight loss in the second week in T1 and another decreased weight in the fourth week in T2. This may either be due to any different and/or abnormal metabolism condition during the stage of the experimental fishes or any stress situation due to nutritional / environmental factors during the period.

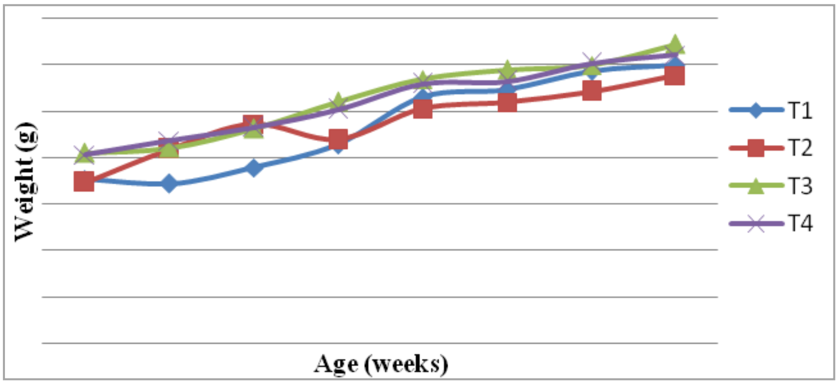


Figure 1. Comparison of body weight of Koi carp fingerlings for different diets

According to the results, the numerically highest weight gain of fingerlings was observed in T1 (1.220±0.089), followed by T3 (1.168±0.053), and T2 (1.150±0.066). Lowest numerical weight gain was observed in the T4, (1.075±0.022). The statistical analysis revealed that the effect of four different diets on weight gain of fingerling during eight weeks was not significant ($P > 0.05$). Further, there was no effect of *C. sativum* as enrichment medium for the feed on the growth of the fishes. Thus, it can be suggested that, in this experiment, there was no effect from the enrichment on their better growth.

Though the growth of the fishes of four treatments were not significant (P> 0.05), the numerically higher growth response shown by the T1 may be due to high protein content in the Diet 1, the commercial feed. Furthermore, young Koi carp fish require a higher percentage of protein (35-40 %) in their diet than older fish because the process of growth demands more amino acid absorption than maintenance of the body (www.fishchannel.com). Our laboratory prepared experimental feeds have comparatively lower protein level than the commercial feed. According to Faturoti *et al.*, (1986), recommended protein level for optimum growth and nutrient utilization in *Cyprinus carpio* should be between 35 – 40 %. In the present study, it was between 26–27 % in the diets and hence, the weight gain has been lowered numerically, compared to the commercial feed fed fish. However, it can be suggested that the low protein levels can also work without affecting the fingerling growth significantly.

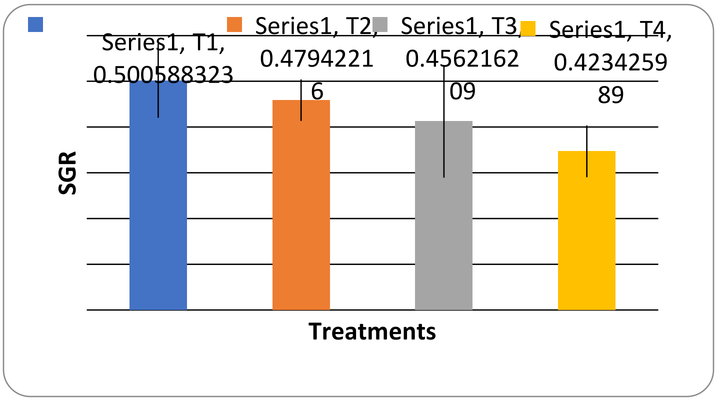
The optimum feeding frequency for maximum growth of fish depends on the fish species, fish size, culture conditions, environmental factors and dietary nutrient (protein). Saeed *et al.*, (2011), indicated that increase in feeding frequency, affect to the high specific growth rate and protein efficiency ratio in the carcass of carps. It was indicated that, protein intake which was highest in three times daily feeding frequency contributed to the best growth in *C. carpio* juvenile (Wang *et al.*, (1998) and Baçınar *et al.*, (2007)). In the present experiment, feeding frequency was twice a day, which might be affecting to the weight gain of Koi carps.

Growth and production in fish culture are generally dependent on the daily feed quality and consumption and feeding frequency (Mookerjee and Mazumdar, 1946). Further, the growth of carp (*C. carpio*) increases with protein levels, and there was an approximately linear increase of growth with feeding level for any given diet. However, during the second and third week, the growth rate of T1 and T2 deviate from being linear.

The overall mean SGR (Figure 2) for Diet 1 was 0.500±0.023, followed by Diet 2 (0.479±0.013), Diet 3 (0.456±0.035) and Diet 4 (0.423±0.016) showing the overall mean values of SGR of different diets were not significantly different from each other (Table 4).

Table 4. Growth performance of Koi carp fingerlings fed on different diets

Parameter		Treatments				
		T1	T2	T3	T4	p value
Initial weight	(g)	3.770±0.201	3.730±0.119	4.050±0.271	4.027±0.128	0.538
Final weight	(g)	4.991±0.272	4.881±0.180	5.219±0.256	5.102±0.123	0.725
Weight gain	(g)	1.220±0.089	1.150±0.066	1.168±0.053	1.075±0.022	0.473
SGR		0.500±0.023	0.479±0.013	0.456±0.035	0.423±0.016	0.201



T1-Diet1,

T2-Diet2,

T3-Diet3,

T4-Diet4

Figure 2. Comparison of SGR for different diet groups

According to Al Hafedh (1999), growth rate of fish increases with increase in the level of dietary protein till the optimum level is reached. Jana and Chakrabarti (1993), suggest that growth, reproductive potential, and survival of each species are affected by the nutrient conditions of the culture media. In the present study, the experiment was conducted in closed condition in the aquaria that were different from any natural environment. There are several factors that influence the precise protein requirements of Koi and Goldfish. Baarvick and Jobling (1990), have reported that the increased competition among fish of similar size possibly resulted in growth retardation. The test fish used in this study were relatively size-sorted, with the least dispersion in the all-diet groups. Furthermore, space requirement of fish also affects the growth. When space was limited, the growth rate declined (Baarvick and Jobling, 1990). In the present study space requirement of fish was limited and they were stocked in small glass tanks. Thus, might not observe higher growth like in ponds as carps prefer ponds than glass tanks. However, there was no sufficient evidence to claim competitive effects on the low SGR in diets.

Thus, needed to further investigate the growth effect of nutrient interrelationship in diet formulation.

As the water quality parameters influence the physical properties and chemical composition of water, its correct management can improve the overall fish performance of health and growth. Water quality parameters; temperature, pH, Dissolved Oxygen (DO), nitrite and toxic ammonia of the present study were shown in the Table 5 and are of acceptable range. Eshchar *et al*., (2006) concluded that the recommended water quality parameters for Koi carp farming are temperature (26-32.7 ºC), pH (5-9), DO (above 5 mg/L), Nitrite (below 0.1 mg/L) and toxic ammonia (below 0.025 mg/L) and water quality of the experiment were within this range. However, lower temperature levels and slight increment in Nitrite levels could be observed in the experimental tanks than the recommended levels.

Table 5: Water quality parameters of different treatments (Each value is the mean of three replicates)

Water quality parameter	T1	T2	T3	T4
Temperature (ºC)	28.80±0.13	28.85±0.14	28.72±0.11	28.87±0.14
DO (mg/L)	4.37±0.00	4.45±0.03	4.47±0.04	4.49±0.05
pH	6.92±0.02	6.90±0.02	6.87±0.01	6.95±0.01
Nitrite (mg/L)	0.141±0.004	0.118±0.001	0.122±0.001	0.130±0.002
Toxic Ammonia (mg/L)	0.0045±0.0003	0.0026±0.0002	0.0017±0.0001	0.0035±0.0002

Mumtazuddin *et al*., (1982) and Rehman *et al*., (1982), has reported that, when temperature changes, it has direct effect on oxygen demand, food requirement and food conversion efficiency. When temperature is high, oxygen and food requirement become higher, as a result, growth rate also increase. In the present study, temperature was in low level, affecting to oxygen and food requirement of fish, that might have decreased the SGR. Several studies have investigated the relationship between oxygen saturation and food intake in fish. When the oxygen content drops below 59% fish starts to lose its appetite. Rainbow trout (*Oncorhynchus mykiss*) reduced its appetite when oxygen saturation fell below approximately 60% (Jobling, 1995). Similar results have been obtained from European sea bass (*Dicentrarchus labrax, L*) (Thetmeyer *et al*., 1999) blue tilapia (*Oreochromis aureus*) (Papoutsoglou and Tziha, 1996) channel catfish (*Ictalurus* *punctatus*) (Buentello *et al*., 2000), juvenile turbot (Pichavant *et al*., 2001) and common carp (*Cyprinus carpio. L*) which showed reduced growth when exposed to low oxygen levels. According to Akinwole & Faturoti (2007), low concentrations of oxygen can produce negative impacts on fish health, like poor growth performance, low feeding rate, and increase risk on potential diseases or even fish death. This could also have happened to the growth rate of koi carp fingerlings in the present study.

The pH ranged from 6.9 to 9.1 which were similar as reported by Swingle (1967), suitable range for koi carps. An increase or decrease in pH disturbs the acid-base balance, ion regulation, and ammonia excretion. According to Wedemeyer and Yasutake (1978), high nitrite concentration indicates reduced feeding and tissue damage. In the present study, as the Nitrite level was slightly high compared to the recommended level, it might have affected the feed intake of koi fingerlings.

Challenge study

After 56 days of feeding, three fish from each group were injected intra-peritoneally with 100 µL of live *A. hydrophila* at a concentration of the 0.8 ppm. Mortality observed for five days period has been shown in Figure 3.

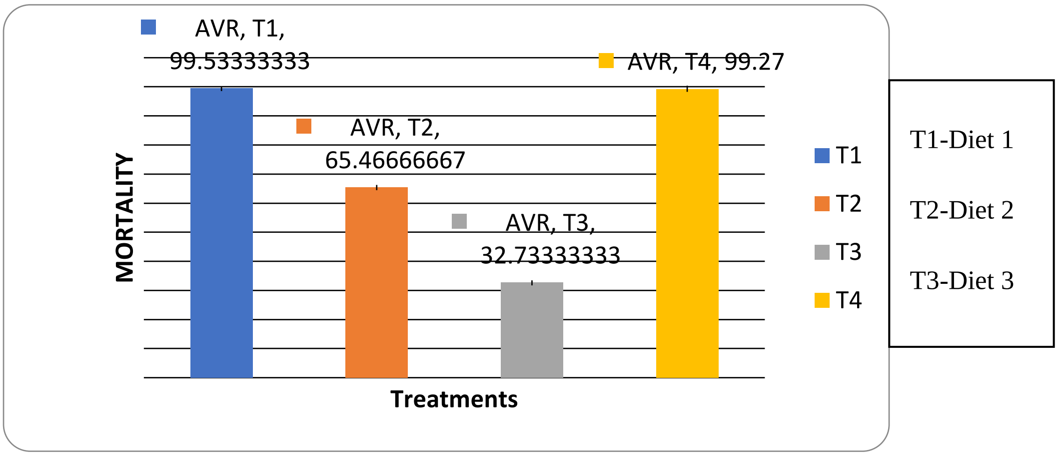


Figure 3. Comparison of mortality percentage of different diet groups

The lowest mortality percentage has been shown in Diet 3 (32.73%) and the highest mortality percentage was observed in Diet 1 and Diet 4. Results indicated that, 10 g of *C. sativum* infusion enriched feed may have some potential to enhance the immunity of Koi carp fingerlings.

Clinical symptoms and pathological changes of Koi carp fingerlings infested with *A. hydrophila*

The fish infected with *A. hydrophila* showed erratic swimming behavior and off feed within 24 hours post infection. Reddening of the skin and bleeding, abdominal distention and severe congestion in ventral area was prominent. Furthermore, enlarged gall bladder engorged with bile was observed and these symptoms were compatible with findings of Rao *et al.*, 2006.

Figure 4. Bleeding from gills

Figure 5. Haemoregic areas in the skin

Currently, bacterial infections in aquaculture, including hemorrhagic septicemia disease are mainly controlled by antibiotics. However, the use of antibiotics in aquaculture has received considerable attention recently because their use can lead to the development of drug resistant bacteria, thereby reducing drug efficacy. Moreover, the accumulation of antibiotics in both the environment and the fish can be potentially risky to the environmental bio diversity. To prevent such problems, use of environmentally friendly natural products has been considered as an alternative to control bacterial infections in aquaculture.

Previous studies revealed that nonspecific immune system of fish can be enhanced by herbal plant extracts. Sahu *et al*. (2007), reported that the survival rate after challenging the fish with *A. hydrophila* was enhanced in *Labeo rohita* fed diets containing *Mangifera indica* kernel. Similar results were also reported after feeding tilapia with two Chinese medicinal herbs, challenging with *A. hydrophila* (Ardo *et al*., 2008). Available reports show that both leaves and seeds contain antioxidants, but leaves were found to have a stronger effect (Helle *et al*., 2004). Britto and Kumar (2004), reported that immune-stimulatory activity of *C. sativum* is evidenced by increased proliferation of lymphocytes and production of interlukin. According to Stephen *et al.* (2006), *C. sativum* enhanced the antibody production in fishes to make antibody response against *Salmonella* sp., which was similar with findings of Innocent *et al.,* (2011). In addition to that, the effectiveness of *C. sativum* is clearly been proven, it causes for increase in hemoglobin content which may enhance the defense 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 to act as a potent immune-stimulant in fish (Innocent *et al*., 2011).

Total Erythrocyte Count (TEC) in immune-stimulant administered to *Catla 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,* thereby enhancing the nonspecific immunity. *C. sativum* enhanced the antibody production in fish to make antibody response against *Salmonella* sp. (Stephen *et al*., 2006).

It was known that, active ingredient of Linalool (LIN) possess antimicrobial, antibacterial and antiviral effects, as well as anti inflammatory, analgesic and local anesthetic activities (Deepa and Anuradha, 2011). According to Çabuk *et al*., (2003) the supplementation of 2% coriander seeds significantly improved daily live weight gain, feed conversion ratio and carcass yield of broilers. These positive improvements could be due to the essential oils present in coriander seed. Coriander seed contains essential oils (up to 1%) such as LIN (60-70%) and other monoterpenoids such as citronellol, geraniol, myrcene, α- and γ-terpinene, limonene, α- and β-phellandrene, p-cymene, α- and β-pinene and camphor. Further, LIN has appetizing properties and stimulating effects on the digestive process. It inhibits pathogenic microorganisms in the digestive system (Güler, 2011). The results of this study are in agreement with the previous results. However, further investigation regarding the immune-stimulator ability of *C. sativum* shall be carried out.

The T3 experiment showed the lowest mortality percentage during the total challenge period, and it might be due to the enhancement of the immunity of koi carp fingerlings. Therefore, it can be suggested that, while comparing the survival of experimental fish and the lowest mortality, Diet 3 was an effective feed type than the other formulated feed in this experiment. Further, 10 g is effective than infusion of 5 g of coriander for the better immunity status of the fingerlings.

Present study revealed that introducing a new low-cost koi carp fish feed to replace the expensive commercial fish feed in semi-intensive ornamental fish farming may be effective.

Table 6. Cost analysis of diet 3 during the experimental period

Ingredient	Amount	Price (Rs)
Fish meal	250 g	75.00
Prawn meal	100 g	25.00
Soybean meal	200 g	20.00
Sunflower oil	120 g	50.00

Rice bran	200 g	10.00
Wheat flour	100 g	10.00
Vit/min pre mix	30 g	15.00
Egg	1	15.00
Gelatin	5 g	5.00
Kotthamalli	10 g	10.00
Total		235.00

The commercial feed seems higher retail price (Rs. 340.00 per kg) during the study period compared to Diet 3 because its cost is Rs. 235.00 (Table 6.) without labor cost and other variable cost. In fish farming, nutrition is critical because feed represent 40-50% of the production cost and assuming it will increase day by day. The general problem of high feed cost associated with fish culture has been addressed by studies on the use of cheaper ingredients as protein sources. Another approach to reduce feed cost is to develop appropriate feeding management strategies and other improvements in husbandry (Abid *et al*., 2009). Keeping in view the economic importance of fish feeding, developing a nutritionally sound, low-cost feed to minimize the aquaculture risks is a timely need.

Conclusion

Study results indicated that *C. sativum* have some immuno-stimulator ability and can be suggested that it induces nonspecific immunity of koi carp fingerlings. Since the growth performances of experimental groups showed a non-significant ($p>0.05$), the quality of the experimental feed must be enhanced.

As suggestions, it is better to conduct Haematological and Serological analysis for identifying the immuno-stimulator ability of Coriander and further research is needed to assess the most effective level of Coriander (by disc diffusion technique) that induce some level of disease resistance and further enhancement of non-specific immunity of koi carps.

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