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Effect of *Mikania cordata* (Burm) B. L. Robins on Non-Specific Immune Response of *Catla catla* (Hamilton, 1822) Against *Aphanomyces invadans*

Vikash Kumar[1]*, Suvra Roy[1] and Debtanu Barman[2]

[1] *Central Inland Fisheries Research Institute, Barrackpore - 700 120, India*

[2] *Center for Aquaculture Research & Development, St. Xavier's Vocational Training Center, Don Bosco, Bishramganj, Tripura - 799 103, India*

Abstract

The study was conducted to evaluate the efficacy of *Mikania cordata* leaf powder on non-specific immune response and disease resistance of *Catla catla* fingerlings against *Aphanomyces invadans* infection. *M. cordata* extract was incorporated in the diet (at 0, 1, 2 and 3%) of *C. catla* fingerlings (19±0.95 g). Blood and serum sampling was carried out on 0^{th}, 14^{th}, 28^{th} and 42^{nd} days of feeding trials to determine Nitroblue tetrazolium (NBT) levels and myeloperoxidase activity. The results revealed that fishes fed with *M. cordata* extract showed significant increased in NBT levels and myeloperoxidase activity when compared to the control group ($p<0.05$). *M. cordata* leaf powder stimulates non-specific immunity and might help *C. catla* to resist fungal infection by *Aphanomyces invadans*.

Keywords: *Mikania cordata, Aphanomyces invadans, Catla catla,* phytotherapy

Introduction

Epizootic Ulcerative Syndrome (EUS) is one of the most destructive diseases among fresh and brackish water fish in the Asia-Pacific region and it is very common in northern and southern India, causing considerable loss to fish farmers. Ulcerative disease syndrome has been used to describe a group of cutaneous diseases of finfish (Hargis, 1985). Among different ulcerative diseases, the first report of a EUS like condition was reported in summer 1971, in farmed ayu (*Plecoglossus altivelis*), in Oita Prefecture, Japan (Egusa & Masuda, 1971). A similar disease was also reported in March 1972 from Central Queensland, Australia, where several species of estuarine fish had developed large shallow circular or irregular skin lesions termed 'red spot disease' (Rodgers & Burke, 1981). India witnessed the first major outbreak of EUS in 1988 in the states of Tripura, Assam, Meghalaya and West Bengal. It gradually spread to states of Orissa, Bihar, Uttar Pradesh, Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Haryana by 1992 (Das, 1997). It is one of the most destructive diseases among fresh water and brackish water fish in the Asia Pacific region causing large economic losses.

Phytotherapy is the oldest form of healthcare known to man-kind. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It is known that more than 4 00 000 species of tropical flowering plants have medicinal properties (Odugbemi, 2006). Herbs are an interesting alternative because they are inexpensive, renewable, locally available and can be easily prepared (Harikrishnan & Balasundaram, 2005). Many of the medicinal plants such as *Ocimum sanctum* (Logambal et al., 2000), *Acalypha indica*, *Phyllanthus niruri, Azadirachta indica, Piper betle, Mentha piperita* (Dinakaran, 2001), *Astragalus membranaceus, Lonicera japonica* (Ardo et al., 2008) *and Withania somnifera* (Sharma et al., 2010) have been shown to trigger innate immune system and enhance disease resistance against pathogenic organisms. *M. cordata* locally known as Refugeelata, Assamlata, Germanlata and Taralata belongs to the family *Asteraceae* (Nayeem et al., 2011).

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* *E-mail: kumar.vika.vikash2@gmail.com*

Aphanomyces invadans the fungal pathogen and causative agent of EUS is almost impossible to control in fish populations and no protective vaccines or effective drug/chemical treatments are available. As the plant *M. cordata* is known to have therapeutic value and there had been limited work on the efficacy of *M. cordata* in controlling *A. invadans* infection in fishes, the present study was designed to evaluate the efficacy of *M. cordata* leaf powder on the non-specific immune responses and disease resistance of *C. catla* fingerlings against experimentally induced *A. invadans* infection.

Materials and Methods

C. catla (Hamilton, 1822), fingerlings of average length 15±0.79 cm and weighing 19±0.95 g, were collected from a fish farm at Bishramganj, India. Fish were acclimatized in FRP (fiber reinforced plastic) circular tanks of approximately 500 liters capacity, at ambient temperature (26-28°C) with continuous aeration. They were fed twice daily with a diet (rice bran and mustard oil cake in the ratio of 1:1) at the rate of 4% body weight at 6.00 am and 6.00 pm. The optimum physico-chemical parameters of water *viz.,* dissolved oxygen (6.88 ± 0.56 mg l^{-1}) and pH (7.14 ± 0.77) were maintained throughout the experimental period.

M. cordata was collected from local farmers of South Tripura District, India. Leaves were washed thoroughly with tap water and dried under shade to make them suitable for grinding. The dried leaves were ground in a mechanical grinder and sieved. The powder obtained was stored in an air tight container for further use (Sharma et al., 2010).

Experimental diets were prepared with locally available ingredients containing 1, 2 and 3% of *M. cordata* leaf powder (Table 1). Initially all ingredients were mixed thoroughly by adding water, and then made into pellets by using a hand pelletizer (Xie et al. 2008) and then dried at 40°C for 12 h. The dried pellets were stored in an air sealed container and stored in cool dry place for further use.

Experiment was performed in 500 l FRP tanks. Fish were divided into four groups *viz.,* Control, T1, T2 and T3 (Table 1) and each group was maintained in triplicate set containing 60 nos. of fish. Control group diet was devoid of leaf powder while the remaining groups T1, T2 and T3 were fed with feed containing 1, 2 and 3% of *M. cordata* leaf powder. Fish were provided with adequate aeration and fed at the rate of 4% of body weight twice daily at 6:00 am. and 6:00 pm. The experiment was conducted for 42 days and the sampling for various immunological parameters was carried out on 0, 14^{th}, 28^{th} and 42^{nd} days of feeding trials. For each sampling, 8 fishes were selected randomly from each tank. Fish were anaesthetized with clove oil (Merck, Germany) and blood was drawn directly from the heart with the help of a sterilized 2 ml hypodermal syringe and 24 gauge needles containing 2.7% EDTA (Qualigens, India) as an anticoagulant. For serum separation, blood was collected without anticoagulant in serological tubes and stored in a refrigerator overnight. The clot was then spun down at 3000 x g for 10 min and serum collected was stored in sterile serum tubes at -20°C until used for assays. All the procedures were carried out in sterilized condition. After drawing blood, fish were given 1% $KMnO_4$ dip treatment and released to the tank.

Nitroblue tetrazolium (NBT) assay was determined by the method of Secombes (1990) as modified by Stasiack & Baumann (1996). Myeloperoxidase activity present in serum was measured according to Quade & Roth (1997) with slight modification as per Sahoo et al. (2005).

Catla (*C. catla*) affected with EUS were obtained from local fish farmer ponds. The affected muscle (approx. 2 mm^3) were dissected and placed on a Petri dish containing the isolation medium (Glucose peptone agar medium) (Lilley et al. 1998). Inoculated media are incubated at approximately 25°C and examined under an inverted microscope within 12 hours. Emerging hyphae tips were repeatedly transferred to fresh plates of GP agar until cultures are free from bacterial contamination. After four days, the agar from the resulting fungal mat was washed out by sequential transfer through five petri dishes containing autoclaved pond water (APW) and mats were kept in a petri dish containing 25 ml of (APW) at 20°C. After about 12 h, the motile secondary zoospores were collected and number of zoospores in the suspension was counted (6×10^4 spores per ml) using haemocytometer (Pradhan et al. 2008).

Challenge study with A. invadans spore

After 42 days of feeding with *M. cordata* supplemented diet, 10 fishes from each treatment group were selected randomly. The experimental fish were injected intramuscularly (into the left flank of fish

Table 1. Composition of control and experimental diets

Ingredients (per 100 g of feed)	Control	1 % (T1)	2 % (T2)	3 % (T3)
Wheat flour (g)	60	59	58	57
Fish meal (g)	35	35	35	35
Vitamin-mineral mix (g)	3	3	3	3
Cod liver oil (ml)	2	2	2	2
Mikania cordata leaf powder (g)	0	1	2	3

just below the middle of dorsal fin region) with 0.1 ml of spore suspension (6 x10^4 spores per ml) of *A. invadans* as described by Chinabut et al. (1995). The control fish groups were treated with 0.1 ml autoclaved pond water at the same time. After injection, experimental and control groups were kept separately in 500 l capacity fiberglass tubs containing 400 l water. The fishes were observed regularly for any overt signs of disease including behavioural abnormalities and mortality. Sampling of the survivors was carried out on the 14th day of *A. invadans* infection (Misra et al. 2006).

Relative percentage survivals (RPS) = 100 x

$$\frac{\text{Number of surviving fishes after challenge}}{\text{Number of fishes injected with } A.\ invadans}$$

Data were analysed using SPSS version 16 in which data were subjected to one-way ANOVA and Duncan's multiple range test (DMRT) was used to determine significant differences between the means. Comparisons were made at 5% significance level.

Results and Discussion

The fishes fed with diet containing *M. cordata* leaf powder at various levels showed significant ($p<0.05$) difference in nonspecific immune responses on 14th, 28th and 42nd days of sampling and relative percentage survival of *C. catla* after challenge with *A. invadans.*

The NBT activity of the experimental groups was found to be significantly ($p<0.05$) different in the treatment groups when compared to control and was observed to be highest in T2 group on 14th, 28th and 42nd days of sampling (Fig. 1). Herbs, which also act as immuno-stimulants, stimulate non-specific defense mechanisms and provide protection in fish (Pandey et al. 2012; Barman et al. 2014). Increase in non-specific immune parameters and resistance against *A. invadans,* in *C. catla* fingerlings after administration of *M. cordata* leaf powder through feed has been reported for the first time. As an alternative to chemotherapy, application of natural products, like plant extracts, in aquaculture is a new and developing venture (Jian & Wu, 2003).

Respiratory burst (NBT) activity measures the quantity of intracellular superoxide radicals produced by leukocytes (Sahu et al., 2007; Ardo et al., 2008; Kumar et al. 2014). In the present experiment, experimental groups fed with *M. cordata* supplemented diet have higher NBT activity as compared to the control group. Superoxide anion production by blood leucocytes was enhanced in *Labeo rohita* after feeding the fish with *Achyranthes aspera* seed (Rao et al., 2006). Feeding Nile tilapia (*Oreochromis niloticus*) with two herbal extracts (*Astragalus membranaceus* and *Lonicera japonica*) alone or in combination significantly enhanced phagocytic and respiratory burst activity of blood phagocytic cells (Ardo et al., 2008).

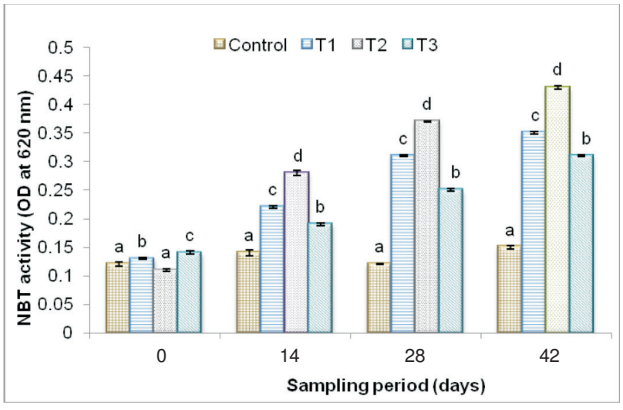

Fig. 1. NBT activity in *C. catla* fingerlings fed with various levels of *M. cordata* during different sampling days (values are mean ± SE). Superscripts indicate significant difference at ($p<0.05$)

The myeloperoxidase activity of the experimental groups increased significantly ($p<0.05$) in the treatment groups and showed an increasing trend from 14th to 42nd day of sampling (Fig. 2). The highest myeloperoxidase activity was found in treatment group T2 followed by T1 and T3. Myeloperoxidase (MPO) is a specific haemeprotein released by neutrophils and becomes functional during activation of neutrophils, which plays an important role in the non-specific defense. In the present study, *M. cordata* supplemented diet fed groups showed higher myeloperoxidase activity in comparison with control. Siwicki (1987) reported that *Cyprinus carpio* injected with levamisole showed increased myeloperoxidase activity. The present findings support the findings of Kumari & Sahoo (2006) and Behera et al. (2011), where *Clarias batrachus* fed with β-1, 3 glucan and *L. rohita* injected with curcumin showed increased myeloperoxidase activity. Similarly, higher myeloperoxidase activity was observed in *Labeo rohita* fed with carrageenan (Kumar et al. 2014).

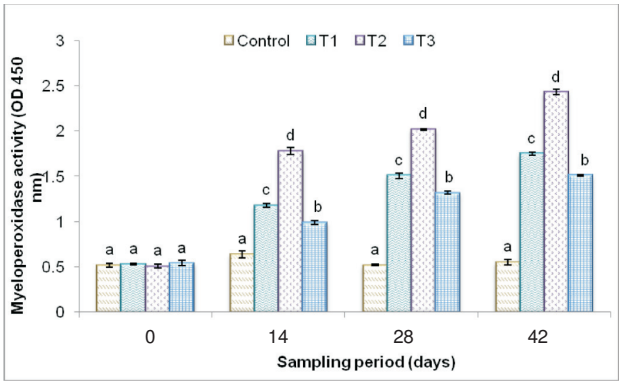

Fig. 2. Myeloperoxidase activity in *C. catla* fingerlings fed with various levels of *M. cordata* during different sampling days (values are mean ± SE). Mean values with different superscript within a column differ significantly ($p<0.05$)

Relative percentage survival of *C. catla* after challenge with *A. invadans* in different experimental groups is presented in Fig. 3. After injection with *A. invadans*, the first mortality was recorded after 8 days. The treatment groups fed with *M. cordata* leaf powder supplemented diet showed significantly ($p<0.05$) high disease resistance against *A. invadans* infection when compared with control group. The highest percentage survival was recorded in T2 (71.06%) followed by T1 (60.95%) and T3 (49.84%) groups. Sahu et al. (2007) reported that survival rate after challenging the fish with *A. hydrophila* was enhanced in *Labeo rohita* which were fed diets containing *Magnifera indica* kernel. Similar results were also reported after feeding tilapia (*Oreochromis niloticus*) with two Chinese medicinal herbs and challenging with *A. hydrophila* (Ardo et al. 2008).

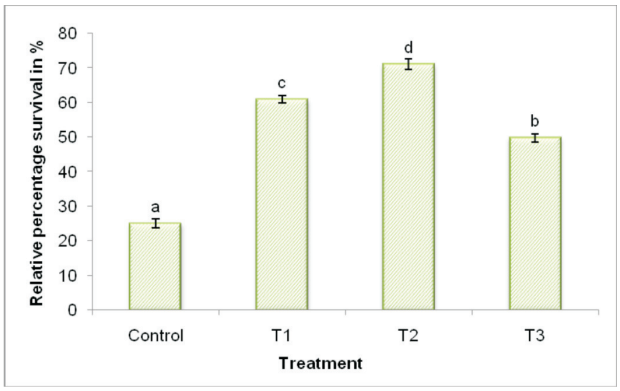

Fig. 3. Relative percentage survival (RPS) under different treatments fed with various levels of *M. cordata* in *C. catla* during different sampling days (values are mean ± SE). Mean values with different superscript with in a column for a parameter is significantly different, ($p<0.05$)

The study concluded that *M. cordata* leaf powder significantly increased non-specific immunity and decreased mortality in *C. catla* experimentally infected with *A. invadans*. The study opens up new approaches for future research on determination of most effective dose under pond conditions, degree and duration of the resistance offered, administrative regime for different age groups of fish and time of application to ensure improved harvest in culture ponds. Moreover, further studies are needed to determine the molecular mechanisms and also isolation and characterization of the bioactive compounds in *M. cordata.*

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References

Ardo, L., Yin, G., Xu, P., Varadi, L., Szigeti, G., Jeney Z. and Jeney, G. (2008) Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila.* Aquacult. 275: 26-33

Behera, T., Swain, P., Sahoo, S. K., Mohapatra, D., Das, B. K. (2011) Immunostimulatory effects of curcumin in fish, *Labeo rohita*. Indian J. Nat. Prod. Resour. 2: 184-8

Barman, D., Kumar, V., Roy, S., Kumar, K. and Rajendran, K.V. (2014) Use of *Mikania cordata* extract to control epizootic ulcerative syndrome disease in Tripura, India. World Aquaculture. 45 (1): 66-67

Chinabut, S., Roberts, R. J., Willoughby, L. G. and Pearson, M. D. (1995) Histopathology of snakehead, Channa striatus (Bloch), experimentally infected with the specific Aphanomyces fungus associated with Epizootic Ulcerative Syndrome (EUS) at different temperatures. J. Fish Dis. 18:41-47

Das, M. K. (1997) Epizootic Ulcerative Syndrome (EUS) in fishes – its present status in India. CICFRI (ICAR), Bull. No. 69, 22p

Dinakaran, M. R. (2001) Scope for using medicinal plant extracts as immunostimulants in finfish aquaculture. National Workshop on Aquatic Medicins. pp 81-82

Egusa, S. and Masuda, N. (1971) A new fungal disease of *Plecoglossus altivelis.* Fish Pathol. 6: 41-46

Hargis W. J. (1985) Quantitative effects of marine diseases on fish and shellfish populations. Transactions of the North American Wildlife and Natural Resources Conference. pp 608-640

Harikrishnan, R. and Balasundaram, C. (2005) Modern trends in *Aeromonas hydrophila* disease management with fish. Rev. Fish. Sci. 13: 281-320

Jian, J. and Wu, Z. (2003) Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker, *Pseudosciaena crocea* (Richardson). Aquacult. 218: 1-9

Kumari, J. and Sahoo, P. K. (2006) Dietary â-1, 3 glucan potentiates innate immunity and disease resistance of Asian catfish, *Clarias batrachus* (L). J. Fish Dis. 29: 95-101

Kumar, V., Kumar, S., Pandey, P. K., Raman, R. P., Prasad, K. P., Roy, S., Kumar, A. and Kumar, K. (2014) Growth and haemato-immunological response to dietary I-carrageenan in *Labeo rohita* (Hamilton, 1822) juveniles. Isr. J. Aquacult. Bamid. 66: 1-10

Lilley, J. H., Callinan, R. B., Chinabut, S., Kanchanakhan, S., MacRae, I. H. and Phillips, M. J. (1998) Epizootic Ulcerative Syndrome (EUS) Technical Handbook. Aquatic Animal Health Research Institute, Bangkok, Thailand. 88 pp

Logambal, S. M., Venkatalakshmi, S. and Michael, R. D. (2000) Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. Hydrobiologia. 430: 113-120

Misra, C. K., Das, B. K., Mukherjee, S. C. and Pattnaik, P. (2006) Effect of multiple injections of â-glucan on non-specific immune response and disease resistance in *Labeo rohita* fingerlings. Fish Shellfish Immun. 20: 305-31

Nayeem, A. A., Khatun, A., Rahman, MdS. and Rahman, M. (2011) Evaluation of phytochemical and pharmacological properties of *Mikania cordata* (Asteraceae) leaves. J. Pharmacognosy and Phytother. 3(8): 118-123

Odugbemi, T. (2006) Medicinal plants as antimicrobials In: Outline and Pictures of Medicinal Plants from Nigeria. University of Lagos press, pp 53-64

Pradhan, P. K., Mohan, C. V., Shankar, K. M. and Mohana, K. B. (2008) Infection experiments with Aphanomyces invadans in advanced fingerlings of four different carp species. In: Diseases in Asian Aquaculture VI (Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M. and Subasinghe, R. P. Eds), pp 105-114, Fish Health Section, Asian Fisheries Society, Manila, Philippines

Pandey, G., Madhuri, S. and Mandloi, A. K. (2012) Medicinal plants useful in fish diseases. Pl. Arch. 12(1): 1-4

Quade, M. J, and Roth, J. A. (1997) A rapid direct assay to measure degranulation of bovine neutrophils primary granules. Vet Immunol Immunopathol. 58:239-248

Rodgers, L. J. and Bruke, J. B. (1981) Seasonal variation in the prevalence of 'red spot' disease in estuarine fish with particular reference to sea mullet, *Mugil cephalus*. J. Fish Dis. 4: 297

Rao, Y. V., Das, B. K., Jyotyrmayee, P. and Chakrabarti, R. (2006) Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immun. 20: 263-273

Siwicki, A. K. (1987) Immunomodulating activity of levamisole in carp spawners, *Cyprinus carpio* L. J. Fish Biol. 31: 245-6

Secombes, C. J. (1990) Isolation of salmonid macrophage and analysis of their killing activity. In: Techniques in Fish Immunology (Stolen, J. S. T .C., Fletcher, D. P., Anderson, B. S. and Van M Winkel, W.B., Eds) pp 137-152, SoS Publication, New jersey

Stasiack, A. S. and Bauman, C. P. (1996) Neutrophil activity as a potent indicator of concomitant analysis. Fish Shellfish Immun. 37: 539-542

Sahoo, P. K., Kumari, J. and Mishra, B. K. (2005) Non-specific immune responses in juveniles of Indian major carps. J. Appl. Ichthyol. 21: 151-155

Sahu, S., Das, B. K., Pradhan, J., Mohapatra, B. C., Mishra, B. K. and Sarangi, N. (2007) Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish Shellfish Immun. 23: 109-118

Sharma, A., Deo, A. D., Riteshkumar, S. T., Chanu, T. I. and Das, A. (2010) Effect of *Withania somnifera* (*L. Dunal*) root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in *Labeo rohita* (Hamilton) fingerlings. Fish Shellfish Immun. 29: 508–512

Xie, J., Liu, B., Zhou, Q., Su, Y., He, Y., Pan, L., (2008) Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. Jian. Aquacult. 281: 5-11