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EVALUATION OF MEDICINAL PLANTS THROUGH FISH FEED AGAINST BACTERIAL FISH DISEASE

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ABSTRACT

Experiments were conducted maintaining under aquarium and pond conditions using feed containing medicinal plants/extracts was evaluated on artificially and naturally infected fish with bacterial pathogens. When the fish were exposed to high bacterial pathogens *Aeromonas hydrophila* Ah-11, *Pseudomonas fluorescens* Pf-13 and *Edwardsiella tarda* Et-70, recovery of the infected fish varied with the pathogens challenged and feed treatments. Fish feed containing bulb extract of *Allium sativum* (3% feeding) showed significantly ($p<0.01$) high therapeutic effect recovering the infected fish (Thai silver barb, *Barbodes gonionotus*) with *A. hydrophila* (100 ± 0% recovery) and *P. fluorescens* (90±0% recovery). A similar result was observed in the case of pangas fish feed containing decoction of leaves of *Calotropis gigantea* where 100 ± 0% *E. tarda* infected Thai pangas (*Pangasius hypophthalmus*) were found to be cured. These two types of herbal feed offered similar result when applied to the naturally ulcer-affected Thai silver barb and Thai pangas fingerling under aquarium condition. In pond condition, the herbal feed containing bulb extract of *A. sativum* was applied experimentally to *A. hydrophila* infected different fish species maintained in different individual ponds where, 91.67 ± 2.35% Thai silver barb, 84.99 ± 2.35% Rui (*Labeo rohita*) and 74.99 ± 2.35% Mrigal (*Cirrhinus cirrhosus*) were found to be recovered. In contrast, plants mixed pangas feed containing decoction of leaves of *C. gigantea* was cured 94.44 ± 3.84% *E. tarda* infected Thai pangas. The other medicinal plants used in this study showed medium to weak effect recovering the bacterial infected fish.

Key words : Medicinal plants, Fish pathogen, Herbal feed, Treatment

INTRODUCTION

Bacterial disease are responsible for heavy mortalities in both culture and wild fishes throughout the world and most of the causative microorganisms are naturally occurringopportunist pathogens which invade the tissue of a fish host rendered susceptible to infection (Roberts, 1989). The resistant bacterial strains could have a microbial resistance due to the use of antibiotics are the possible impacts on human health resulting from the emergence of drug-resistance bacteria in animals caused by the prolonged use of low-level antibiotics in animal feed (Sorum *et al*., 1992). Antibiotic

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residues may persist in sediments for a long time. Traditionally a number of herbs have been widely used in veterinary and human therapy. The World Health Organization (WHO) estimated that 4 billion people, 50% of the world population presently use herbal medicine for some aspect of primary health care. Herbal medicine is a common element in Ayurvedic, Homeopathic and Naturopathic treatments. Herbs or herbal products also have a role in aquaculture at present time (Direkbusarakom, 2000). In Thailand, during the outbreak of EUS in 1983 farmers who cultured snakehead fish in Uthaitance province used the bark of cork wood (*Sesbania grandiflora*) for the treatment haemorrhage lesions. The bulbs of *A. sativum* are used both for medicinal and culinary purposes (Vallachira, 1998) and it is a natural antiseptic agent (Anawer, 2001). *Calotropis* is used as a traditional medicinal plant (Rastogi and Mehrotra, 1991; Oudhia and Dixit, 1994). Traditionally calotropis is used alone or with other medicinals to treat common disease such as fevers, rheumatism, indigestion, caugh, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrohea (Das, 1996). *Calotropis* is also a reputed Homeopathic drug (Ferrington, 1990).

Many kinds of medicinal herbs are available in Bangladesh which grow naturally in roadside, fallow lands or in small jungles and most of them are cultivatable with very low cost. Many species of these herbs are used directly as human food or as medicine. Therefore, their use mixing with fish feed would be safe for aquatic environment as well as for human beings. So, it was of keen interest to observe whether herbal fish feed might be used as an alternative treatment for bacterial fish disease. The Government of Bangladesh has also given emphasis on herbal treatment not only for human beings but also for other animals including fish. Considering the above, the objective of the study was to evaluate the effect of feed prepared from indigenous medicinal herbs applying on artificially and naturally bacterial infected fish maintained under laboratory and pond condition.

MATERIALS AND METHODS

Trial for therapy under laboratory condition

Selection of herbs and bacteria

High effective five herbs viz., *Allium sativum*, *Calotropis gigantea*, *Momordica charantia*, *Polygonum hydropiper*, *Psidium guajava* and three high virulent bacteria (*Aeromonas hydrophila* Ah-11, *Pseudomonas fluorescens* Pf-13 and *Edwardsiella tarda* Et-70) were selected for this study based on the *in vitro* result obtained by Muniruzzaman and Chowdhury (2004). The herbs were applied to fish through feeding.

Preparation of fish feed containing herbs

The feed containing herbs for therapy was prepared according to the method described by Liping (1994) with minor modification.

Extracts collected from 1.0 kg garlic (bulb) mixed with 0.5 kg salt (NaCl) and 1.0 kg SABINCO fish powder feed. Require amount of sterilized water was mixed to make it

paste. Then pellet feed was prepared from this paste using feed preparation hand machine and air dried at room temperature with the help of an electric fan. The feed prepared in this way was named as "**Herbal Fish Feed**". Similarly, other herbal feed was prepared using 2.0 kg fresh leaves of *Momordica charantia*, 3.0 kg fresh leaves of *Polygonum hydropiper* and 3.0 kg fresh leaves of *Psidium guajava*. The leaves of individual medicinal plant were boiled in water for 30 min. and the decoction was mixed with the powder feed. An amount of 5.0 kg fresh leaves of *Calotropis gigantea* were boiled for 30-45 min. and the decoction was mixed with 3.0 kg pangas powder feed. The prepared feed was designated as "**Herbal Pangas Feed.**"

Application of herbal fish feed against artificially infected fish in aquarium

Thai silver barb, *Barbodes gonionotus* (15 to 20g in weight) were artificially infected by the selected virulent isolates *A. hydrophila* Ah-11 and *P. fluorescens* Pf-13 whereas Thai pangas, *Pangasius hypophthalmus* (40 to 50g in weight) were infected by virulent *E. tarda* Et-70 and maintained in the separate aquaria. For infection, the experimental fish were injected intramuscularly with 0.1ml of individual bacterial suspension of the pre-fixed dose ($2.5x10^6$ CFU/ml) at the base of dorsal fin. Control fish received only sterile physiological saline (o.85% NaCl). Infected ten fish were kept in 10-litre of laboratory maintained tap water in a 15-litre glass aquarium with aeration. The fish were first fed with herbal feed one hour after exposure to the pathogens. Thereafter herbal feeding continued once a day for 7 days of 10 days experimental period. The recovered fish in aquarium were released into clinical fish pond and kept there for 15 days to check the recurrence of disease in the treated fish under pond condition and confirmed the recovery by applied herbal feed.

Application of herbal feed against naturally ulcer-affected fish

The ulcer-affected Thai silver barb (15 to 20g in weight) were collected from bacterial affected pond under Sadar upazilla of Mymensingh district and fingerling of Thai pangas were collected from Al-Falah Agro Farm situated at Trishal upazilla. After acclimatization, ten fish of each species were maintained in each aquarium and 3 replications were done for each species. Fish feed with *A. sativum* mixture was applied on Thai silver barb and pangas feed with *C. gigantea* mixture was applied on fingerling of Thai pangas once a day for 7 days of 10 days experimental period at a dose of 3% body wt. of fish. Control fish were fed with no herb feed.

Application of herbal feed against artificially infected fish maintained in pond condition

Treatment 1

Thai silver barb, Rui (*L. rohita*), Mrigal (*C. cirrhosus*) were artificially infected by *A. hydrophila* Ah-11 and *P. fluorescens* Pf-13 separately. Two treatment ponds for each species along with control were maintained. One hour after exposure to bacterial pathogen in aquarium condition fish were released into these ponds at a stocking density of 0.75 fish/m^2 (30 fish/40m^2) and fed with herbal fish feed (fish feed + *A. sativum*) once a day for 10 days at a dose of 3% body weight of fish only in treatment ponds.

Treatment 2

Thai pangas were artificially infected by *E. tarda* Et-70 and maintained in four ponds (three treatments and one control pond). One hour after exposure to bacterial pathogens in aquarium condition fish were released in these ponds at a stocking density of 0.75 fish/m^2. Herbal pangas feed was applied once a day for 10 days at a dose of 3% body weight of fish only in three treatment ponds.

Statistical analysis

The analyses of data were done following one way analysis of variance (ANOVA) using MSTAT and SPSS statistical programme. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (Gomez and Gomez, 1984.

RESULTS AND DISCUSSION

Therapy under laboratory condition

In laboratory condition when the fish were exposed to *A. hydrophila* Ah-11, *P. fluorescens* Pf-13 and *E. tarda* Et-70, recovery of fish varied with bacterial pathogens, doses of feed and treatments. Results obtained from different treatments are shown in Table 1 and Table 2.

Values (mean ± SD) of the percentage of recovery with different superscripts (a, b, c, d) differ significantly in each treatment and with same superscripts do not differ significantly ($p<0.01$).

Treatment 1 (Fish feed + bulb extract of A. sativum)

Among the four doses (1%, 2%, 3% and 4% feed/kg body wt. of fish) of herbal fish feed applied on Thai silver barb, infected by *A. hydrophila* Ah-11, 3% feed was found to be most significantly ($p<0.01$) effective where 100 ± 0% (mean ± SD) fish were recovered. In case of 4% feed, 83.33 ± 5.77% fish were observed to be cured. In case of *P. fluorescens* Pf-13 infection, 3% feed was significantly ($p<0.01$) effective where 90.00±0% fish were found to be recovered.

Treatment 2 (Fish feed + leave extract of M. charantia)

In case of *A. hydrophila* Ah-11 infected fish, 80.00±0% fish were observed to recover at a dose of 3% feed whereas 23.33 ± 5.77% fish at a dose of 1% feed. Recovery was found to be 73.00±5.77% when the dose was 4%. No significant difference was observed between recoveries of fish at the dose of 3% and 4% feed. When herbal fish feed was applied on the fish infected by *P. fluorescens* Pf-13, 3% feed was found to be more effective (76.67 ± 5.77%) than the other three doses.

Treatment 3 (Fish feed + leave extract of P. guajava)

In case of A. hydrophila Ah-11 infected fish, high percentage (73.33 ± 5.77%) of fish were found to recover at a dose of 3% feed whereas the low percentage (33.33 ± 5.77%) at a

dose of 1% feed. When 3% and 4% feed applied on P. fluorescens infected fish, 70.00 ± 0% and 63.33 ± 5.77% fish were observed to be recovered and there was no significant ($p<0.01$) difference between recoveries of fish.

Table 1. Effect of herbal fish feed on Thai silver barb artificially infected with *Aeromonas hydrophila* Ah-11 and *Pseudomonas fluorescens* Pf-13 maintained under aquarium condition

Treatment	Dose (% feed/kg body wt. of fish)	Infection with *A. hydrophila* Ah-11		Infection with *P. fluorescens* Pf-13	
		Clinical signs of infection (%)	Recovery (%) of infection (mean ± SD)	Clinical signs of infection (%)	Recovery (%) of infection (mean ± SD)
T_1	1	50-70	33.33±5.77^d	50-70	33.33±5.77^d
	2	20-40	63.33±5.77^c	20-40	60.00±0.00^c
	3	0-10	100.00±0.00^a	0-10	90.00±0.00^a
	4	10-30	83.33±5.77^b	10-30	73.33±5.77^b
T_2	1	50-70	23.33±5.77^c	50-70	26.67±5.77^d
	2	30-50	50.00±10.00^b	30-50	46.67±5.77^c
	3	0-20	80.00±0.00^a	0-20	76.67±5.77^a
	4	10-30	73.00±5.77^a	20-40	63.33±5.77^b
T_3	1	50-70	33.33±5.77^c	50-70	26.67±5.77^c
	2	30-50	46.67±5.77^b	30-50	50.00±10.00^b
	3	10-30	73.33±5.77^a	10-30	70.00±0.00^a
	4	20-40	63.33±5.77^a	20-40	63.33±5.77^a
T_4	1	60-80	16.67±5.77^d	70-90	13.33±5.77^c
	2	30-50	43.33±5.77^c	40-60	36.67±5.77^b
	3	10-30	66.67±5.77^b	20-40	56.67±5.77^a
	4	0-20	83.33±5.77^a	30-50	43.33±5.77^b
Control with no herb		80-100	None	80-100	None

T_1 : Fish feed + *A. sativum*, T_2 : Fish feed + *M. charantia*, T_3 : Fish feed + *P. guajava*, T_4 : Fish feed + *P. hydropiper*

Treatment 4 (Fish feed + decoction of P. hydropiper)

The dose of 4% feed per kg body wt. of fish was found to be significantly ($p<0.01$) medicative than the other three doses where 83.33 ± 5.77% fish were found to be recovered. The herbal fish feed was less effective on *P. fluorescens* Pf-13 infected fish. The recovery of fish was 56.67 ± 5.77 at a dose of 3% feed.

Treatment with C. gigantea through pangas fish feed

The dose of 3% pangas herbal fish feed was found to be significantly ($p<0.01$) effective where 96.67 ± 5.77% fish were recovered while 80.00±0% at a dose of 4% feed (Table 2). When 2% feed was applied 60 ± 0% fish were found to recover. In all the cases, no fish of clinical pond was found to be further infected rather they became healthy and fresh.

Table 2. Effect of *C. gigantea* through feed on Thai pangas artificially infected with *Edwardsiella tarda* Et-70 maintained under aquarium condition

Dose (% feed/kg body wt. of fish)	Clinical signs of infection (%)	Percentage of recovery (mean ± SD)
1	50-70	33.33 ± 5.77^{d}
2	20-40	60.00 ± 0.00^{c}
3	0-10	100 ± 0^{a}
4	0-20	80.00 ± 0.00^{b}
Control with no herb	80-100	None

Data mean of 3 replications
Values (mean ± SD) of the percentage of recovery with different superscripts (a, b, c, d) differ significantly ($p<0.01$)

Effect of herbal fish feed against naturally ulcer-affected fish under laboratory condition

When herbal fish feed (feed + *A. sativum*) was applied on naturally ulcer-affected Thai silver barb, 100 ± 0% fish were found to recover whereas none from the control group where no herbal feed was applied (Table 3). Herbal pangas feed also detected as effective. In this case, 100 ± 0% pangas fingerlings of treated group were found to be recovered. The therapeutic effect of four types of herbal fish feed applied on experimental fish, *B. gonionotus*, artificially infected with *A. hydrophila* Ah-11 and *P. fluorescens* Pf-13 varied from one type feed to another and one dose to another. Among the four treatments, treatment 1 (fish feed + *A. sativum*) showed significantly ($p<0.01$) strong effect (100.00±0% recovery) on experimental fish, infected with *A. hydrophila* Ah-11 followed *C. fluorescens* by Pf-13 infected fish (90.00 ± 0% recovery) at dose of 3% feed/kg body wt. of fish. This 3% herbal feed also found to be most effective in reducing disease of naturally affected Thai silver barb. In most cases, 3% feed was found to be the most suitable dose in reducing the infection. The percentage of recovery was lower at a dose of 4% feed than that of 3% feed. It occurred due to pollution of aquarium water. Only one case (Treatment 4) experimental fish infected with *A. hydrophila* Ah-11 were found to be recovered more at a dose of 4% feed than 3% feed. In all cases, herbal fish feed were found to be more effective on *A. hydrophila* Ah-11 infected fish than *P. fluorescens* Pf-13 infected fish. In both cases of *A. hydrophila* Ah-11 and *P. fluorescens* Pf-13 infected fish, the treatment 4 (fish feed + *P. hydropiper*) was observed comparatively less effective than other three treatments. In all cases, none of the fish of control group was found to be cured. Yulin (1996) used *A. sativum* to control bacterial diseases and *P. hydropiper* to control bacterial enteritis and gill rot. Rajandra (1990) used *P. hydropiper* for treatment of enteritis or gill rot with feed. The findings correlate with ths present study. In case of herbal pangas feed (pangas feed + *C. gigantea*), 3% feed showed significantly ($p<0.01$) best effect both on *E. tarda* Et-70 infected Thai pangas and naturally affected Thai pangas fingerling. A powder of dried leaves of *C. gigantea* is an efficacious local application for ulcer, eczema and other skin diseases (Anawer, 2001). A decoction of leaves is also a useful washing and rapidly healing agent

for ulcer disease in human. The findings mentioned above support the present study. Use of the herb, *C. gigantea* is most probably first time in aquaculture.

Table 3. Effect of herbal fish feed against naturally ulcer-affected fish under laboratory condition

Feed	Fish exposed	No. of fish treated	Percentage of recovery (mean ± SD)
Herbal fish feed	Thai silver barb	10	100 ± 0
Control with no herb	Thai silver barb	10	None
Herbal pangas fish feed	Thai pangas	10	100 ± 0
Control with no herb	Thai pangas	10	None

Application of herbal fish feed against artificially infected fish maintained in pond

When fish were exposed to *A. hydrophila* Ah-11 and *P. fluorescens* Pf-13, recoveries of fish in treated group varied with bacterial pathogen and fish species exposed. Results are shown in Table 4. In case of *A. hydrophila* Ah-11 infected Thai silver barb, 91.67 ± 2.35% (mean ± SD) fish in the treated ponds were found to recover which was significantly ($p<0.01$) higher than the control ones where 8.33 ± 2.36% fish were recovered. When the fish exposed to *P. fluorescens* Pf-13, the mean percentage of recoveries was 78.33 ± 2.36 in the treated pond and 3.33 ± 2.36% in the control ponds. The experimental fish Rui (*L. rohita*), infected with *A. hydrophila* Ah-11 were found to recover 84.99 ± 2.35% in treated pond whereas 9.99 ± 4.71% in control pond which were significantly ($p<0.05$) different. On the other hand, recoveries of Rui fish, infected with *P. fluorescens* Pf-13 were 78.00 ± 2.83% and 3.33 ± 4.70% for treated and control pond, respectively.

Table 4. Effect of *Allium sativum* through fish feed on the experimental infection of different fish species with *A*eromonas *hydrophila* Ah-11 and *Pseudomonas fluorescens* Pf-13 maintained under pond condition

Fish exposed	Treated/ control pond	Clinical signs of infection (%)	Mean percentage of recovery (± SD)	Level of significance
A. Hydrophila	Treated pond	0-10	91.67 ± 2.35	**
infected Thai silver barb	Control pond	70-90	8.33 ± 2.36	
P. fluorescens	Treated pond	0-20	78.33 ± 2.36	*
infected Thai silver barb	Control pond	80-100	3.33 ± 4.70	
A. Hydrophila	Treated pond	0-20	84.99 ± 2.35	*
infected Rui	Control pond	70-90	9.99 ± 4.71	
P. fluorescens	Treated pond	0-20	78.00 ± 2.83	*
infected Rui	Control pond	80-100	3.33 ± 4.70	
A. Hydrophila	Treated pond	0-20	74.99 ± 2.35	*
infected Mrigal	Control pond	70-90	9.99 ± 4.71	
P. fluorescens	Treated pond	0-20	73.33 ± 0.00	*
infected Mrigal	Control pond	80-100	5.00 ± 7.07	

** : Significant at 1% level ($p<0.01$); * : Significant at 5% level ($p<0.05$)

Statistically significant ($p<0.05$) difference was observed in recovery of fish between treated and control pond. In the case of Mrigal (*C. cirrhosus*), challenged with *A. hydrophila* Ah-11, the percentage of recovery was significantly ($p<0.05$) higher (74.99 ± 2.35%) in treated pond than that of the control ponds where recovery was only 9.99 ± 4.71%. Similar result was found when Mrigal was challenged with *P. fluorescens* Pf-13 where recoveries were 73.33 ± 0% and 5.00 ± 7.07% in treated and control ponds, respectively and the variation is significant ($p<0.05$) differed. Herbal pangas feed was effective when applied on Thai pangas, infected with *E. tarda* Et-70 (Table 5). The percentage of recovery was found to be 94.44 ± 3.84 in treated ponds whereas 1.19 ± 2.06% in control ponds and the variation is statistically significant ($p<0.01$).

Table 5. Effect of *Calotropis gigantea* through pangas fish feed on the *Edwardsiella tarda* Et-70 infected Thai pangas maintained under pond condition

Experimental pond	Clinical signs of infection (%)	Recovery (%)	Percentage of recovery (mean ± SD)	Level of significant
1	0-10	96.66	94.44 ± 3.84	*
2	0-10	96.66		
3	0-10	90.00		
Control with no herb	80-100	0	1.19 ± 2.06	
Control with no herb	80-100	3.57		

* : Significant at 1% level ($p<0.01$)

Many kinds of herbs had been introduced to shrimp farms suffering from infections diseases in Thailand since 1990. For example garlic (*A. sativum*) or onion has been mixed to the shrimp pellet and fed every day to protect the bacterial infection (Direkbusarakom, 2000). Garlic contains acroline, crotonic, aldehyde and allyl sulphide, which act as a powerful germicide (Anawer, 2001). Chowdhury *et al*. (1991) found that the aqueous extract of garlic (*Allium sativum*) and allicin, a naturally occurring antibiotic from garlic both showed significant *in vitro* antibacterial activity against isolates of multiple drug-resistant *Shigella dysenteriae* 1, *Sh.flexneri* Y, *Sh. sonnei* and enterotoxigenic *Escherichia coli.* Liping (1994) mixed 1.0 kg root of garlic (*A. sativum*) with 0.5 kg NaCl, 5.0 kg powdered feed, 1.0 kg binder and some water and their herb-mixed feed was used for 100 kg fish to prevent and treat bacterial and fungal disease. The feed was fed once a day for 3-6 days. This is directly correlating the present study. In case of herbal pangas feed, no relevant finding was found. Dung (1990) applied *Eclipta alba* for treatment of necrosis in catfish caused by *A. hydrophila* and *E. tarda* and *Phyllanthus urinaria* for bacterial disease of pangas fish caused by *A. hydrophila* and *E. tarda*.

CONCLUTION AND RECOMMENDATIONS

In the recent years, the application of herbs to prevent and control microbial diseases has received increasing attention as an alternative treatment of chemotherapeutics. The

present study revealed that some herbs have an important role when use mixing with feed to control bacterial disease in fish. Further detailed studies are necessary before applying herbal therapy by herbal feed in field condition.

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