



Validation of an Indigenous Technical Knowledge (ITK) against the Epizootic Ulcerative Syndrome (EUS) of Fish

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ABSTRACT

Epizootic ulcerative syndrome (EUS) is a major setback in Fisheries with almost no cure. A farmer of Sabroom, South Tripura District developed a concoction of 8 kg mustard oil cake, 4 litre kerosene and 1½ kg soil for a hectare pond against this disease with encouraging result. We have visited the site, collected samples and validated the finding. The mixture was found enhancing the immunity and disease resistance of fish without affecting the water (temperature, dissolved oxygen, alkalinity, ammonia, nitrate, phosphate, nitrite and transparency), soil (pH, OC, available N and available P), plankton and proximate composition of fish. Blood glucose was significantly minimized, whereas, serum protein, enzyme, respiratory burst (NBT), lysozyme and bactericidal activity were increased ($p \leq 0.05$). Erythrocytes, leukocytes and hemoglobin content of the blood were also improved ($p \leq 0.05$). The mixture is easy to prepare and use; it requires single dose at last week of November. It is further eco-friendly and cost-effective (Rs. 690/ha), thus, suggested against the EUS.

1. Introduction

Epizootic ulcerative syndrome (EUS) is one of the most deadly fish diseases occur during winter due to an oomycete fungus, *Aphanomyces invadans*. Large hemorrhagic cutaneous ulcers, epidermal degeneration, necrosis and sloughing of scales are the principal symptoms of the EUS (Kar *et al.*, 2000). Tripura was the first State to report the losses due to the EUS in India during September, 1988 (ICSF, 1992) and currently, North East India has become a hub for EUS (Kar *et al.*, 2006). After almost three decades since its first occurrence, farmers of North-East and North India still today consider EUS as the most devastating in fish farming. CIFAX (1-liter/ha-m) is an effective preventive medicine against the EUS but its curative application is ineffective. Furthermore, timely availability of the CIFAX is a matter of serious concern in the remote places of Tripura and other NE states where livelihood heavily relies on Fisheries. Therefore, there is a search for cost-effective alternative medicines against the disease.

A number of indigenous technical knowledge (ITK) was evolved against the EUS (Kalita *et al.*, 2004; Goswami *et al.*, 2006; Das *et al.*, 2013). Recently a farmer of Tripura (Mr. Arun Patari, 45) awarded twice for his ITK of using kerosene-mustard oil cake-soil to control the EUS. Therefore, it was found worth reporting in this article so that relevant information can be refined further to combat the disease more effectively.

2. Materials and Methods

The study was conducted during December at Sabroom (23.00°N lat., 91.73°E long.), South Tripura. Mr. Arun Patari, the developer of the ITK was interviewed and information collected.

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The preparation of the concoction of kerosene, mustard oil cake and soil and its application in the pond was photographically documented (Plate 1-6). Information was also collected from the neighbouring farmers.

Blood samples were collected from the fish and analyzed for biochemical (glucose, protein, albumin, globulin, albumin: globulin), enzymatic (GOT-glutamate oxaloacetate transaminase, GPT-glutamic pyruvic transaminase and ALP-alkaline phosphatase), immune (Respiratory burst activity, lysozyme and bactericidal activity) and haematological parameters (erythrocyte, leucocyte and hemoglobin) using the standard protocols. Water and soil samples were collected from the ponds and analyzed for temperature, dissolved oxygen, total alkalinity, ammonia, nitrate, phosphate, nitrite, transparency, phytoplankton and zooplankton level in water and pH, organic carbon, available nitrogen and available phosphorus level in soil following the Standard Methods.

Flesh samples were collected from the fish and analyzed for the proximate composition using the methods of AOAC. Samples were also assessed from the ponds infected with EUS and compared. The data were analyzed in SPSS 11.2 using the one-way ANOVA and expressed as Mean \pm S.D. The difference was considered significant at 5% level ($p \leq 0.05$).

3. Results and Discussion

EUS is a serious problem in Tripura; farmers account 50-100% loss in fish farming due to it. It is difficult to identify in earlier stages and by the time it detects, mass mortality already occurs (Mohan and Bhatta, 2002). The livelihood of Mr. A. Patari is solely relies on fish farming. He is a progressive fish farmer of South Tripura. He is associated with fish farming since last fifteen years and every year encounters the EUS. During 2004-05, there was a severe EUS outbreak in his locality but he astonishingly noticed that his pond is unaffected. He was curious, started digging the matter and found that

the water of his pond is partially use for cleaning automobiles and receiving the wash-outs. He realized that might be the reason of no disease in his pond. He started doing experimentation. He mixed kerosene with mustard oil cake and applied it into pond and saw, disease intensity has been reduced in the pond. Gradually, he found that mixing some amount of soil with mustard oil cake and kerosene mixture and maturing it for a day further enhance its efficiency. He tried several combinations of the kerosene, mustard oil cake and soil and ultimately found that 8 kg mustard oil cake, 4 liter kerosene and 1½ kg soil gives best result in a hectare of pond. The infected fish shows healing symptoms after 10-15 days of application of the mixture.

The water and soil sample analysis has showed insignificant difference between the infected and non-infected ponds (Table 1); however, there was improvement in the immunity and disease resistance of fish with the use of the mixture. The glucose content of blood was reduced by 49.72% and A/G by 18.44%, whereas, protein content was increased by 39.77%, albumin by 54.05% and globulin by 25.56% (Figure 1), GOT activity by 81.82%, GPT by 62.12% and ALP by 53.8% (Figure 2), respiratory burst activity (NBT) by 44.44%, lysozyme activity by 56.9%, bactericidal activity by 73.3% (Figure 3), erythrocyte count by 75.88%, leukocyte count by 70.66% and hemoglobin content by 71.08% (Figure 4) with the application of the mixture. It clearly indicated that kerosene has immunostimulatory effect on fish. There is no information on using kerosene as fish immunostimulant, however it has been reported in the folk remedies against the problems of stomach, inflammation and ulcer. Furthermore, a recent study showed that about 70% Nigerians uses petroleum products medicinally against infectious diseases, autoimmune diseases, cancer, arthritis

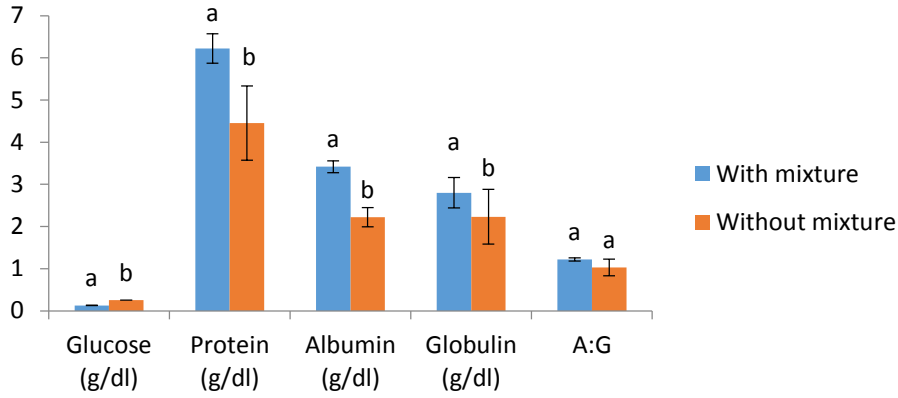


Figure 1. Biochemical responses of fish to kerosene-oilcake-soil mix. Bars having the same superscripts in same column are not significantly different ($p \leq 0.05$)

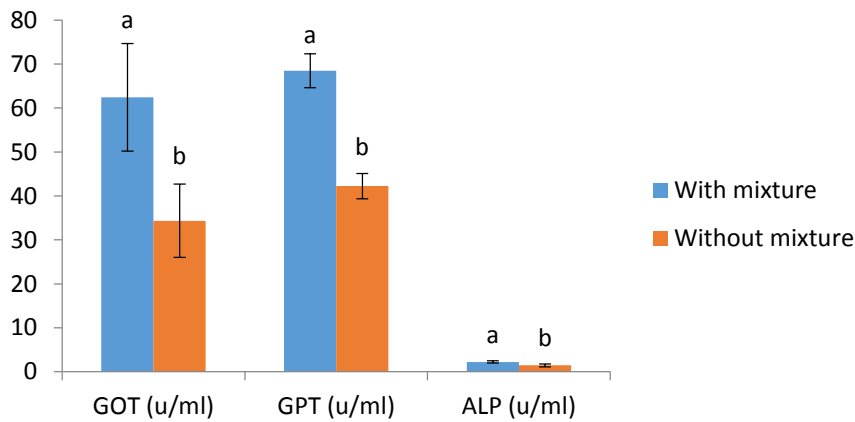


Figure 2. Enzymatic responses of fish to kerosene-oilcake-soil mix. Bars having the same superscripts in same column are not significantly different ($p \leq 0.05$)



Plate 1 & 2. Mixing of kerosene with mustard oil cake



Plate 3. Mixing of soil with kerosene-oil cake mixture

Plate 4. Broadcasting of the mixture into the pond



Plate 5. Fish (*Labeo bata*) showing wound healing



Plate 6. Fish (*Catla catla*) showing wound healing

and rheumatic diseases (Arikpo, 2010). During EUS, there is an overgrowth of *A. invadans* and secondary microbes in the infected sites; as a result, fish became immunologically depressed. Kerosene eliminates the microbes and parasites from the blood, bowel and infected organs (Last, 2012) and up-regulated the host defence mechanism by facilitating the function of phagocytes, natural killer cells, complement system, bactericidal and lysozyme activities, and antibody responses. Thus, the disease intensity is reduced and wounds healed. There is no report of using kerosene against the fish pathogenic fungi but it has been reported against the human pathogenic fungus, *Candida sp.* without altering the activity of normal gut bacteria (Awodele *et al.*, 2007).

The quantity of kerosene used in the present study, was much lower than acute toxic level, i.e., 5000 mg/kg (LD₅₀). Thus it didn't influence the water quality, soil quality and proximate composition of fish (Table 2).

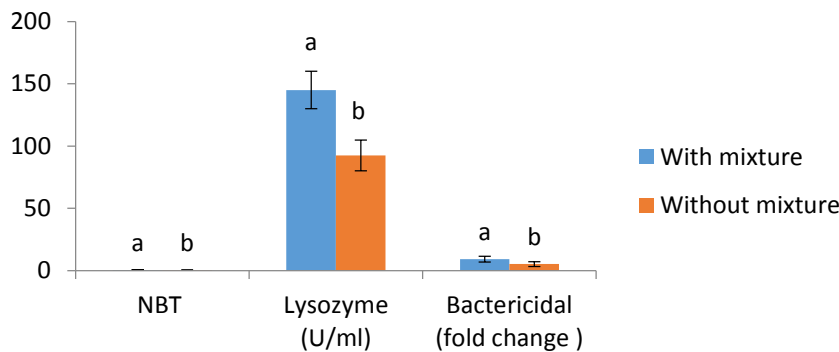


Figure 3. Immunological responses of fish to kerosene-oilcake-soil mix. Bars having the same superscripts in same column are not significantly different ($p \leq 0.05$)

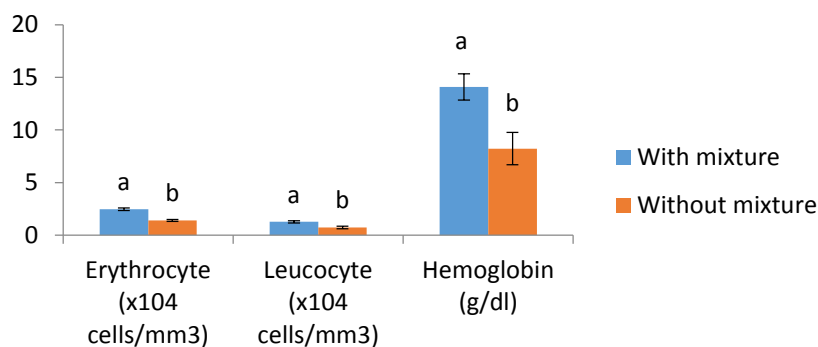


Figure 4. Hematological responses of fish to kerosene-oilcake-soil mix. Bars having the same superscripts in same column are not significantly different ($p \leq 0.05$)

Table 1. Water and soil quality parameters of the ponds. Figures in the same row having the same superscripts are not significantly different ($p \leq 0.05$)

Parameters	With mixture	Without mixture
Water quality		
Temperature ($^{\circ}$ C)	18.7 \pm 0.15a	18.6 \pm 0.11a
pH	7.16 \pm 0.05a	7.13 \pm 0.05a
Dissolved oxygen (mg/l)	4.63 \pm 0.15a	4.73 \pm 0.11a
Total alkalinity (mg/l)	62.25 \pm 0.95a	62.22 \pm 1.22a
TAN (mg/l)	0.56 \pm 0.01a	0.58 \pm 0.02a
NO ₃ -N (mg/l)	0.36 \pm 0.01a	0.36 \pm 0.01a
PO ₄ -P (mg/l)	0.33 \pm 0.03a	0.35 \pm 0.005a
NO ₂ -N (mg/l)	0.03 \pm 0.00005a	0.03 \pm 0.00005a
Transparency (cm)	42.44 \pm 1.10a	42.53 \pm 0.62a
Phytoplankton (x10 ³ cells/L)	6.32 \pm 0.12a	6.22 \pm 0.25a
Zooplankton (x10 ³ individuals/L)	1.82 \pm 0.11a	1.85 \pm 0.12a
Soil quality		
pH	6.52 \pm 0.10a	6.56 \pm 0.12a
Organic carbon	0.56 \pm 0.08a	0.55 \pm 0.11a
Available N	25.23 \pm 2.35a	24.3 \pm 1.05a
Available P	3.24 \pm 1.15a	3.54 \pm 1.12a

Another reason of reduced disease intensity was due to sanitization effect of kerosene on pond soil which is suspected to carry the agent of the EUS.

EUS is untreatable once occurs in the fish (Haniffa, 2011). Therefore, farmers are suggested for following the scientific management strategies in the form of de-silting, stocking of good quality fingerlings, regular water exchange, proper feeding, water quality management, health management etc. But, it is difficult to follow particularly by the marginal and small category of fish farmers. As a result, the pond environment became deteriorated and fish prone to the diseases. In that backdrop, kerosene-oil cake-soil mix can be suggested against the EUS.

The mixture is very effective. Further, the formulation and use of the mixture does not require any sophisticated technique, thus, farmers can adopt this to combat the EUS. The cost of the mixture is just Rs. 690/ha (mustard oil cake @ Rs. 30/kg, kerosene @ Rs. 50/l and one man-day @ Rs. 250/man-day) compared with Rs. 1000/ha requires in conventional treatment.

The only suggestion is to delaying the harvest by 10-15 days to avoid the off-flavour of kerosene from the fish. Finally, it could be concluded that, mustard oil cake (8 kg), kerosene (4 lit.) and soil (1½ kg) mixture is effective against the EUS. It is easy to prepare using locally available materials and apply. It requires single application preferably in last quarter of November. It fits well into the economy of marginal and small farmers. Therefore, the ITK is suggested for use. Further research is recommended for refinement and up-gradation of this ITK by blending modern techniques so that the problem of EUS could be more effectively managed for sustainable fishery production.

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Table 2. Proximate composition of fish (*Labeo rohita*). Figures in the same row having the same superscripts are not significantly different ($p \leq 0.05$)

Components (%)	With mixture	Without mixture
Moisture	70.43±1.36 ^a	70.71±1.38 ^a
Protein	17.15±1.31 ^a	17.41±1.22 ^a
Lipid	4.63±0.50 ^a	4.70±0.51 ^a
Ash	2.44±0.11 ^a	2.61±0.17 ^a
Nitrogen free extract (NFE)	5.33±3.08 ^a	4.55±2.02 ^a

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