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Toxicity of Ulcer disease of freshwater fishes and control aspects by tropical medicinal plant extracts in India

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ABSTRACT

Most of the fish diseases are originated from the polluted water. To control the Ulcer disease of the Koi fish (*Anabas testudineous*), twelve (12) Plant extracts were tried. It is revealed that the treated Koi fishes of the Eucalyptus, Arjun and Muchkunda treated pots were alive up to fifty (50) days. Whereas, the Koi fishes of the Control potare expired within ten (10) days. Immunity power of the treated Koi fishes of Eucalyptus, Arjun and Muchkunda had been increased in many times by collecting the nutrients from the broth of the pots. These plant extracts are biogenic, biodegradable and least toxic to the Koi fishes as well as to the human beings. These plants are produced locally. Plant extracts are simple, unfixed formulation and can be prepared easily. It is an eco-friendly control measure of the toxic Ulcer disease of the Koi fishes. Eucalyptus is the best performer followed by Arjunand Muchkunda. Plant extract chemicals might have an active role as a fish vaccine substratum in the immunisation mechanism of the treated Koi fishes in their lengthy lives.

Key word: *Fish Vaccine, Immunity, Plant extracts, Toxicity, Ulcer*

Introduction

Water of this planet is polluted mostly by environmental, industrial, agricultural and sewage pollutants which are produced by the human beings itself (Aremu *et al*., 2010). These pollutants have both direct and indirect influences on the ecosystem and behaviour of the aquatic living beings. Water pollution could affect the fish health by changing their biochemistry of the body, respiratory system, population structure, developmental growth and reproductive system (Arizo *et al.,* 2022).

Most of the fish diseases are created from the foul water quality. Higher quantities of organic materials, heavy metals, Nitrogen, Phosphorous, Hydrocarbons, Industrial wastes, Sewage, Pesticides, Insecticides in water which are the reasons of the Oxygen depletion. It changes the pH value and enhances the infectious microorganisms (Austin, 1998). Pesticides and heavy metals which enter the fishery water may damage or kill the fishes. Synthetic pesticides which are used in agricultural field for the control of weeds or harmful parasites are severe toxic in even very small quantity. Exposure to the heavy metals may damage the fish's capabilityto smell, disrupting its ability to locate the food, to protect itself from the attack from the predatory animals and also from the attack of the larger fishes (Ndhalala *et al.,* 2015).

Nitrogen and Phosphorus enter into the ponds,

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tanks, streams, lakes, canals, rivers and fisheries from the nearby agricultural crop fields where fertilizers are used randomly without proper thoughts. Nutrients wash into the water bodies through the storm, runoff which causes the exhaustion the oxygen in the water which is very essential for the survival of the fishes. These nutrients also encourage the huge, growth of the algae, fern and other watery vegetations. When they decay, they diminish the Oxygen level in the fishery water and kill the fishes (Saenphet *et al.*, 2001). The agricultural pesticides, industrial chemicals, sewage are the main sources of the water pollution which are the main causes of the diseases of the fishes (Passapan *et al*., 2004; Akter *et al*., 2006). Many fish diseases are also originated from the polluted water ofthe fishery. The pollutants in the water of the fishery encourages the enhancement of microbial population growth which invites the notorious fish diseases, such as: Gill disease, Liver damage, Epidermal papilloma, Wart, Finor Tail rot, Hyperplasia, Neoplasia, Scale loss and Ulcer toxicity (Ahmed *et al.,* 2007). The intensive culture systems, higher stocking densities and poor water qualities of the fisheries are the main reasons of the various toxicities of many diseases of the fishes (Chaudhuri, 1971; Barua *et al.,* 1989; Hossain, 2014).

Mucus layer on the outer body of the fishes hold the abundance immunity power which keeps the fishes aloof from the attack of the enemies like bacteria, fungus etc. The pathogens are not capable enough to enter into the external skin layers of the fishes. The water conditioners that contain Polyvinyl pyrilodone (PVP) may increase the Slime coat of the fishes. It also enhances the wound healing of the fishes. Use of the pharmaceuticals and antibiotics are very costly as well as hazardous to the living beings (Koski and Scott, 2003). Whereas, locally available traditional plant extracts are very cheaper, effective, economical, biodegradable, least toxic and ecofriendly (Biswas, 1982; Waterman *et al.,* 2010). The use of the plant extracts to control the Ulcer disease of the Koi fishes is an alternative method of the chemotherapy (Reverter *et al*., 2014; Stratev *et al*., 2018). Considering the above factors the present research project was taken up to search out the suitable plant extract or extracts out of twelve (12) to control the Ulcer disease of the Koi fish (*Anabas testudineous*).

Materials and Methods

Materials Required

1) Plastic Pot (5 lit size) - 13 Pcs.
2) Koi fishes (*Anabas testudineous*)
3) Water measuring bottles (1 lit, 500 ml, 250ml, and 100 ml) - 4 pcs.
4) Tested plant materials.
5) Tap water
6) Electric Waring –Blendor- 1 Pc.
7) Sharpen Chopping Knife- 1pc.
8) Wooden Block - 1pc.
a) Nylon Strainer - 1pc.
10) Small Balance - 1pc.

Plant parts which are used in this experiment were collected from the local area of Tamluk (Latitude - 22.2858° N, Longitude - 87.9189 E), East Medinipur, West Bengal, India. The collected Medicinal plants are:

Procedure for plant extraction

All the collected plant materials were washed off,

Table 1. List of used tropical medicinal plant parts against Ulcer disease of Koi fish (*Anabas testudineous*).

Sr. no.	Common Name	Scientific Name	Family	Plant Parts
	Marigold	*Tagetes erecta*	Asteraceae	Leaf
	Kadamba	*Neolamarckiacadamba*	Rubiaceae	Leaf
	Sheuli	*Nyctanthesarbortistis*	Oleaceae	Leaf
	Tulsi	*Ocimumsanctum*	Lamiaceae	Leaf and Seed
	Aloe Vera	*Aloe barbadensis*	Liliaceae	Leaf
	Casuarina	*Casuarina equisetifolia*	Casuarinaceae	Leaf
	Arjun	*Terminalia arjuna*	Combretaceae	Leaf
	Eucalyptus	*Eucalyptus globulus*	Myrtaceae	Leaf
	Guava	*Psidium guajava*	Myrtaceae	Leaf
	Gulancha	*Tinospora cordifolia*	Menispermaceae	Leaf
	Muchkunda	*Pterospermumacenifolium*	Sterculiaceae	Leaf
	Pineapple	*Ananus comosus*	Bromeliaceae	Leaf base

cut into small pieces and weighed 100 gm by a small balance and grinded by Waring - Blendor with mixing tap water, strained through nylon netstrainer and made up the volume up to 100 ml. This prepared plant extract was considered to be 50% concentration. The selected plant extracts of 100 ml is mixed with the additional tap water and made, the volume upto 1 l. In this way, the 12 plastic pots of 5 litre capacity and one control pot with plain tap water were made ready for the individual assay of the different plant extracts against the five diseased Koi- fishes (*Anabas testudineous*) of each pot (Biswas, 1982; Nargis *et al.,* 2011). The Koi-fishes were freed in the separate labelled pots filled with plant extracts for the studies of the potential power of the individual plant extracts. The readings of the results were recorded at a fixed interval of time of one to five days (Joshi *et al.,* 2011). The experiment was conducted in the Summer season (38 °C) where the evaporation of water from the treated pots created the problem. To solve this problem, an additional 250 ml tap water per pot had added per week against the evaporation to maintain the water level of the treated pots uniformly upto one litre for the easy movements of the treated Koi fishes.

Results and Discussion

Aquatic pollution of the fisheries causes the serious diseases of the Koi fishes (*Anabas testudineous*) (Fig. 1). The results in Table 2 demonstrated that on the third day of the experiment, single treated Koi fish in pot of the Pine apple (*A. comosus*) extract and two untreated Koi fishes of Control pot are died due to the toxic Ulcer disease (Fig. 2). Noother fish is affected.

On the fifth (5th) day, the treated Koi fishes of five (5), pots such as: Pineaplle (*A. comosus*) (4), Casuarina (*C. equisetifolia*) (3), Guava (*P. guajava*) (3), Gulancha (*T. cordifolia*) (2), Sheuli (*N. arbortistis*) (1), control (4) which are partially infected by the Ulcer disease died partially. Other seven treated pots are not affected at all, they are Alovera (*A.barbadensis*), Arjun (*T. arjuna*), Eucalyptus (*E. globulus*), Kadamba (*N. cadamba*), Marigold (*I. erecta*), Muchkunda (*P. acerifolium*) and Tulsi (*O. sanctum*).

Fig. 1. Aquatic Pollution causes serious illness of fishes at Tamluk.

Fig. 2. Ulcer disease of Koi fish(*Anabas testudineous*) at Tamluk.

At the age of 10th day of the experiment, the treated Koi fishes of the three (3) plant extracts such as: Guava (*P. guajava*); Pineapple (*A comosus*) and Sheuli (*N. arbortistis*) and the all fishes of the Control, pot, all are expired totally. The treated Koi fishes of other four (4) such as Casuarina (*C. equisetifolia*) (4), Kadamba (*N. cadamba*) (3), Gulancha (*T. cordifolia*) (3) and Tulsi (*O. sanctum*) (1) all died partially. But the treated Koi fishes of the rest five pots are not affected at all. They are: Aloevera (*A.barbadensis*), Arjun (*T. arjuna*), Eucalyptus (*E. globulus*), Marigold (*T.erecta*) and Muchkunda (*P. acerifolium*).

On the 20th day, it is observed that the treated Koi fishes of Two pots: Aloevera (*A. barbadensis*)(3) and Tulsi (*O. sanctum*)(3) died in the treated pots partially. The treated Koi fishes of the other six pots died fully. They are Casuarina (*C. equisetifolia*),

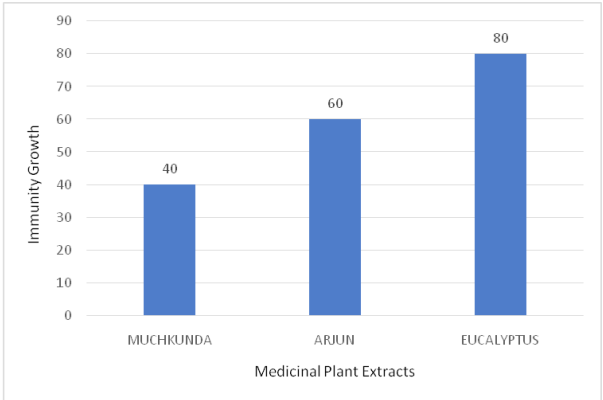

Fig. 3. Comparative study of the immuinity growth produced by the three medicinal plant extracts against the Ulcer disease of Koi fish (*Anabas testudineous*).

Table 2. Study on the potentiality of the Effectiveness of the twelve Crude Plant Extracts against the Ulcer Disease of Koifish (*Anabas testudineous*) at Tamluk.

Sl. No.	Plant Extracts	No. of Dead Koi Fishes Period (Days)													
		1	2	3	4	5	10	15	20	25	30	35	40	45	50
1	Aloe Vera	0	0	0	0	0	0	2	3	4	5	5	5	5	5
2	Arjun	0	0	0	0	0	0	0	0	0	0	1	1	1	2
3	Casuarina	0	0	0	2	3	4	5	5	5	5	5	5	5	5
4	Eucalyptus	0	0	0	0	0	0	0	0	0	0	0	0	0	1
5	Guava	0	0	0	2	3	5	5	5	5	5	5	5	5	5
6	Gulancha	0	0	0	1	2	3	5	5	5	5	5	5	5	5
7	Kadamba	0	0	0	0	0	3	5	5	5	5	5	5	5	5
8	Marigold	0	0	0	0	0	0	0	0	1	2	3	5	5	5
9	Muchkunda	0	0	0	0	0	0	0	0	0	1	1	2	2	3
10	Pineapple	0	0	1	3	4	5	5	5	5	5	5	5	5	5
11	Sheuli	0	0	0	0	1	5	5	5	5	5	5	5	5	5
12	Tulsi	0	0	0	0	0	1	2	3	4	4	5	5	5	5
	Control	0	0	2	3	4	5	5	5	5	5	5	5	5	5

Guava (*P.quajava*), Gulancha (*T. cordifolia*), Kadamba (*N.cadamba*), Pineapple (*A. comosus*) and Sheuli (*N.arbortistis*). But the rest other, treated four pots are not affected at all. They are: Arjun (*T. arjuna*), Eucalyptus (*E.equisetifolia*), Marigold (*T.erecta*) and Muchkund[1] (*P.acenifolium*).

On the 50th day, remarkable data were recorded. The treated Koi fishes of the nine (9) plant extracts, all expired except the three. They are: (1) Eucalyptus (*E. globulus*) (2) Arjun (*T. arjuna*) and (3) Muchkunda (*P. acerifolium*) .On the basis of performances of the plant extracts against the toxic Ulcer disease of the Koi fish it is revealed that the Eucalyptus. (*E. globulus*) is the best performer followed by Arjun (*T. arjuna*) (2) and Muchkunda (*P. acerifolium*) (3). The mortality 20% against Eucalyptus, 40% in Arjun and 60% in Muchkunda (Fig. 4). On the other hand, the immunity power of the treated Koi fishes developed

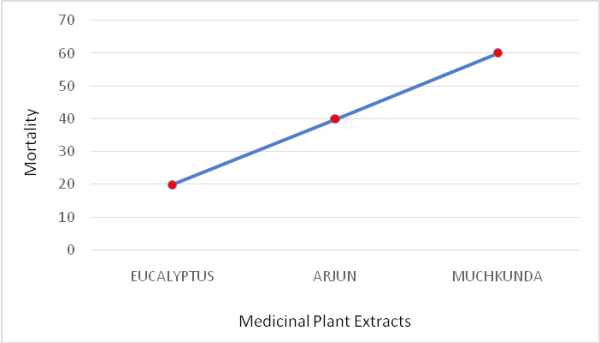

Fig. 4. Comparative study of the three medicinal plant extracts against the mortality due to the Ulcer disease of Koi fish (*Anabas testudineous*).

80 percent in Eucalyptus, 60 percent in Arjun and 40 percent in Muchkunda (Fig. 3) (Ravikumar *et al.*, 2010; Jeba *et al.*, 2011; Mahapatra and Biswas, 2023 (b)). It is apprehended that the resistant Koi fishes acquired their nutrients from the broths of the pots which were placed under the open sun. The feeds of the treated Koi fishes such as: algae, fungi, bacteria, phytoplankton, zooplankton, protozoa; ete. were grown sufficiently in the water of the pots profusely with the support of the plant extracts, sun light, water, and the excreta of the Koi fishes (Mahapatra and Biswas, 2023 (a). It is perceived that the Biofloc Technology might have taken the active role in this process (Ray *et al.*, 2010 and De Schryver *et al.*, 2008, Hargreaves, 2006). This process acts as a Water Treatment Remedy of the pot. It is also, the active suspension pondtechnology for the fish culture which reproduces the natural feeds of the koi fishes in the broth of the pots.

Plant extracts are least toxic, cheaper, and easily available in the local rural areas. These are biogenic, biodegradable and easily available (Biswas, 1982). It is produced locally in simple and unfixed formulation which could be prepared easily. This method is an ecofriendly control measure of the toxic Ulcer disease of the Koi fish. Eucalyptus plant extract is the best performer followed by Arjun and Muchkunda (Olusola *et al.*, 2013; Stratev *et al.*, 2018, Waterman *et al.*, 2010; Mahapatra, and Biswas 2024). The treated Koi fishes might have been immunised by oral vaccine due to the plant extract materials were the main source of the production of the feeds

for their consumption. Plant extract ingredients might have an active role as a" Fish Vaccine" substrates in the immunisation mechanism of these treated Koi fishes. The treated Koi fishes are till active even after the period of 50 days which is a remarkable record of this project (Jeba *et al.,* 2011; Nahak and Sahu, 2014; Aremu e*t al*., 2012).

It is presumed that the plant extract chemicals would have some vaccinated role in the body of the treated Koi fishes which conducted the enhancement of resistance power against the invasion of the Ulcer disease of the Koi fishes (Vinitnantharat, 1999), Su *et al*., 2022, Meachasompop *et al.,* 2023). Koi fishes responded to the Vaccine activities in their bodies under the direct sunlight upto the temperature of 38 °C. The higher the water temperature, the faster the immune response of the Koi fishes in their bodies. The fish vaccine method might be significantly reduce the specific Ulcer diseased related loss (Vinitnantharat *et al.,* 1999; Horne *et al*., 1984; Kumwan *et al.,* 2023; Kembou *et al*., 2023.

Efficiency of the fish Vaccines are superiorover the Antibiotics because they are natural, biological, live - materials which leave no residual effect in the body of the treated Koi fishes or in the enviroment. Alsoit would not induce any resistant strain of the Ulcer disease organism. (Vinitanantharat *et al*., 1999; Horne *et al*., 1984; Chakraborty 2019; Larsen *et al*., 1997; Gudding *et al*., 1999, Marana *et al*., 2019). Pharmaceuticals and antibiotics for the treatment of the Koi fish diseases are diminishin gowing to the bioaccumulation of poisonous chemical compounds in the body of the fishes which ultimately harm the human beings. Industrially produced medicines are too expensive. The antibiotics recommended for the fish diseases are very costly besides having other harmful effects to the human body (Koski and Scott, 2003; Hargreaves, 2006).

Use of plant extracts to control the fish diseases are an alternative to chemotherapy (Reverter *et al*., 2014; Stratev *et al*., 2018; Joshi *et al*., 2011). Immunity power of the treated Koi fishes had been developed many times. Plant extrats have mostly zero chances of residual toxicity. It is an ecofriendly treatment of the fish diseases which will save our next generations from different health hazards (Aremu *et al*., 2010; Jeba *et al*., 2011; Olusola *et al*., 2013; Citarasu, 2010; Chai *et al*., 2005).

Koi fishes control the mosquitoes by consuming eggs, larvae, and pupa which in directly protects the human being from the invasion of the mosquito borne diseases such as Encephalytis, Dengue etc. (Mahapatra and Biswas, 2023(c); Mahapatra and Biswas, 2024).

The Koi fish is rich in iron and copper which is the vital component for the haemoglobin synthesis. It contains an easily digestible poly- unsaturated fatty acids (PUFAs) and essential amino acids (EAA) (Sarma *et al*., 2010). Koi fish is an important food diet for the convalescent patients (Saha *et al*., 2009). Demand of Koi fish is more and Market value is high. More scope for employment in this Koi fish culture business (Panda, 2016; Chakraborty, 2020).

Conclusion

Due to severe water pollution many notorious diseases are noticed in the Koi fishes. Here, twelve (12) plant extracts were tried to control the Ulcer disease of Koi fish (*Anabas testudineous*). Three plant extracts such as Eucalyptus, Arjun and Muchkunda are the most prospective out of twelve (12). Out of the three (3), Eucalyptus is the best performer followed by Arjun and Muchkunda. These are easily available, locally prepared in simple and unfixed formulation which are biogenic, biodegradable and least toxic to the Koi fish as well as to the human beings. It is an ecofriendly technique to control the toxic Ulcer disease of the Koi fish which will be helpful to our next generations to maintain their good health. Plant extract chemicals might have an active role as a fish vaccine substratum in the immunisation mechanism of the treated Koi fishes.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

Ahmed, G.U., Dhar, M., Khan, N.A. and Choi, J.S. 2007. Fish Diseases. Bangladesh Agl. University. *J. of Life Science*. 17(10): 1309-1314.

Akter, S., Ahmed, G.K., Roy, M.K. and Akhter, N. 2006. Investigation of the diseases of some small indegenous fish species from Ailee Beel, Mymensingh. *Progress Agric*. 17(1): 219-225.

Aremu, A.O., Ndhlala, A.R., Fawole, O.A., Light, M.E., Finnie, J.F. and Van Staden, J. 2010. Ten South African Medicinal plants used as Antihelmintics. *South African J. of Botany.* 76 (3) : 558-566.

Aremu, A.O., Finnie, J.F. and Staden, J.V. 2012. Potential of South African Medicinal plants used as antihelmintics - their efficacy, safety concernsand reappraisal of current screening methods. *South African J. of Botany. Elsevier.* 82: 134-150.

Arizo, J., Tasadac, H.S. and Nighat, U.N. 2022. Impact of aquatic pollution on fish fauna. *Bacterial Fish Diseases.* Chapter V, pp. 103-112.

Austin, B. 1998. The effects of pollution on fish health. *J. Appl. Microbiol.* National Institute of Health. Dept, of Biol. Sc. Heriot Watt University, Ricearton, Edinburgh, U.K. Dol : 10. 1111/J.1365-2672.

Barua, G., Banu, A.N.H. and Khan, M.H.1989. An investigation into prevalence of fish diseases in Bangladesh during 1988-1989. *Bangladesh J. of Agriculture.* 11(2): 75-79.

Biswas, P. 1982. *Studies on the controlof some plant diseases with crude plant extracts.* Ph. D. Thesis. Deptt. of Pl. Path,, Faculty of Agril., Bidhan Chandra Krish Viswa Vidyalaya, Mohanpur, Nadia, W. B.,India.

Chai, J.Y., Murrell, K.D. and Lymbery, A.J. 2005. Fish borne parasitic zoonoses :Status and issues. *Int. J. Parasitol.* 35: 1233-1254.

Chakraborty, S., Cao, T., Hossain, A., Gnanagobal, H., Vasquez, I., Boyce, D. and Santander, J. 2019. Vibrogen - 2 vaccine trial in lumpfish (*Cyclopteruslumpus*) against *Vibrio anguillarum. J. Fish Dis.* 42(7): 1057- 1064.

Chakrabarti, S. 2020. Composite fish culture - ecologically viable and economically profitable fish culture technique- A Review. *International J. of Life Sciences*. 8(2): 397-403.

Chaudhuri, H. 1971. Stocking pond management, In : *Combined workshop on AICRP on Air Breathing Fishes for swamps and composite culture of Indian and Exotic Carps. CIFRI* Cuttack. p8.

Citarasu, T. 2010. Herbal Biomedicine: a new opportunity for Aquaculture Industry. *AquaInt*. 18: 403-414.

De Schryver, P., Crab, R., Defoirolt, T., Boon, N. and Verstraete, W. 2008. The basis of bioflocs technology: the added value for aquaculture. *Aquaculture.* 277: 125-137.

Gudding, R., Lillehaug, A. and Evensen, O. 1999. Recent developments in fish vaccinology. *Vet. Immunopathol*. 72 (1-2) : 203-212

Hargreaves, J.A. 2006. Photosynthetic suspended growth system in aquaculture. *Aqua. Eng.* 34: 344-363.

Horne, M.T., Tatner, M.F. and Ward, P.D. 1984. Vaccination of fish: a practical view. *Vet. Rec.* 114 (22): 537-539.

Hossain, M.Y. 2014. Threatened Fishes of World: *Mystusvittatus* (Bloch, 1794) (Siluriformes: Bagridae). *Croatian J. Fish.* 72: 183-185

Jeba, C.R., Vaidyathan, R. and Kumar, R.G. 2011.Immuno modulatory activity of aqueous extract of *Ocimum sanctum* in Rat. *Int. J. Pharm. Biomedical. Res.* 2: 33-38.

Kembou, R.J.E., Steinhagen, D., Readman, J., Daly, J.M., Adamek, M. 2023. Tilapia Lake Virus Vaccine Development : A Review on the Recent Advances. Vaccines (Basel). 11 (2): 251.

Joshi, B., Saha, G.P., Basnett, B.B., Bhat, M.R., Sharma, D. and Subedi, K. 2011. Phyto chemical extraction and antimicrobial properties of different medicinal plants. *J. Microbil. Antimicrob.* 3:1-7.

Koski, K.G. and Scott, M.E. 2003. Gastrointestinal nematodes, trace elementsand immunity. *J. Trace Element Exp. Medicine.* 16: 237-251.

Kumwan, B., Bunnoy, A., Chatchaiphan, S., Kayansamruaj, P., Dong, H.T., Senapin, S. and Srisapoome, P. 2023. First investigation of the Optimal Timing of Vaccination of Nile Tilapia (*Oreochromis niloticus*) larvae against *Streptococcus agactiae*. *Vaccines (Basel).* 11(12): 1753.

Larsen, J.L. and Pedersen, K. 1997. Vaccination strategies in freshwater salmonid aquaculture. *Dev. Biol. Stand.* 90: 391-400.

Mahapatra, A. and Biswas, P. 2023. Studies on the culture practices and performances of the Gangetic Koi fish, *Anabas cobojius* (Hamilton, 1822) at Tamluk, Purba Medinipur, West Bengal India. *Inter. J. of Creative Res. Thoughts (IJCRT).* 11 (1) : 144-150.

Mahapatra, A. and Biswas, P. 2023. Study on the fish polyculture practices in accordance with economic aspect at Tamluk, Eart Medinipur, West Bengal, India. *Inter. J. of Fisheries and Aquatic Studies.* 11 (3): 28-34.

Mahapatra, A. and Biswas, P. 2023. Studies on the mixed fish culture in the aquarium at Tamluk, East Medinipur, West Bengal, India, *Inter. J. Adv. Biol. Science.* 10 (8): 34- 45.

Mahapatra, A. and Biswas, P. 2024. Wart Disease of fresh water fishes due to Water pollution and control by Plant extracts at Tamluk, East Medinipur, West Bengal, India. *J. of Chemical, Biological and Physical Sciences*. 14 (2) : 201- 211.

Marana, M.H., Sepulaveda, D., Chen, D., Al Jubury, A., Jaffar, R.M., Kania, P.W., Henriksen, N.H., Krossoy, B., Dalsgaard, I., Lorenzen, N. and Buchmann, K. 2019. A pentavalent vaccine for rainbow trout in Danish aquaculture. *Fish Shelfish. Immunol.* 88: 344-351.

Meachasompop, P., Bunnoy, A., Kaeswejjareansuk, W., Dechbumroong, P., Namdee, K. and Srisapoome, P. 2023. Development of Immersion and Oral Bivalent Nanovaccines for *Streptococcosis* and Columnaris Disease Prevention in Fry and Fingerling of Asian Seabass (*Lates calcarifer*) Nursery Farms. *Vaccines (Basel).* 12 (1): 17.

Nahak, G. and Sahu, P.K. 2014. Immunostimulatory effect of *Ocimum sanctum* Linn. Leaf extract in *Clariasbatrachus* Linn. *Asian J. Pharma, Clin Res.* 7: 157-163.

Nargis, A., Khatun, M. and Talukder, D. 2011. Use of Medicinal plants in the remedy of fish diseases. *Bangladesh Res. Pub. J.* 5(3): 192-195.

Ndhalala, A.R., Ghebrehiwol, H.M., Ncube, B., Aremu, A.O., Gruz, J., Subrtova, M., Dolezal, K., Du Plooy, C.P., Abdelgadir, H.A. and Vanstaden, J. 2015. Antimicrobial, Antihelminthic activities and characterisation of functional phenolic acids of *Achyranthes aspera* Linn. *Frontiers in Pharma*. 6:274

Olusola, S.E., Emikpe, B.O. and Olafia, F.E. 2013. The potential of medicinal plant extracts as bio- antimicrobial in aquaculture. *Int. J. Med. Aromat Plants*. 3: 404-412.

Panda, S. 2016. Composite fish culture forgainful employment. *Inter. J. Bioassay*. pp. 4593-4596.

Passapan, L., Wongsawad, C., Kumchoo, K. and Sripalwit, P. 2004. Survey of helminths in Climbing Perch (*Anabas testudineous*) from San Sai district. Chiang Mai Province, Thailand. 35(1): 288-290.

Ravikumar, S., Selvan, G.P. and Gracelin, A.A. 2010. Antimicrobial activity of medicinal plants along the Kanyakumari Coast, Tamilnadu, India. *Afr. J. Basic. Appl. Sc*. 2 (5-6): 153-157.

Ray, A.J., Gloria, R., John, S., Susan, W.L., Alisha, B.W. and Craig, L.B. 2010. Characterization of microbial communities in minimal exchange, intensive aquaculture systems and the effects of suspended solid management. *Aquaculture.* 310 (1-2): 130-138.

Reverter, M., Bontemp, N., Lecchini, D., Banaigs, B. and Sasal, P. 2014. Use of plant extracts in fresh culture as an alternative to Chemotherapy: Current status and future perspectives. *Aquaculture.* 433: 50-61.

Saenphet, S., Wongsawad, C. and Saenphet, K. 2001. A survey of helminths in fresh water animals from some areas in Chiang Mai, Thailand, South East. *Asian J. Trop. Med. Public Health.* 32: 210-213.

Saha, A., Kabir, M.R. and Ali, M.M. 2009. Breeding performances of Thai Koi, *Anabas testudineous* (Bloch, 1792) in different months of the breeding season under two sex ratios. *Bangladesh Res. Pub. J.* 2: 667-673.

Sarma, 2010. Acclimatisation of *Anaba°testudineous* (Bloch) to three test temperatures influences thermal tolerance and oxygen consumption. *Fish Physiologyand Biochemistry.* 36: 85-90.

Stratev, D., Zhelyazkov, G., Noundou, X.S. and Krause, R.W.M. 2018. Beneficial effects of medicinal plants in fish diseases. *Aquaculture International.* 26 : 289-308.

Su, F.J. and Chen, M.M. 2022. Protective Efficacy of Novel Oral Biofilm Vaccine against *Phobacterium damselae* subsp; *damselae* infection in Giant Grouper, *Epinephelus lanceolatus*. Vaccines (Basel). 10(2): 207.

Vinitnantharat, S., Gravningen, K. and Greger, E. 1999. Fish Vaccines. Alpharma NW, Aquatic Animal Health Div. Belevue, Washington, USA. *Adv. Vet. Med.* 41: 539-550.

Waterman, C., Smith, R.A., Pontiggia, L. and Marderosian, A.D. 2010. Antihelminthic screening of Sub -Saharan African plants used in traditional medicine. *J. of Ethno Pharmacology.* 127(3): 755-799.