EFFECT OF FERTILIZER ON PROTEIN CONTENT OF FRESH WATER FISH GAMBUSIA AFFINIS

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Abstract

Gambusia affinis is a small fish native to the fresh water. Fishes are considered as a rich source of protein, good quality fat and micronutrients. The percentage composition of four major constituent of fish is water, protein, lipid and ash are referred to as proximate composition. These four nutrients account for about 96 to 98 percent of total tissue in fish. Carbohydrates, vitamin, nucleotide, other non-protein, nitrogenous compound etc. are also present in small quantities. Proteins composed approximately 70 % dry weight of the organic material in fish tissue, therefor protein content is one of the most important nutritional compounds of fish feeds. A fertilizer is any material of natural or synthetic origin that is applied to soil or to plants tissue to supply one or more plant nutrients essential to growth of plants. We are using the NPK fertilizers. At present the grade I-10:26:26. Fertilizers are commonly used in agricultural activities. Because of drift, atmospheric transport, agricultural and residential runoff, individual misuse, and improper disposal, fertilizers also found in aquatic habitat. This study confirms that Gambusia affinis showed the decrease in the biochemical composition i.e., Protein when expose to fertilizer toxicity. Effect of fertilizer on protein content of Gambusia affinis was analysed in the present study.

Keywords: *Gambusia affinis, Protein, Fertilizer, LC_{50}.*

INTRODUCTION

Water pollution is increasing day by day, different pollutants like heavy metals, pesticide, antifouling agent, fertilizers etc. has adverse effect on growth and survival of aquatic animals. These pollutants directly indirectly enter in the body of aquatic animals and effect on them and disturbs biochemical and physiological pattern of animals. The heavy metals like copper, chromium, iron etc. cause maximum effects on non-target organisms resulting in the imbalance of ecosystem. Fertilizer pollutes the aquatic ecosystems and effect the aquatic biota. Fertilizer accumulates in the soil and pollutes the water which affects the flora and fauna (Kulkarni *et.al.*, 2015). Rapid industrialization and drainage of toxicants in water is affecting the target organ of the aquatic animals. Pesticides reach to aquatic environment by direct application, spray Drift, aerial spraying and washing from the atmospherically precipitation and runoff from agriculture lands where they ravage the biotic life (Thongnipon *et.al.*, 1995). Water quality is one of the major environmental issues. The fertilizers are used for the healthy plant growth and optimum yield but they adversely effect on the non-target organism. Chemical fertilizers loke a urea and NPK are industrially manufactured through process. Increased interest in the use of chemical fertilizers (urea and

NPK) in aquaculture and agriculture in general, necessary investigation on the toxic effects of these chemical fertilizers on aquatic organism (Asuquo *et.al*., 2016).

Frequent use of chlorpyrifos pesticide in fields and its disposal in water bodies is very hazardous and unwarranted to the fresh water ecosystem. Indiscriminate use of chlorpyrifos effect on biochemical content of fish. Biochemical constituents are serving as potential bio marker for a variety of different organism. Fish act as a bio indicator of aquatic contaminants. Fish play an important and significant role in the food chain (Sneha Varma & Anurag Rawat 2017). The high protein requirement for the growth of carnivorous fish gives rise to high food cost and elevated discharge of nitrogenous waste into the environment. The protein sparing effect of the different sources and levels of carbohydrates has been debated, evidence suggest that dietary levels of digestible starch improve protein utilization efficiency in rainbow trout. (Barauge *et.al*., 1995). Abundant use of fertilizers became essential for better agricultural practices in most of the developing countries including India. Environmental pollution cause by fertilizers, especially in aquatic ecosystems has become a serious problem. These fertilizers even when applied in farm are washed and carried away by rains and floods to large water bodies like ponds and rivers and thereby alter the physiochemical properties of water and effects on aquatic organism Pesticides produce many biochemical changes in the fresh water fauna by influencing the activities of enzyme and metabolites (Koundinya *et.al*., 1982). The pesticides affect the survival, growth rate, fecundity and reproductive activity of fish. The *Gambusia affinis* are ubiquitous inhabitants of all types of water bodies are in Ahmednagar District. (Pawar *et.al*., 2016). Fish are largely being use for assessment of the quality of aquatic environment and such as can serve as bio indicators of environment pollution. Now a Days there is a considerable increase in the use of agro chemicals. Chemical Fertilizers become one of the leading polluting agents of aquatic ecosystems. The contamination of water due to toxic chemical known as cyanide, have large application in industries and kill the aquatic organism. Fish is one such a type of aquatic organism (Prashant *et.al*., 2011)

Experiment on bio assay was undertaken to find out the toxic effect of Ethepon on proximate composition of Rosy Barb (*Puntius conchonius*). Ethephon [(2 - Chloroethyl) Phosphoric acid] is a major plant growth regulator that promotes fruit ripening, abscission, flower induction, and other response by releasing ethylene gas, a natural plant hormone. In India, it is being extensively use to accelerate the post-harvest ripening of bananas, mangoes, pineapples etc. Ethylene diffuses easily through the air from one plant to another and its residual effect in soils remains and come to the aquatic environment during rainy period. They effect on the Zebra fish, Rosy Barb, Indian Medaka, *Gambusia*, Gappi etc. The utilization of all the energy components when fish is under stress. Fish needs more energy to detoxify the stress toxicants. (Sana K. M. Patel and S. T. Idulkar 2017). Industrial, domestic and agricultural effluents generally contain a wide variety of organic and inorganic pollutants such as solvents, oils, heavy metals, suspended solids and fertilizers which are invariably discharged into river, canal and streams without scientific treatment. The toxicity of trivalent arsenic lies in its ability to bind to the Sulphur group of essential cysteine amino acid in proteins (S. Ananth and U. Mathivanan 2013). Pesticide is a common pollutant of freshwater ecosystem where they cause adverse effect on aquatic biota. The alteration in biochemical contents in different tissue of fish due to toxic effect of pesticide and heavy metals. The sublethal concentration of

chlorphyrifos cause the decrease in protein content of fish, *Channa striatus*. (Revathi. T and Krishnamarthy 2008). The *Gambusia affinis* is used for the preparation of sauce in most parts of Southeast Asia, it is called by different names in different countries. The *Gambusia affinis* is a small freshwater species and it is not normally used for human consumption. Therefor the main object of this work was to utilise the *Gambusia affinis* for sauce production. Fish sauce contains proteins, essential amino acids and it is safe for human consumption as well. (Sayed Mekawy Ibrahim 2010)

Smaller mosquitofish fed mostly on zooplankton whereas larger *Gambusia affinis* are greater quantities of terrestrial fauna and large invertebrate. These differences are probably correlated with gap size and visual acuity, although ecological factors may also be important. Mosquitofish are generalist predator whose diet composition reflects temporal and spatial changes in prey availability. There is also a relationship between fish size and prey size that is probably linked to both anatomical characteristics and ecological factors (S. Mansfield and B. H. Mcardle 1998).

The generic name *Gambusia* is derived from a Cuban word Gambusino, meaning nothing, or a joke or farce. In addition to that, in Latin the *Gambusia* also means nothing (Pyke 2005) suggesting the genus was viewed as a somewhat irrelevant group of fishes. The common name applied to *Gambusia* appears to reflect aspects of species behaviour for example the name top minnow reflects it's tendency to swim near the surface, plague minnow referring to species negative impact on native fauna and mosquitofish referring to it's reputation for controlling mosquito population .(Jed Macdonald and Zeb Tonkin 2008).There is extensive array of literature available on western *Gambusia* (*Gambusia affinis*) due to their high abundance, widespread distribution, ease of capture and maintenance and mixed attitudes.*Gambusia* are generally considered to have yolk dependent embryos (lecithotrophic). Growth rate in *Gambusia* has been found to be influenced by water temperature, salinity, and rate of food consumption, diet and density. Greater the growth and developmental rate in *Gambusia*. *Gambusia* are able to store fats and lipid. The stored fats and lipid are shifted to ovaries, eggs and developing embryos. The lipid is stored in somatic cell are more in non-reproductive state (Graham H. Pyke 2005).

MATERIALS & METHODS

Materials:

1) Biological Material: *Gambusia affinis*
2) Fertilizer: N: P: K - 10:26:26
3) Glassware: Beaker, Bowls, Measuring cylinder, Test tube.
4) Other Requirements: Colorimeter, Cotton, Pond water, Variable volume Micro pipette, Fishing net, Distilled water, Muslin cloth, Test tube stand, Aluminum foil.

Preparation of chemicals:

1) Lowery – A: - a. 0.1 N NaOH = 0.2 gm of NaOH was dissolve in distilled water and made volume up to 50 ml.
   b. 2% Na_2CO_3 = 0.8 gm of Na_2CO_3 was dissolve in distilled water and made volume up to 40 ml.
2) Lowery – B: - a) 2% Sodium Potassium tartarated = 1gm. of Sodium Potassium tartarated was in dissolve in distilled water and made volume up to 50 ml.
b) 1% $CuSO_4$ dissolved in 2% Sodium Potassium tartrate = 0.5 gm. of $CuSO_4$ dissolve in 50 ml of 2% Sodium Potassium tartrate.
3) Lowery – C: - 40 ml of Lowery A mixed with 0.8 ml of Lowery B.

4) Folin phenol: - 3.5 ml of Folin phenol was mixed in 3.5 ml of distilled water.
5) BSA; - Bovine Serum Albumin, 0.01 gm of BSA dissolved in 100 ml of water (100 µg/mL)

Methods:

1) **Collection:** Fishes were collected by using the fishing net. The fishes were collected randomly in plastic container and transported to laboratory.

2) **Maintenance of Culture in laboratory condition:** The *Gambusia affinis* were acclimatized under laboratory condition. In the present investigation almost equal sized mosquitofish *Gambusia affinis* were maintained in the laboratory. The fishes were cleaned by using 0.1% $KMNO_4$, to avoid dermal infection and to prevent from disease outbreaks. The fishes were fade with pond water containing zooplankton the dishes were identified by using identification key.

3) **Experimental setup for LC_{50} value:** This bio assay test was carried to calculate LC_{50} agents *Gambusia affinis*. This step will help to minimize further animal killing. The fish were not feed on the day before the beginning of the experiment. The experiment was carried out in 6 sets. At 1st 6 beakers were taken.

4) **Experimental Setup for effect of fertilizer on bio chemical composition of *Gambusia affinis*:** After determining the LC_{50} value a batch of 10 fishes were exposing to sub lethal concentration of fertilizer. The fishes were expose to 2 different sets. First set was control (without fertilizer) and second set with sub lethal concentration of fertilizer. After 24 hours and 96 hours of exposure period, the fishes were dried in natural condition and make fish powder by using mortar and pestle. Fishes were sacrificed to estimate protein content. The whole-body tissues were taken from the controlled and treated fishes for the analysis of bio chemical composition i.e., proteins. Total protein was estimated according to Lowry's *et al* method.

Estimation of Proteins (Lowry *et al.*, 1951)

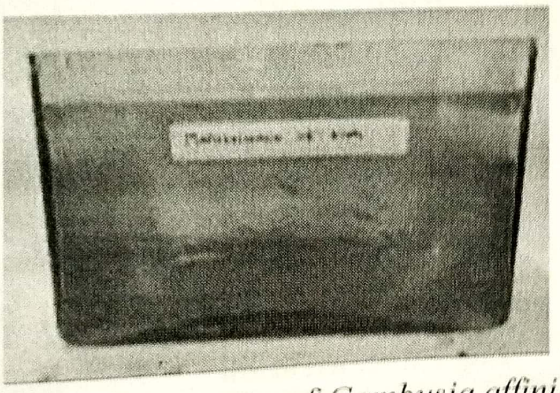


Figure 5: Maintenace of *Gambusia affinis*

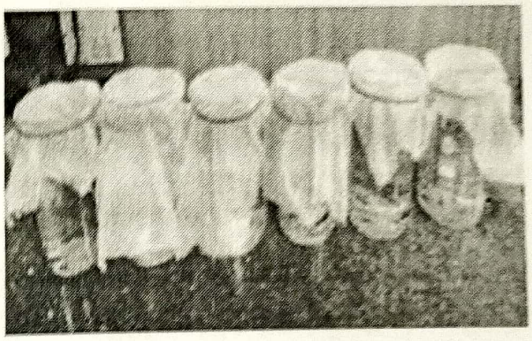


Figure 6: Experimental set up for LC_{50}

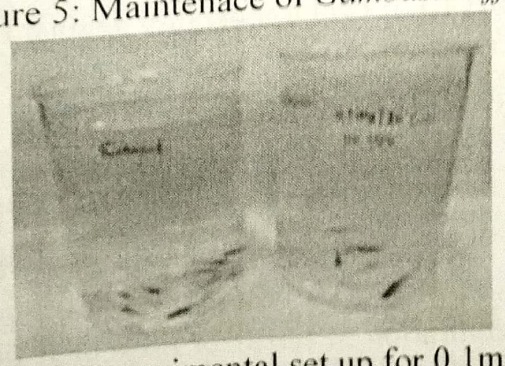


Figure 7: Experimental set up for 0.1mg/lit. concentration of NPK fertilizer

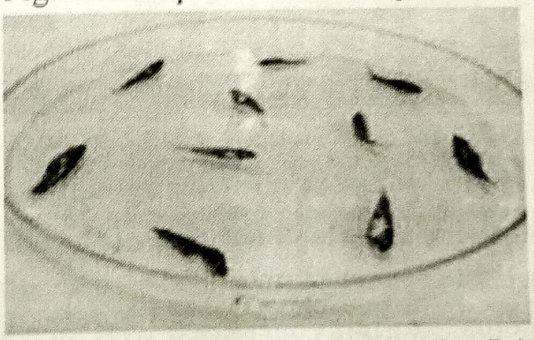


Figure 8: Experimental set up for fish drying

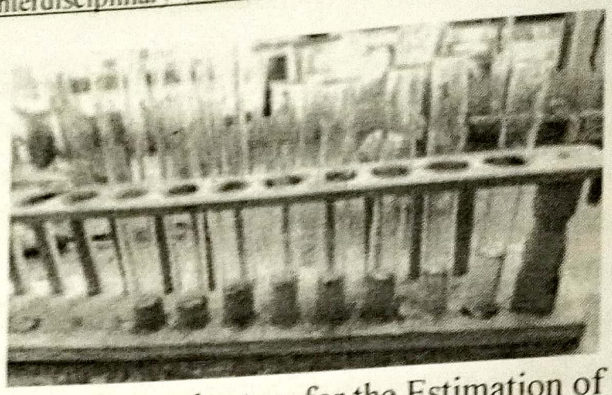


Figure 9: Experimental set up for the Estimation of Proteins

RESULT

Table 1.1- Mortality of *Gambusia affinis* in different concentration of NPK fertilizer at 24 hours exposure period: -

Sr. No.	Conc. Of fertilizer in mg/Lit	Log of conc.	No. of fishes exposed	No. of fishes alive	No. of fishes dead	Percent mortality (%)	Probit mortality
1	1.0	0	10	10	0	0	-
2	1.2	0.079	10	7	3	30	4.48
3	1.4	0.146	10	6	4	40	4.75
4	1.6	0.204	10	4	6	60	5.25
5	1.8	0.255	10	3	7	70	5.25
6	2.0	0.301	10	1	9	90	6.28

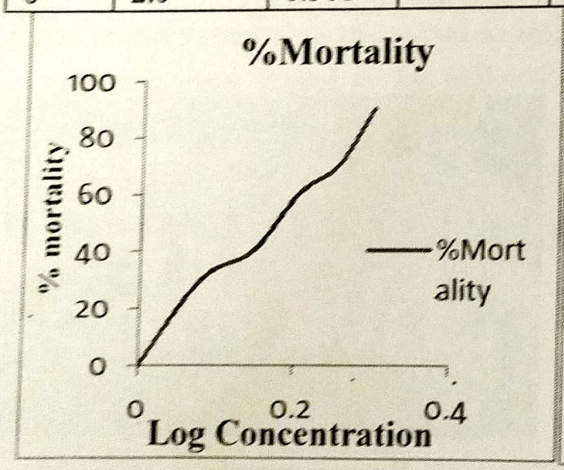


Chart 1.1.1: % Mortality against Log Conc.

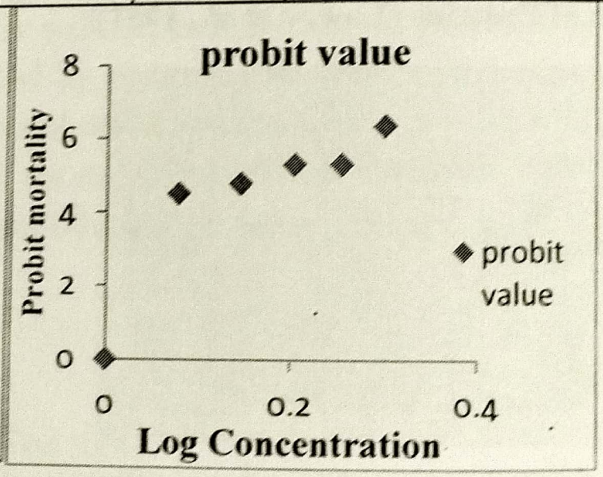


Chart 1.1.2: Probit mortality against Log Conc.

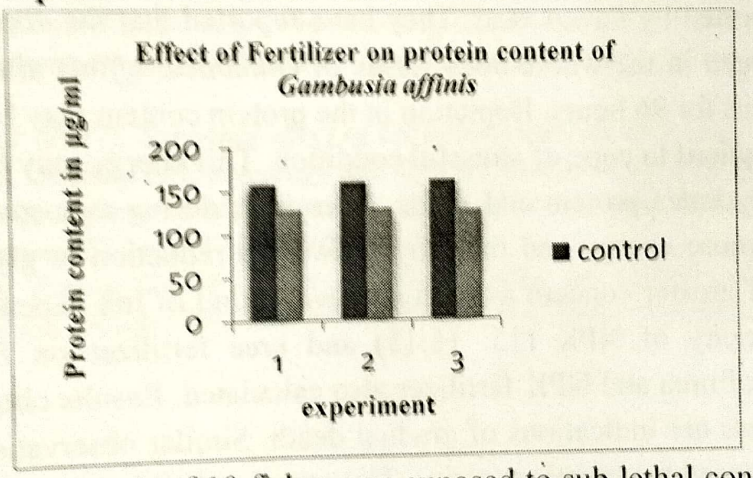
Initially, a range find in test was conducted to ascertain the range of NPK fertilizer to be selected in the definitive test. In the present experiment observation, LC_{50} value of *Gambusia affinis* which is exposed to fertilizers was calculated. Median tolerance limit (LC_{50}) of NPK fertilizers to fish was estimated by exposing six groups of fishes (10 fishes/group) to different concentration of the fertilizers. The first set served as control that is without fertilizer. The concentration of fertilizers was given variously to fishes in each set-in ascending order in mg/lit. The concentration ranging from 1.2, 1.4, 1.6, 1.8, 2.0 mg/lit for the period of 24 hours. Mortality increased with increasing concentration of fertilizer presented in table 1.1. The 50% mortality at log of concentration is 0.17. The result of 24 hours median lethal concentration value

for *Gambusia affinis* was graphically represented in chart 1.1.1 and 1.1.2, there for the LC50 value of NPK fertilizer for *Gambusia affinis* was found to be 1.5mg/lit. at 24 hours of exposure period. The LC50 value differs from species to species for the same fertilizer a well as for different fertilizer due to their mode of action on fish.

Table 1.2: - Effect of fertilizer on protein content of *Gambusia affinis*.

Sr. No	Protein content in (µg/ml)	
	Control	Experiment (Exposed to 0.1mg /lit Conc. of N.P.K.)
1	157.14	128
2	160.12	130
3	162.08	128
Mean	159.78± 2.487489	128.66± 1.154701

Chart 1.2: - Changes in the protein content over the control in whole body tissue of *Gambusia affinis* exposed to sub lethal concentration of fertilizer for 96 hr.



After determining the LC_{50}, a batch of 10 fishes was exposed to sub lethal concentration of NPK for 96 hours. In the sub lethal experiment, concentration such as 0.1 mg/lit. was selected ($1/10^{th}$ 0f LC_{50} for 96 hours). The sub lethal effect of NPK on biochemical content of *Gambusia affinis* is given in Table 1.2. The first set is kept as control that is without concentration of fertilizer. The experimental set show significant decreased in protein content of *Gambusia affinis* when compared to control set. The control group did not show any significant changes in protein 159.78±2.487489 µg/ml. The experimental set show decreased in the protein 28.66±1.154701 µg/ml. The graph was plotted between the control and experimental value (Chart 1.2). 19.47% protein was reduced in fish, *Gambusia affinis* which is exposed to 0.1 mg/lit concentration of NPK fertilizer.

DISCUSSION

The present data highlights an important role of *Gambusia affinis* or mosquitofish in the aquatic eco-system for the control of mosquito population. The fertilizer may influence the various physiological and biochemical mechanism and finally death may be due to the precipitation of toxicants in the fish body.

The fish death may be due to the metabolic inhibition. The fresh water fish, *Gambusia affinis* showed varied degrees of mortality with different concentration of NPK fertilizer. The mortality data of *Gambusia affinis* in different concentration of NPK fertilizer is presented in table 1. The commercial grade of fertilizer has an LC_{50} value of 1.5 Mg/lit. Acute toxicity of NPK fertilizer on fish, Gambusia affinis was carried out. The control fishes show maximum amount of protein 159.78±2.487489. The experimental set show decreased in the content of protein as compared to control that is 128.66±1.154701. The protein content was estimated by using the Lowrey's et.al method. The fishes were expose to fertilizer toxicity had decrease in the amount of protein content as compared to control fish. Similar results were reported by Sneha Varma and Anurag Rawat. They have reported that the protein and the carbohydrate concentration of ovary tissue were varied with the concentration of pesticide, chlorpyrifos. The minimum potein and carbohydrate concentration were noticed in the fish tissue expose to highest concentration of CPF and maximum content were observed in lowest concentration of CPF after 96 hours of exposure. Kulkarni et.al have also been reported the effect of urea fertilizer on the sodium and potassium content in the haemolymph of fresh water field crab, Barytelphusa Guerini. Sujatha et.al have also been reported as fish also contain significant amount of all amino acid particularly lysine. Fish protein contains all essential amino acid which contribute to their nutritional and biological value.

Similar result was reported by Pawar et.al. They have reported that the changes in biochemical content viz. protein and glycogen in the whole-body tissue of *Gambusia affinis* after the treatment of sublethal concentration of rogorus for 96 hours. Depletion in the protein content may be due to the metabolic stress i.e., more energy is required to cope of stressful condition. This energy may be obtained from the organic constituents as carbohydrates protein and lipids. Therefore, during exposure, fishes try to detoxify the toxicant by spending more energy and there by showing a reduction in glycogen and protein content. Aquatic pollution is of greater concern as each and every kind of life depends on water. Asuquo et. al., observed that the toxicity of NPK (15: 15:15) and urea fertilizer on *Heterobranchus biodorsalis* fingerlings. The Lc50 of urea and NPK fertilizer also calculated. Results obtained from this study reveal that behavioure response are indications of gradual death. Similar observation was noted by Ananth et. al., when fish were exposed to Arsenic toxicity. The arsenic trioxide Lc50 (89 mg/lit) was found out for 96 hours respectively as sublethal concentration of study. The fishes were sacrificed and required fishes were collected for protein estimation. The protein levels in gills, kidney, liver and brain of Grass carp, Ctenopharyngodon Idella showed significant decrease when compared to control fish at 28 day of exposure period.

REFERENCES

N. Kulkarni, J. V. Balkhande and C. S. Bhowate (2015), Prevention of urea toxicity on Na+ and k+ contents of a fresh water field crab,barytelphusa guerini by using sulphur contaning amino acid methionine as an additive. Asian Journal of Biochemical and Pharmaceutical Research. (vol.5) Issue3; 33-37.

Asuquo IE, Essien -Ibook MA & Abiaoba No. (2016), The effect of some agricultural fertilizer on fingerlings of Heterobranchus bidorsalis. Journal of Aquaculture and Marine Biology. Volume 4, Issue 2: 1-7.

A. Pawar, P. S. Shinde, A. K. Pandarkar and A. N. Shendge (2016), Toxicity and impact of rogorus on protein and glycogen content of fresh water fish Gambusia affinis (BAIRED & GIRARD) . J. Exp. Zool. India Vol.19 ; 267-271.

Brauge ,G. Corraze , F. Medals (1995), Effect of dietary levels of lipid and carbohydrate on growth performance, body composition, nitrogen excretion and plasma glucose levels in rainbow trout reared at 8 or 18 °C. Repod Nutr Dev (35) :277-290.

Graham H. Pyke (2005), A review of the biology of Gambussia affinis and Gambusia Holbrooki. Review in fish Biology and Fisheries. Volume 15:339-365.

Jed Macdonald and Zeb Tonkin (2008): A review of the impact of estern gambusia on native fishe of the Murray-Darling Basin. 1-52

K. Sujatha, A. Anitha Joice and P. Senthilkumaar (2013), Total protein and lipid content in edible tissues of fishes from Kasimodu fish landing center, Chennai, Tamilnadu Europian Journal of Experimental Biology Vol.3(5), pp. 252-257.

Koundinya, P. R., & Ramamurthi, R. (1982). Effect of organophosphorus pesticide sumithion (fenitrothion) on alkaline phosphatase activity of freshwater teleost, Sarotherodon mossambicus (Peters). Current science.

M. S.Prashanth, H. A. Sayeswara and Mahesh Anand Goudar (2011), Effect of Sodium Cyanide on Behaviour and Respiratory Surveillance in Freshwater Fish , Labeo rohita (Hamilton) . Recent Research in science and Technology, 3(2) :24-30.

Revathi. T and Krishnamurthy. R. (2008), Impact of pesticide chlorophyrifor on protein alternation in a freshwater fish, Channa striatus. International Studies of Pharmacy and Bioscience volume 8 Issue (3):926-931

S. Ananth and U. Mathivanan (2013), Studies on Biochemical changes in the fish Ctenopharyngodon idella in Relation to Arsenic toxicity. International Journal Of Pharmaceutical and Biological Achiever Vol. 4 Issue (6): 1226-1232

S. Mansfield and B.H. Mcardle (1998), Dietary composition of Gambusia affinis (family Poecilidae) population in the northern Waikato region of New Zealand . New Zealand Journal of Marine and Freshwater Research, Vol.32 :375-383

Sana km Patel & ST Indulkar (2017), Sublethal effect of Ethiopian pesticide on Carcass composition of rosy barb. Punitus conchonius. Journal of Entomology and Zoology Studies Vol. 5 Issue(6) : 1365-1368

Sayed Mekawy Ibrahim (2010), Utilization of Gambusia (Affinis affinis) for fish Sauce Production. Turkish Journal of Fisheries and Aquatic Science Volume 10:169-172

Sneha Verma and Anurag Rawat (2017), Effect of chloropyrifos on protein nd carbohydrat content of Heteropneustes fossilis (Bloch,1794) International Journal of Fisheries and Aquatic Studies 5(1):pp. 463-466.

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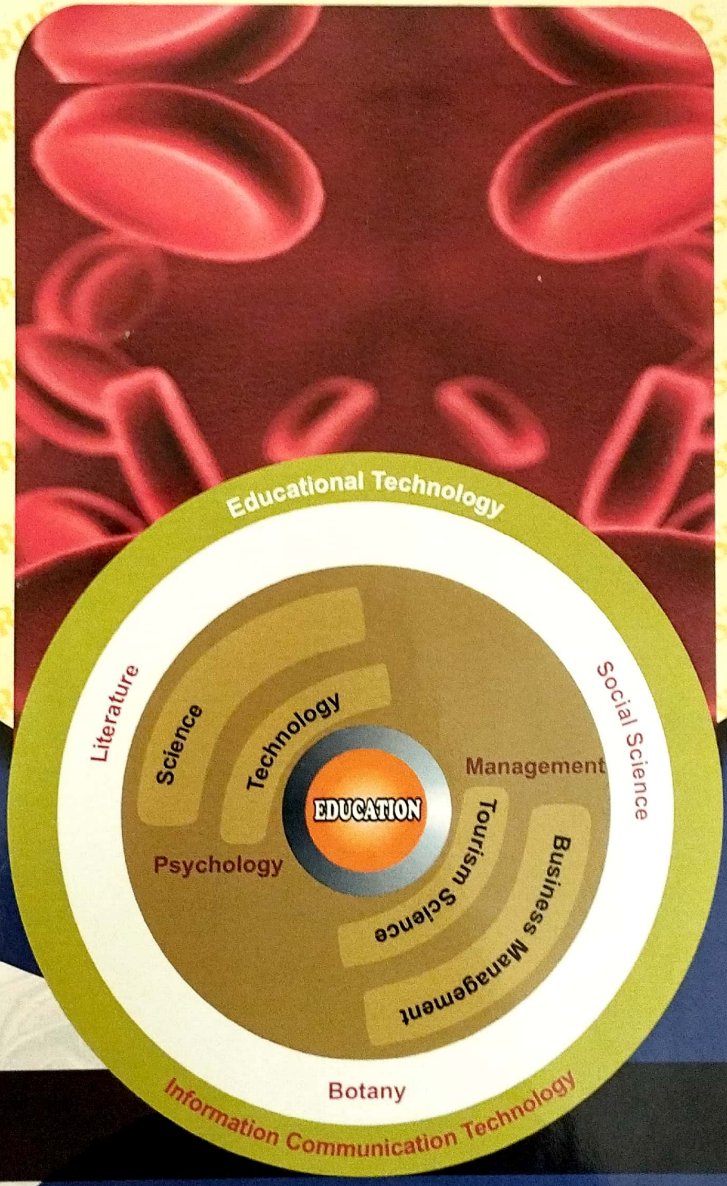

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