

**A STUDY ON THE USE OF MICROBIAL CONSORTIUM  
FOR PRODUCING HEALTHY FISHES**

BY

**SREEJA . A**

Reg. No. 97 PLS 08

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE  
AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF  
**MASTER OF SCIENCE IN LIFE SCIENCES.**

MAY 1999

**A STUDY ON THE USE OF MICROBIAL CONSORTIUM  
FOR PRODUCING HEALTHY FISHES**

BY  
**SREEJA . A**  
Reg. No. 97 PLS 08

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE  
AND HIGHER EDUCATION FOR WOMEN - DEEMED UNIVERSITY, COIMBATORE - 641 043  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF  
**MASTER OF SCIENCE IN LIFE SCIENCES.**

MAY 1999

**CERTIFIED AS BONAFIDE RESEARCH WORK**

*Sarjini Sukumar*  
12.5.99.

Signature of the  
Head of the  
Department

*Rajammal Thirumobesan*

Signature of  
the Guide

# **ACKNOWLEDGEMENT**

## **ACKNOWLEDGMENT**

I wish to express my gratitude to Dr. (Tmt.) Rajammal P. Devadas, M.A., M.Sc., Ph.D. (Ohio State), D. Sc. (Madras), Hon. D.H.L. (Oregon State), Hon. D.H.L. (Ohio State) Hon. D. Sc. (C. Azad Agri. University, Kanpur), Hon. D.Sc. (University of Ulster, Northern Ireland), Chancellor, Avinashlingam Institute for Home Science and Higher Education for Women – Deemed University, Coimbatore for providing me the opportunity to undertake this investigation.

I wish to express my heartfelt thanks to Dr. (Tmt.) Lakshmi Santa Rajagopal, M.S. (Tennessee), Ph.D. (Madras), Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women – Deemed University, Coimbatore for all the encouragement given to carry out the study.

I express my heartfelt gratitude to Dr. (Mrs.) Saroja Prabhakaran, M.A., Dip. in Ed., Ph.D. (Mother Teresa), Registrar, Avinashlingam Institute for Home Science and Higher Education for Women – Deemed University, Coimbatore for providing the needed facilities for the study.

I extend my profound thanks to Dr. (Tmt.) S. Sivakamasundari, M.Sc (Annamalai) M.Phil., Ph.D. (Madras), Dean, Faculty of Science, for all her encouragement to bring out this work successfully.

I wish to express my heartfelt thanks to Dr.(Tmt.) Sarojini Sukumar, M.Sc., Dip. Ed.(Madras), Ph.D., Head of the Department of Life Sciences, for her continuous help and guidance to complete my dissertation.

I would like to express my profound gratitude to Dr.(Tmt.) Rajammal Thirumalnesan, M.Sc., Dip. Ed., M.Phil., Ph.D., Reader, Department of Zoology, Avinashlingam Institute for Home Science and Higher Education for Women – Deemed University, Coimbatore for her guidance and constant encouragement from the selection of this topic to the completion of this project.

Sincere thanks to Dr. D. Purushothaman, Centre for Advanced studies in Agriculture, Microbiology, Tamil Nadu Agricultural University, Coimbatore, for all his timely help, pertinent suggestions and incessant inspiration for the successful completion of this project.

My sincere thanks to my parents and my friend Priya for their support and encouragement.

I wish to express my heartfelt thanks to all the staff members in the Department of Life Sciences for their encouragement and timely advice given during the course of study.

# **CONTENTS**

## CONTENTS

| CHAPTER<br>NO. | TITLE                  | PAGE<br>NO. |
|----------------|------------------------|-------------|
| 1.             | INTRODUCTION           | 1           |
| 2.             | REVIEW OF LITERATURE   | 7           |
| 3.             | MATERIALS AND METHODS  | 14          |
| 4.             | RESULTS                | 19          |
| 5.             | DISCUSSION             | 24          |
| 6.             | SUMMARY AND CONCLUSION | 26          |
|                | BIBLIOGRAPHY           |             |

## LIST OF TABLES

| TABLE NO. | TITLE   |
|-----------|---|
| I         | Food and MC composition for different experimental groups.        |
| II        | Mortality of the fish during experimental period.                 |
| III       | Total weight of each group during experimental period (in grams). |
| IV        | Mean weight gain during experimental period.                      |
| V         | Growth characteristics based on mean weight.                      |

## LIST OF FIGURES

| FIGURE NO. | TITLE   |
|------------|---|
| 1.         | % Mortality in experimental period.                                     |
| 2.         | Weight gain by each group during experimental period.                   |
| 3.         | Total weight gain by the groups at the end of experimental period.      |
| 4.         | Mean weight gain by different groups during experimental period.        |
| 5.         | Mean weight gain by different groups at the end of experimental period. |
| 6.         | Growth rate of different groups.  |
| 7.         | % Weight gain by different groups.                                      |
| 8.         | Specific growth rate in different groups.                               |
| 9.         | % Survival in experimental groups.                                      |

## LIST OF PLATES

| PLATE NO. | TITLE  |
|-----------|--|
| I         | Fish feed (pelleted bengal gram powder),<br>Fortified feed and Microbial consortium. |
| II        | Acclimatization of fish.   |
| III a     | Control group and Experimental group<br>of fishes.                                   |
| III b     | Experimental group of fishes.  |
| IV        | Fishes grown with feed fortified with MC.  |

# **INTRODUCTION**

## I INTRODUCTION

The term aquaculture means the growing of aquatic organisms under controlled conditions. Aquaculture or the farming of aquatic organisms is known to be practiced in various farms and the varying degrees of intensity for over 2000 years.

Aquaculture is defined variously. The most recent and internationally accepted definition has been arrived at by Food and Agriculture organisation of the United Nations organisations (UNO, FAO, 1990). "The farming of aquatic organisms include fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding protection from predators etc. Farming also implies individual or corporate ownership of the stock being cultivated.

Aquaculture can also be defined as tending the confined waters for growing aquatic organisms and harvesting the production for human benefits (Kutty, 1999).

One of the main objectives of aquaculture is to attain maximum yield by employing modern scientific management techniques.

Aquaculture has emerged as an important industry during last decade and is practiced in more than 150 countries in the world. Global aquaculture industry is worth about 30 billion US \$ and is growing at the rate of about 10 percent ( De Silva and Anderson, 1995).

Asia is considered to be the cradle for aquaculture. Asian countries contribute 85 percent of the total production and Japan shares a major part of it. Considerable part of Japanese economy relies on the fish market.

Aquaculture and agriculture are not strictly parallel developments in food gathering hunting and fishing might have started at about the same time in human history. The aquaculture boom and increased socio-economic benefits such as increase in employment and social well being, concomitant with the increase in the extent and intensity are alleged to have created several problems, particularly those of deteriorating environment owing to worsening water and soil quality, as well as owing to incursions by aquaculture into the other sectoral activities, such as agriculture, capture, fisheries, forestry, public health, tourism, urbanization and housing. The impacts can be two-fold, one affecting aquaculture itself by autopollution and consequent out-break of diseases.

In recent years commercial fish farming has assumed greater attention. Several hundreds of fresh water and marine fish farms have come up in recent years. The marine and aquaculture techniques employed in modern farms are quite scientifically based and the yield of carps are very much encouraging.

Newer techniques and approaches are developing year after year to improve the productivity. One of the main aspects in fisheries research is the study of feed and feeding habits along with growth.

Factors influencing the development of European aquaculture towards the year 2000 comprise of environmental issues, development of new types of feed and successful disease management strategies. (Ackefors, 1996).

One of the major problems in the nurseries where fingerlings are reared is the high % of mortality. Due to pathogenic proliferation in the nurseries or due to improper hygienic conditions or through contaminated feed. Feed and feeding are crucial elements in the culture of aquatic animals.

The problems associated and encountered in feed and feeding differ from those found in other farms of husbandry. These arise because of the large number of aquatic species cultured, which differ drastically in anatomy, behavior and physiology. Other problem arises because of the nature of the aquatic medium in which the animals need to spend some or all of their life.

Feed cost ranges from 30 to 60 percent of the total culturing cost depending upon the type of fish and culture system. Various rotifers, plant extracts, stout's viscera, soybean meal etc. are normally employed as the feed.

There are reports of microorganisms causing mortality of fish fauna for the last few years. Zoosporic fungi, bacteria, viruses are constant and ubiquitous components of the aquatic environment and pose a continual challenge to fish, but still thrust has been on the use of microorganisms as the feed. (Bisht *et al.*, 1996)

For growing healthier fishes, Japanese company has come out with a novel preparation. Over years of research in Japan marine scientists have developed a consortium of useful microorganisms. The concept of inoculating fishponds with the consortium of beneficial microorganisms will ward off fish pathogens and will help producing healthy fishes. Many modern aquaculture units have started using the microorganisms in rearing tanks. If the ponds and other water bodies maintain a high load of beneficial bacteria they keep fishes healthy and disease free.

The introduction of beneficial microorganisms into rearing pond at appropriate time and through fish feed will eliminate pathogenic bacteria and viruses and fishes will grow healthier.

## EFFECTIVE MICROORGANISMS (EM)

Basically Microbial consortium is a mixture of several microorganisms like Actinomycetes, Bacillus, Bifidobacterium, Lactobacillus, Leuconostoc, Clostridium and Ruminococcus.

All these microorganisms can co-exist in mixed culture and their also compatible physiologically. When these cultures are introduced into the natural environment, their individual beneficial effects are greatly magnified in a synergistic manner.

## PRINCIPAL MICROORGANISMS IN THE EM

### 1. Photosynthetic bacteria

These are autotrophs and they synthesise very useful substances as secretions using light energy and the heat.

### 2. Lactic acid bacteria

Lactic acid bacteria produce Lactic acid from sugars and other oxidisable carbohydrates produced by photosynthetic bacteria and yeast. It suppresses harmful pathogenic microbes and maintains the health of fishes.

### 3. Yeast

Yeast has been reported to synthesise antimicrobial substances and growth harmones. These promote rapid growth of fishes. Lactic acid bacteria and actinomycetes depend on the secretions of yeast for their activity.

#### 4. Actinomycetes

These are essentially bacteria but they look like fungi because of their filamentous structure. The actinomycetes are known to produce several antibiotics. They synthesize a plethora of substances of antibiotic nature and also several amino acids. These organisms can co-exist with yeast and photosynthetic bacteria. These antimicrobial substances suppress pathogenic bacteria and fungi.

#### 5. Fungi

Effective microbial consortium also includes filamentous fungi like Aspergillus and Penicillium. These fungi go a long way in the decomposition of organic matter and produce alcohol by fermenting sugars. They also produce esters and antimicrobial substances, which play a role in keeping off the pathogen.

The off-odour of water of the fish tank is prevented. The fungi destroy maggots of insects and egg masses in water bodies. Each species of microorganism has its own role and important function in suppressing the pathogens.

It is reported that the microorganism contained in MC have been so carefully selected that all of them can co-exist without any antagonism among them. This product has emerged out of several years of laboratory testing.

When the populations of effective microorganisms reach high levels, the total bacterial flora of the water body becomes comprised of only beneficial microorganisms. The most striking phenomenon is that the fish pathogens occurring in the waterbody, hatcheries and nurseries are eliminated by the microbial consortium to the extent that no disease or health hazards appear on the fingerlings.

Microbial consortium is added in appropriate quantity to the waterbodies or rearing ponds in intervals and this consortium is also given along with fish feed. Various algae, yeasts and bacteria have been employed as primary or secondary feed in the recent years. Algae such as Chlorella, Haematococcus, Spirulina were found to be useful for growing young fish. ( Rajammal and Indira, 1997).

Use of microorganisms as fish feed is found safe for human consumption. Microbial cells as food supply additional nucleic acids, proteins, vitamins and various minerals along with carotenoids. Thus microorganisms employed as the fish feed agent present a bright prospects for fish industry on general as for our country in particular to enhance fish production in fish culture and to combat diseases in nurseries.

It was therefore and is still the right strategy to concentrate on aquaculture to increase farmed fish production.

Hence the objectives of the present study include

1. To select and study the suitable Microbial consortium in controlling the fingerling mortality.
2. To eliminate bacterial pathogens often causing high mortality in fish nurseries.
3. To produce healthy fishes by using effective microorganisms.
4. To evolve a biological method of preventing disease causing pathogens and thereby to maintain healthy fishes.

**REVIEW  
OF  
LITERATURE**

## II REVIEW OF LITERATURE

A group of Effective microorganisms or Microbial consortium constitutes beneficial microorganisms, which have been so carefully selected that all of them can co exist without any antagonism among them.

Microbial consortium

Microbial consortium is added on to the water body and also incorporated with fish feed. Food is considered as an important ecological factor influencing the population dynamics and growth of fishes. Food intake is a vital factor for fish growth.

Paloheimo and Dickie (1969) have postulated that the quality of food bears the greatest potential effect of food consumption and growth efficiency of fishes.

A field trial was undertaken to evaluate the efficacy of Spirulina alga and groundnut cakes on the growth of the fish, Tilapia mossambica and it was observed that the weight gain was remarkable in the Spirulina fed batch of fishes. (Raja,1979).

Algae are receiving increasing attention as a possible protein source for fish diets, particularly in tropical developing countries where algal production rates are high. (Venkataraman et al., 1980). Many authors have reported the utilization of algae in fish feeds. (Appler and Jauncey, 1983; Juario and Storch, 1984).

An assessment of the use of rice bran as a fish feed in the four areas of Udonthani province in the Northeast Thailand was done by Thomas (1989). The survey confirmed that rice bran was the most commonly used fish feed being used by 65 percent .

An assessment of the nutritive value of Cassava leaf meal (CLM) as a dietary protein source in pelleted feed for Nile Tilapia, Oreochromis niloticus was carried out by Keong and Wee (1989) and it was found that cassava leaf meal was a viable partial dietary protein source for Nile Tilapia.

The influence of diets [(Earthworm, Chara plant, combined diet (earthworm :charaplant, 50:50)] on feed utilization was carried out in Oreochromis mossambicus and Cyprinus carpio var communis. Among the three diets, fishes fed on earthworm exhibited the highest consumption, absorption, conversion and protein conversion efficiency. (Elankumaran et al., 1992).

An experiment was conducted to study the influence of irradiated sludge pellet as herbivore fish feed in red niloticus (Oreochromis sp.) and it was found that these fishes fed with irradiated sludge pellet were worse based on the weight gain and growth. (Harsojo et al., 1992).

Vonder and Lied (1993) investigated the effect of soybean protein on growth and muscle metabolism in fish cod, Gadus morhua. Fingerlings were fed on a fish feed formula with the high quality fish meal protein having been replaced with 100, 200 or 300g soybean protein/kg fish mealprotein and it was concluded that 200g/kg or less of the high quality fish meal protein may be replaced by soybean protein in a fish feed formula.

Sherief and James (1994) investigated the extent to which a fish meal based pelleted diet for Etilopius susratensis (Bloch) could be successfully replaced with dried Azolla powder. The study revealed that the inclusion of dried Azolla powder in the diet is possible only upto a level of 25 percent.

Das et al . (1994) found that fish growth rate has been seen to be most suitable while using both conventional fish feed (rice bran, groundnut oil cake and fish meal in 1:1:1 proportion) and digested Azolla in 4:1 proportion.

Attempts were made by Islam and Hossain (1994) to find out the optimum level of protein requirement in formulated diets for Tilapia, Oreochromis mossambicus. Tilapia fed on 35 percent protein grew to 9.3 g and yielded 101.7 percent feed efficiency within 8 weeks.

An observation using coconut oil as a dietary lipid source and feed additive was conducted to evaluate its effect on the growth and body composition of carp Cyprinus carpio .L. The addition of coconut oil at a level of 10% proved optimum for growth. (Aliah, 1996).

Efficacy of formulated fish feed incorporating silages fish and poultry offals was tested against fish feed prepared with conventional fish meal and it was observed that growth and overall performance was better in feed with fish and poultry viscera silage (Chakrabarthy, 1996).

Hilge and Stephens (1996) observed that the growth of fry and fingerlings was strongly temperature dependent. Growth was rather poor at 16 – 18<sup>0</sup> C and best between 26 – 30<sup>0</sup> C .

The chemical weedicide “Glyphosate” was evaluated for use as a novel management tool to improve the efficiency of intensive carp Cyprinus carpio production in Poland. The survival and growth of carp fry was greatest in ponds in which natural vegetation had been treated with glyphosate (Krueger et al., 1996).

A 10 week feeding trial was conducted in the laboratory during which channel catfishes Ictalurus punctatus were fed five practical diets containing either 0, 500, 1000, 2000 or 4000 units of microbial phytase / kg diet. Fish fed diets containing 500 or more units of microbial phytase / kg consumed more feed and gained more weight than fish fed the basal diet without supplemental phytase (Scott et al., 1996).

Gopal et al. (1996) investigated the effect of a biogas plant effluent based pelleted diet on the growth of Oreochromis mossambicus fingerlings. Cattle dung based biogas plant effluent was evaluated as a dietary ingredient in pelleted feed in the place of rice bran for Tilapia culture over a period of 42 days. The mean growth rate and muscle protein showed no change when the biogas plant effluent was used. It was recommended that dried bio gas plant effluent could replace rice bran in pelleted fish feed formulations.

Park (1997) explained that certain algae and microorganisms are the cause for the off-flavor and several associated problems in fish rearing. This appears to be a severe threat to fish farming.

Li-Meng and Robinson (1997) found that fish fed with diets containing 250 units of microbial phytase /kg and above consumed more feed and gained more weight.

A study was conducted to determine the effects of different dietary concentrations of air dried whole krill meal and on the growth of juvenile chinook salmon, Oncorhynchus tshawytscha. The test diets contained 50, 150 or 250g air dried krill meal. The diet containing 250g air dried krill meal grew better. (Anderson et al., 1997).

Basudha and Vishwanathan (1997) made a comparison between fish feed A and fish feed B on Osteobrama belangeri (valenciennes) a medium carp. Feed A contained Azolla powder, fish meal, mineral and vitamin supplements and feed B contained rice bran and mustard oil cake. % weight gain of fish fed with feed A

has better FCR and PER when compared to feed B, suggesting that for rearing fry of this fish, feeding with supplementary diet feed A was better.

Bhosale (1997) reported that the disease resistance of fish can be strengthened by using better quality fish feed and can minimize the use of antibiotics like chloramphenicol in the fish. He also reported that the use of microorganisms as fish feed is found safe for human consumption.

Sidhu and Khulbe (1998) observed that in the reservoirs during winter, moderate temperature ( $15 - 20^{\circ}\text{C}$ ) and higher values of organic matter facilitated the growth of water molds and their inoculum potentiality which attracted the fish population caused their mortality.

Otta *et al.* (1998) reported that the most significant pathogens that are commonly associated with cultured penaeid shrimps belong to viruses, bacteria, fungi, parasites and algae. Fungal infection causes mass mortality particularly in hatcheries where it affects the larval stage.

#### **USE OF EFFECTIVE MICROORGANISMS (EM) IN FISH FARMING**

The concept of introducing a consortium of beneficial microorganisms to soil, marine or aquatic habitats to create a favourable microbiological environment to soil, plant growth and fish farming is of recent origin. A few Indonesian and Korean scientists have developed a consortium of beneficial microorganisms under the trade name 'MC' (Effective microorganism) for fish farming. The use of this effective microorganisms to fish farming process is claimed to hasten the growth of fishes, feed uptake and more essentially to keep the fingerlings practically away from the onslaught of devastating diseases. This product is in use with great success in Thailand, Indonesia, South Korea, Malaysia, Singapore. Since last two years the efficacy of the MC is being studied in our country as well.

A literature on the performance of MC on fish culturing is scarce and not much information could be presented on effective microorganisms.

**MATERIALS**  
**AND**  
**METHODS**

### III MATERIALS & METHODS

Aquaculture means the growing of aquatic organisms under controlled conditions. The aim of aquaculture is to attain maximum yield by employing modern scientific management techniques. The present study is concerned with the growth of fingerlings by using effective microorganisms.

#### COLLECTION OF FINGERLINGS

Fingerlings of Tilapia mossambicus measuring about 4 – 5 cms in length with a weight ranging from  $1.028 \pm 0.14$  -  $1.543 \pm 0.48$  gms were collected from a pond at Palakkad – Kerala and brought to the laboratory for acclimatization.

#### ACCLIMATIZATION PERIOD

The fingerlings were acclimatized to laboratory conditions in aquaria for 15 days at  $28 \pm 2^0$  C. During acclimatization period water in the aquaria was changed every alternate day to ensure sufficient oxygen supply to the fingerlings.

Fingerlings of Tilapia mossambicus were selected because they were sturdy and get easily acclimatized to laboratory conditions. General health of these fingerlings was recorded during acclimatization period (Plate II).

#### MORTALITY

Due to change in temperature and also due to transportation, 5 – 10 fingerlings died during the first two days of acclimatization.

#### MICROBIAL CONSORTIUM (MC)

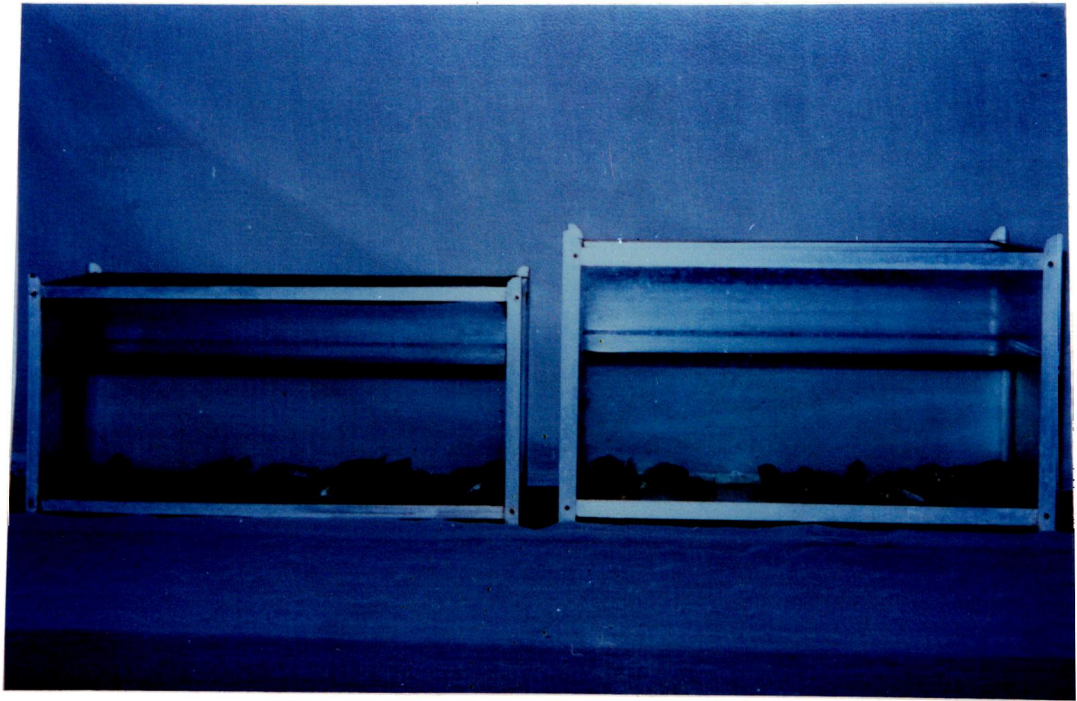


Plate II. Acclimatization of fishes.

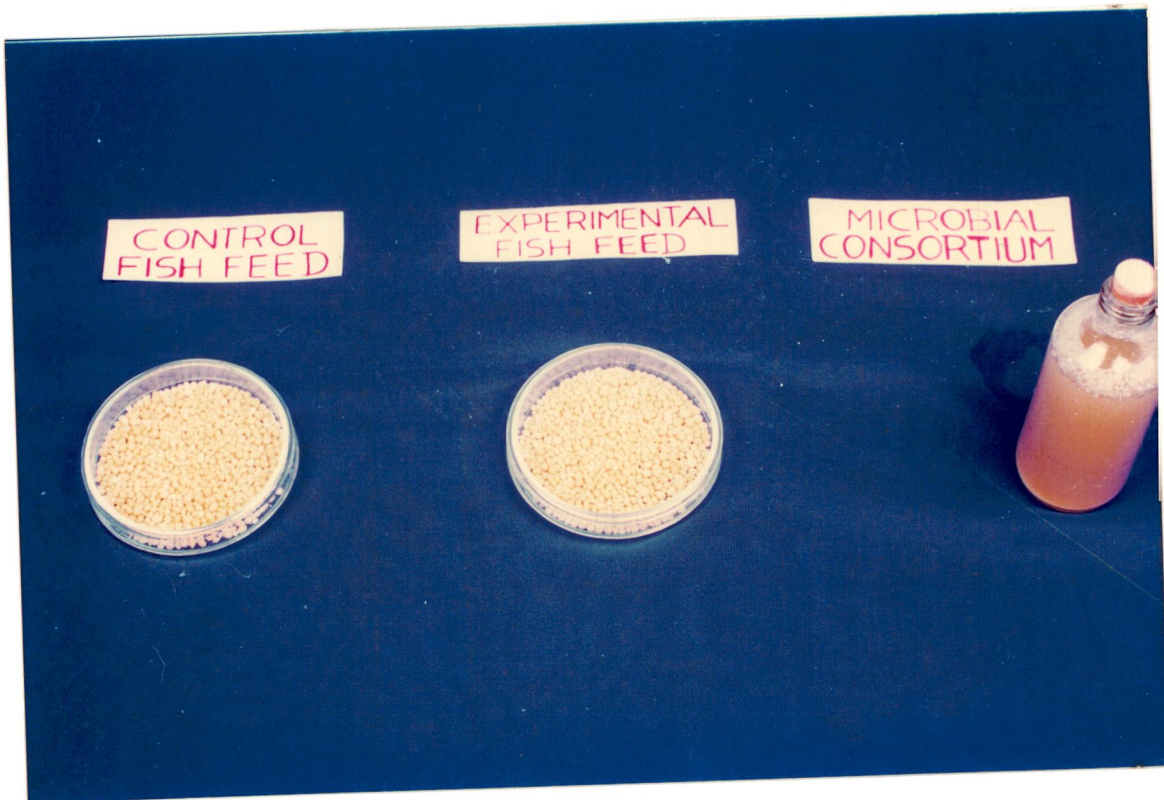


Plate I.

Commercial preparation of Effective Microbial consortium obtained from the Japanese company being marketed at Mumbai is purchased as liquid preparation (Plate I). Effective Microbial consortium (EM) is available in the market as concentrated cell suspension of the microbes. It is a brown colored pleasant smelling viscous liquid storable at room temperature.

#### **METHOD OF APPLICATION OF MC**

MC preparations at 1:10,000 ratio is to be used i.e. for every 10,000 litre of water a quantity of one litre of effective microorganisms is required. Whenever fresh water is let into the ponds or troughs, the effective microorganism preparation is applied. This is the usage recommended by Japanese company.

In the present study, the MC preparation was applied at different concentrations as 0.5 ml, 1.0 ml, 1.5 ml and 2 ml to different plastic tubs containing 5 litre of water and also MC preparation was given as fish feed fortified with a base. MC preparation was added to the plastic tubs every alternate day when the water was changed.

#### **MODE OF ACTION OF MC**

There are photosynthetic, autotrophic bacteria in the consortium. These can fix carbondioxide from the atmosphere. The autotrophic bacteria produce many aminoacids and bioactive substances. The lactic acid bacterial component is very important. From the sugars manufactured by the photoautotrophs, lactic acid bacteria produce lactic acid in water. Lactic acid is a powerful microbicide. The fish pathogens are wiped out by lactic acid.

### **METHOD OF PREPARATION OF FORTIFIED FISH FEED**

Microbial cells as fish feed supply additional nucleic acids, proteins, vitamins and various minerals along with carotenoids. Fortified fish feed was prepared by mixing 100 ml of Microbial consortium (MC) with 250 g of Bengal gram powder. The mixture was mixed well and made into pellets, and these pellets were sun dried (Plate I). Fish normally prefers feed in granulated form. This fortified fish feed was stored at room temperature.

### **METHOD OF PREPARATION OF NORMAL FISH FEED**

The normal fish feed was prepared by mixing 300 gms of Bengal gram powder with tap water and was made into dough. The dough was made into small round pellets. These pellets were spread on a white sheet of paper sun dried and stored (Plate I).

### **PROCEDURE**

Six plastic tubs of capacity seven litre were used for the experiment (Plate III a,b). In each tub 5 litres of water was filled and 14 fingerlings were introduced after taking the initial weight of each fingerling. Among the six tubs, one tub was maintained as control. The treatments for each tub were as follows:

#### **CONTROL**

To the tub maintained as control, 500 mg of normal fish feed (Bengal gram flour + water) pellets is used in feeding the fingerlings.

#### **TREATMENT - 1**

A quantity of 500 mg of normal fish feed and 0.5 ml of MC were added to the water.

**TREATMENT – 2**

A quantity of 500 mg of normal fish feed and 1 ml of MC were added to the water.

**TREATMENT – 3**

An aliquot of 1.5 ml and 500 mg of normal fish feed were added to the water.

**TREATMENT – 4**

An aliquot of 2 ml of Microbial consortium and 500 mg of normal fish feed comprised the treatment 4.

**TREATMENT – 5**

A quantity of 500 mg of fortified fish feed (Bengal gram + MC) pellets was used as fish feed.

After the initial weight, the weight of the fingerlings was recorded on 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day, to record an increase in weight of the fingerlings.

General observations like change in color, malformation in fins, skin eruption if any were noted. The fish fingerling mortality in each treatment, onslaught of any diseases particularly viral disease, active movement of the fingerlings were observed.

Following growth parameters were also studied

**AVERAGE WEIGHT GAIN**

Average weight gain was calculated using the formula

$$\text{Average weight gain} = W_2 - W_1$$



Plate III a. Control group and experimental group of fishes.



Plate III b. Experimental group of fishes.

**GROWTH RATE**

Growth rate was calculated using the formula

$$\text{Growth rate} = (W_2 - W_1 / \text{No.of days}).$$

**SPECIFIC GROWTH RATE (SGR)**

$$\text{SGR} = 100 (\text{Log}_e w_2 - \text{log}_e w_1) / \text{No.of days}.$$

**PERCENTAGE WEIGHT GAIN**

$$\text{Percentage weight gain} = (W_2 - W_1) \times 100 / W_1.$$

Depending on the mortality the % survival was also calculated.

# RESULTS

## IV RESULTS

The results of the present study on the “Use of Microbial consortium (MC) for producing healthy fishes” are given in Tables I, II, III, IV and V.

Table – I shows the fish feed and MC composition of different experimental groups. As per the table the pelleted bengal gram powder was the fish feed used and it was also incorporated with MC which constituted the fortified food used for T<sub>5</sub> group. Control group was fed with pelleted bengal gram powder. T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> were fed with pelleted bengal gram powder and to the medium, MC was added 0.5, 1.0, 1.5, 2 ml respectively.

Table – II shows mortality of the fingerlings during experimental period and also the % mortality. It was found that in the control group the mortality was higher. Two out of 14 fingerlings died in the first stage of experimental study and 3 fishes died within 30 days, while in the experimental groups T<sub>1</sub> showed lesser mortality level one in the first 15 days and another one in 30 days and 3<sup>rd</sup> one on 45<sup>th</sup> day. Overall a mortality of 3 fishes were recorded. in T<sub>1</sub> group. In T<sub>2</sub> group, one fish mortality was recorded after 30 days. Similarly for T<sub>3</sub> only one mortality was recorded after 45 days and in T<sub>4</sub> and T<sub>5</sub> there were no mortality during experimental period of 60 days. Fig:1 illustrates the highest % mortality of control group followed by T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> groups. Table – II also shows the % survival of fingerlings.

The results recorded in Table – III show the total weight of each group depending on the level and use of MC during experimental period. Every group including control has shown a continuous growth gain phenomena by comparatively the growth gain between the groups is increasing depending on the

**Table – I**

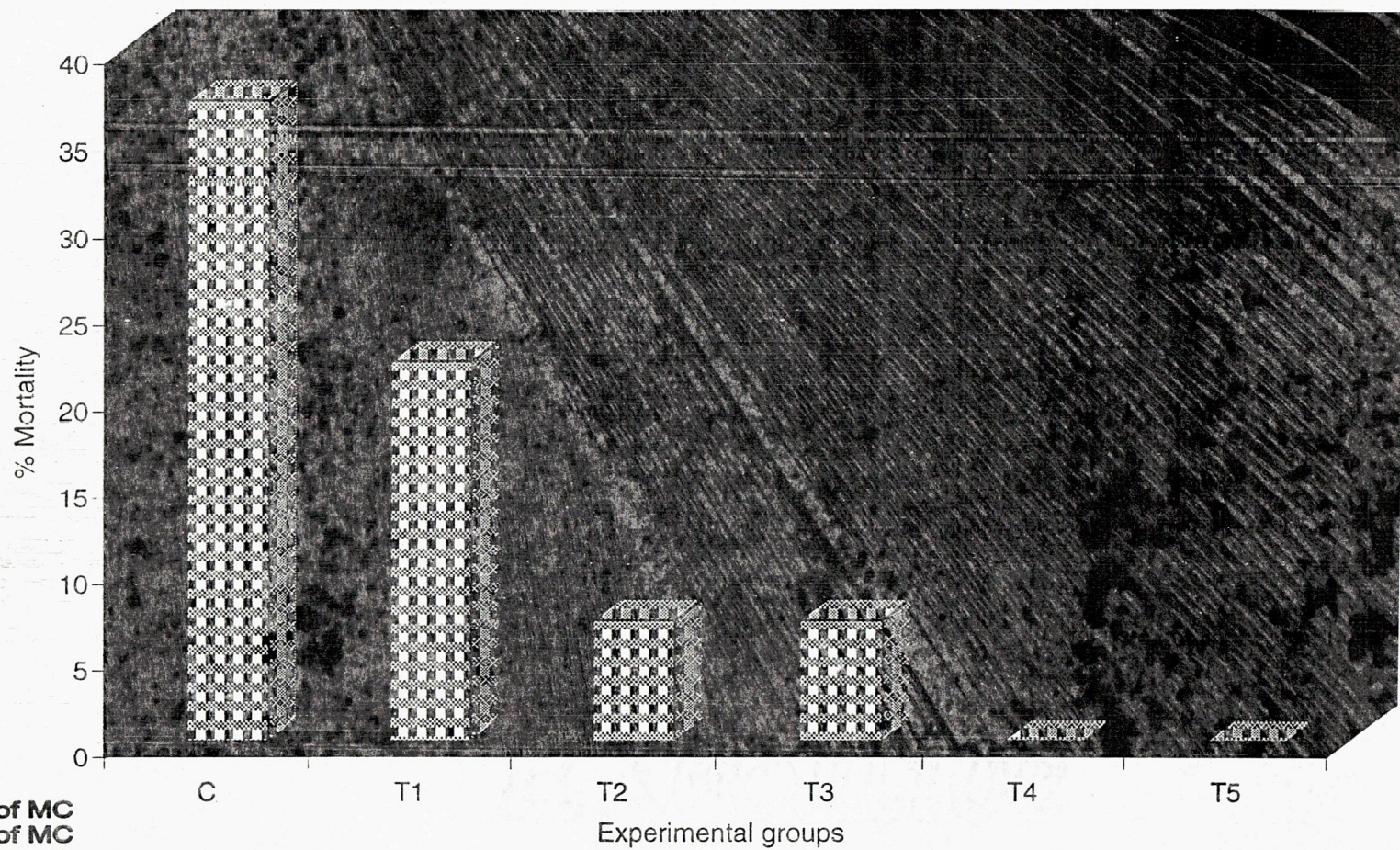
**Food and MC Composition for different experimental groups.**

| S.No. | Groups         | Food                        | Quantity in mg (for group) | Nature of feeding | Microbial Consortium (MC) in ml |
|-------|----------------|-----------------------------|----------------------------|-------------------|---------------------------------|
| 1.    | Control -C     | Pelleted Bengal gram Powder | 500 mg                     | Adlibitum         | -                               |
| 2.    | T <sub>1</sub> | Pelleted Bengal gram Powder | 500 mg                     | Adlibitum         | .05                             |
| 3.    | T <sub>2</sub> | Pelleted Bengal gram Powder | 500 mg                     | Adlibitum         | 1.0                             |
| 4.    | T <sub>3</sub> | Pelleted Bengal gram Powder | 500 mg                     | Adlibitum         | 1.5                             |
| 5.    | T <sub>4</sub> | Pelleted Bengal gram Powder | 500 mg                     | Adlibitum         | 2.0                             |
| 6.    | T <sub>5</sub> | Pelleted Bengal gram Powder | 500 mg                     | Adlibitum         | -                               |

**Table II**

**Mortality of fish during experimental period.**

| S.No. | Groups         | 15 days | 30 days | 45 days | 60 days | Total | % mortality | % Survival |
|-------|----------------|---------|---------|---------|---------|-------|-------------|------------|
| 1.    | C              | 2       | 3       | -       | -       | 5     | 36.00       | 64.00      |
| 2.    | T <sub>1</sub> | 1       | 1       | 1       | -       | 3     | 21.00       | 79.00      |
| 3.    | T <sub>2</sub> | -       | 1       | -       | -       | 1     | 7.00        | 93.00      |
| 4.    | T <sub>3</sub> | -       | -       | 1       | -       | 2     | 7.00        | 93.00      |
| 5.    | T <sub>4</sub> | -       | -       | -       | -       | -     | 0           | 100        |
| 6.    | T <sub>5</sub> | -       | -       | -       | -       | -     | 0           | 100        |



**C = Control**  
**T1 = 0.5 ml of MC**  
**T2 = 1.0 ml of MC**  
**T3 = 1.5 ml of MC**  
**T4 = 2.0 ml of MC**  
**T5 = Fortified feed with MC**

**Fig.1. % Mortality in experimental groups**

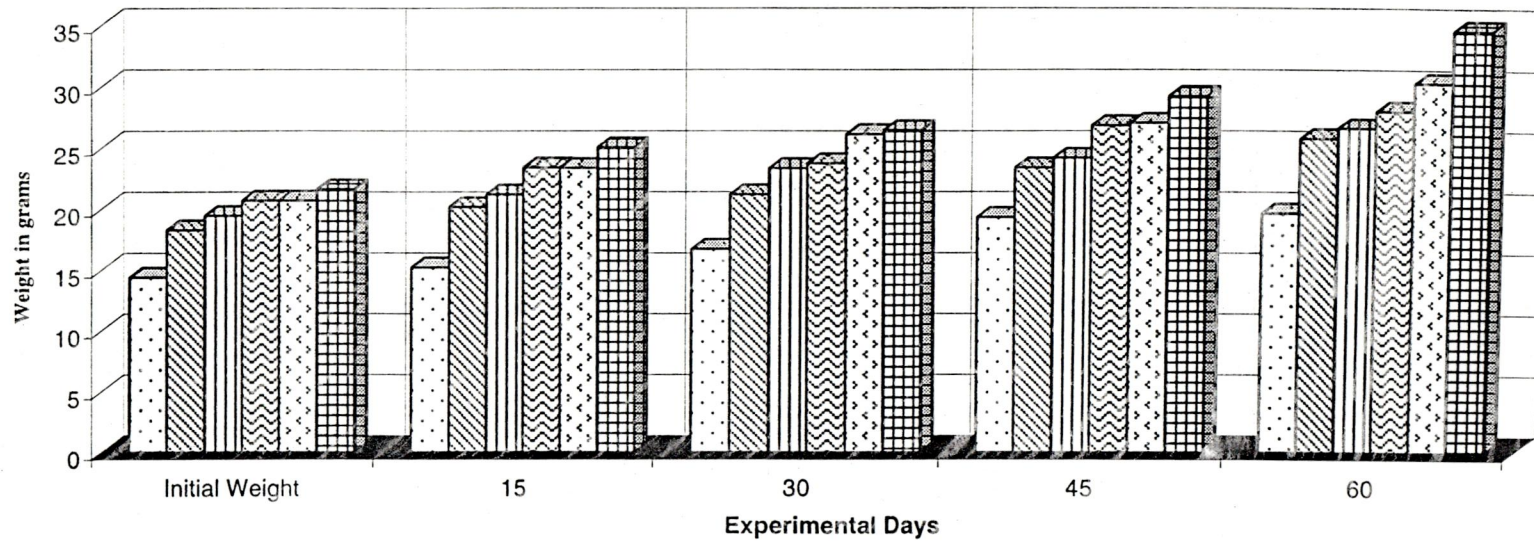
**Observe the high % mortality in control and no mortality in T4 & T5**

**Table III**

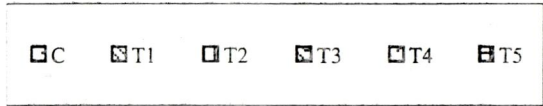
**Total weight of each group during experimental period (in gms)**

| S.No. | Groups         | Initial Weight | Increase in 15 days | Increase in 30 days | Increase in 45 days | Increase in 60 days | Total weight gain by the group during experimental period(gm) |
|-------|----------------|----------------|---------------------|---------------------|---------------------|---------------------|---|
| 1.    | C              | 14.392         | 15.209              | 16.727              | 19.321              | 19.655              | 5.263   |
| 2.    | T <sub>1</sub> | 18.267         | 20.141              | 21.168              | 23.380              | 25.764              | 7.497   |
| 3.    | T <sub>2</sub> | 18.283         | 20.622              | 23.323              | 24.173              | 26.609              | 8.326   |
| 4.    | T <sub>3</sub> | 19.438         | 23.048              | 23.722              | 26.855              | 28.004              | 8.566   |
| 5.    | T <sub>4</sub> | 20.686         | 23.339              | 26.134              | 27.054              | 30.299              | 9.613   |
| 6.    | T <sub>5</sub> | 21.608         | 25.050              | 26.455              | 29.244              | 34.580              | 12.972  |

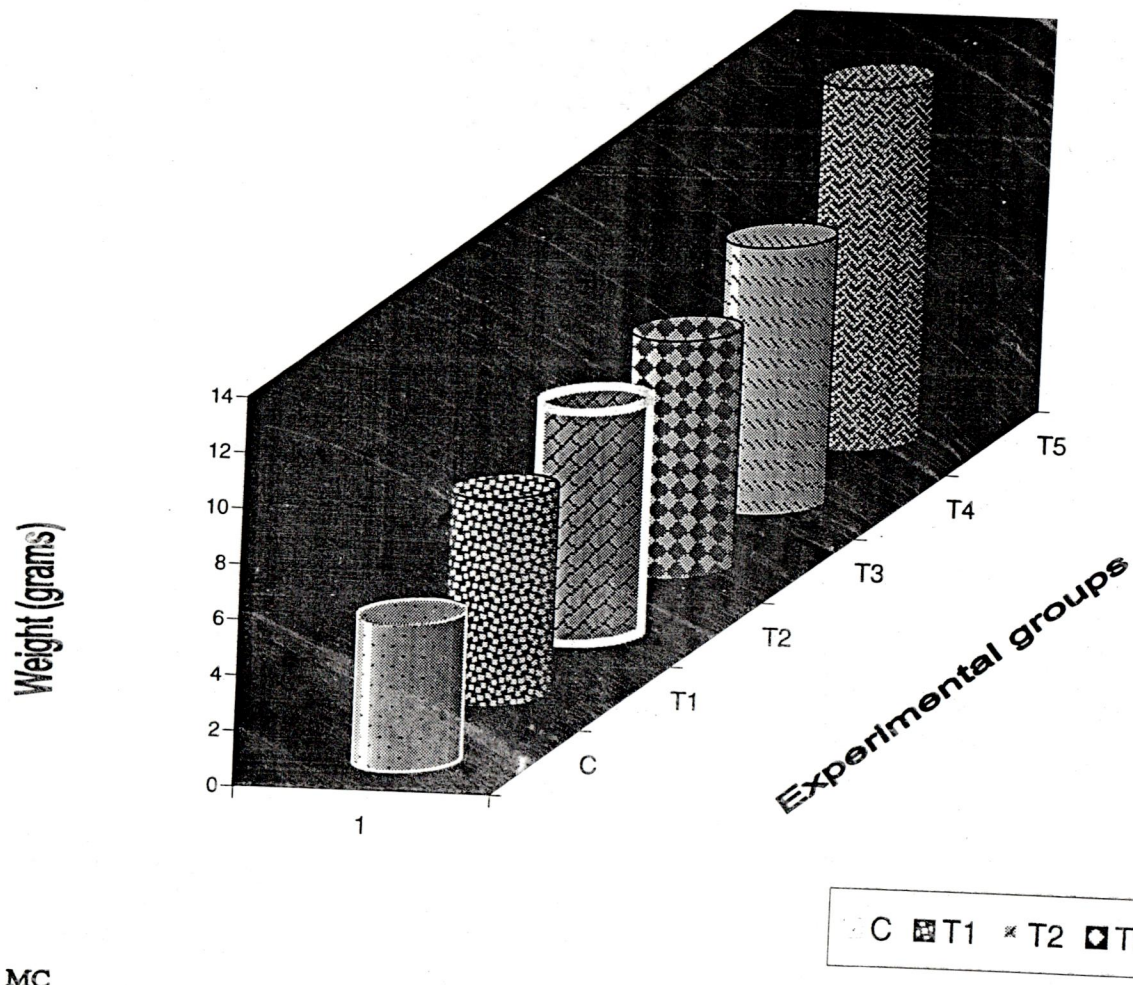
N = 14/Treatment



**C = Control**  
**T1 = 0.5 ml of MC**  
**T2 = 1.0 ml of MC**  
**T3 = 1.5 ml of MC**  
**T4 = 2.0 ml of MC**  
**T5 = Fortified feed with MC**



**Fig.2. Weight gain by each group during experimental period**  
 Observe the tremendous increase in T5 after 60 days



**Fig.3. Total Weight gain by the groups at the end of experimental period**  
 The weight gain by the T5 group is the maximum

level and use of consortium. Total weight gain by the control group over a period of 60 days was 5.263 g and that of T<sub>1</sub> – 7.497 g, T<sub>2</sub> – 8.326 g, T<sub>3</sub> – 8.566 g, T<sub>4</sub> – 9.613 g and T<sub>5</sub> – 12.972 g. Weight gain by each group during experimental period is represented in Fig:2. It also shows that there is a steady weight gain in all groups in every fortnight time, in that the T<sub>5</sub> group has recorded a maximum weight gain. Fig. 3 explains the total weight gain by different groups which also points out the maximum weight gain by T<sub>5</sub> group.

The mean weight gain for every fortnight is recorded in Table – IV. Mean initial weight of control fish was  $1.028 \pm 0.14$  and after 60 days it was  $1.403 \pm 0.36$ . The mean initial weight was  $1.304 \pm 0.34$ ,  $1.305 \pm 0.38$ ,  $1.388 \pm 0.37$ ,  $1.477 \pm 0.34$ ,  $1.543 \pm 0.48$  respectively for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and finally after 60 days it was  $1.840 \pm 0.37$ ,  $1.900 \pm 0.40$ ,  $2.000 \pm 0.40$ ,  $2.164 \pm 0.31$  and  $2.470 \pm 0.42$ .

Fig: 4 illustrates the mean weight gain by different groups every fortnight during experimental period and the T<sub>5</sub> group showing maximum mean weight gain during the period.

Table – V gives the growth characteristics of fish such as mean weight gain, growth rate, % weight gain and specific growth rate (SGR).

### MEAN WEIGHT GAIN

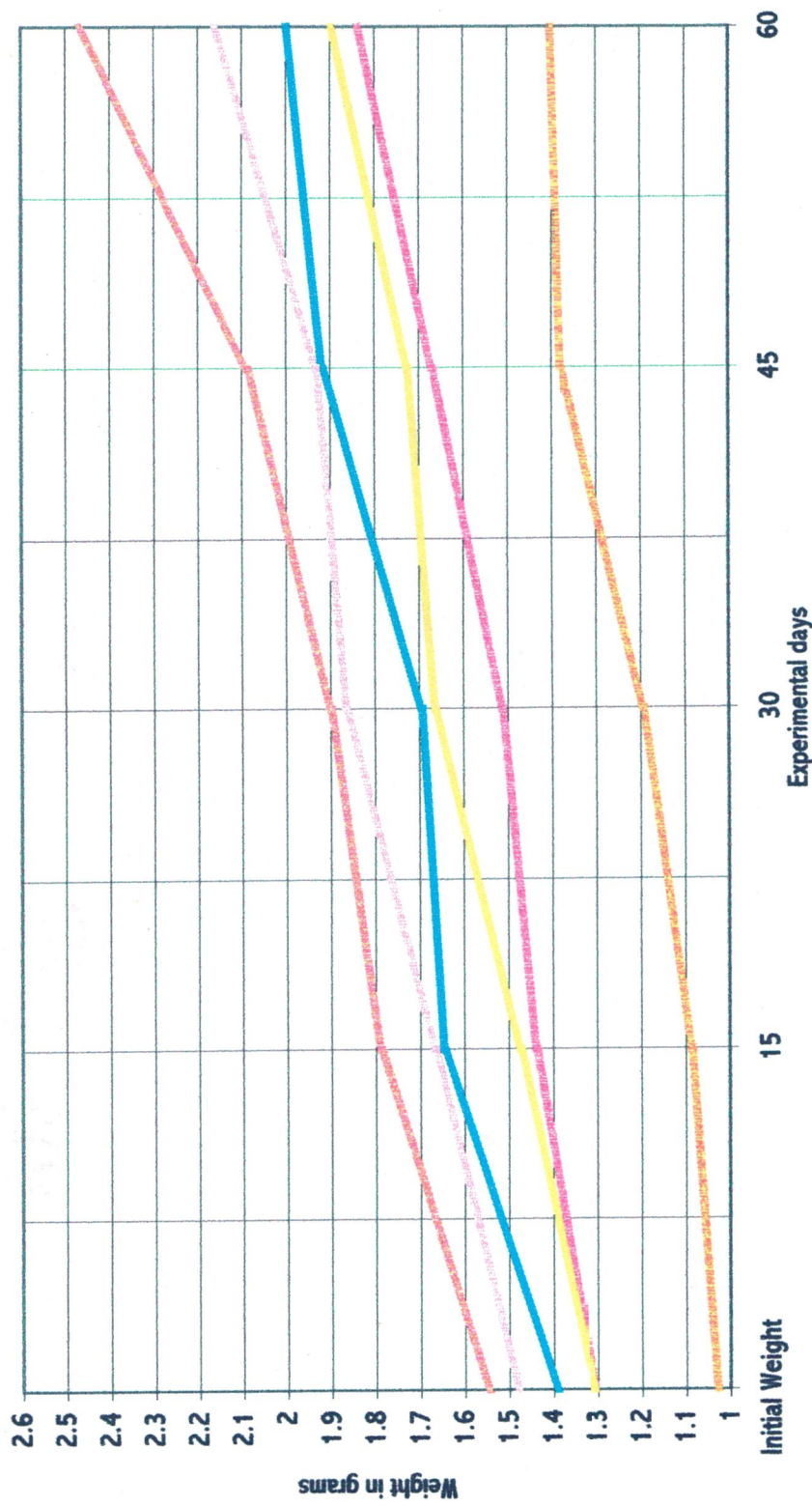
In the comparison between control and experimental groups, all experimental groups have shown an increase over the mean weight gain of the control group as revealed in Fig.5 and the order of increase is T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and the maximum by T<sub>5</sub>.

**Table IV**

Mean weight gain during experimental period

| S.No. | Groups         | Initial Weight | Increase in 15 days | Increase in 30 days | Increase in 45 days | Increase in 60 days |
|-------|----------------|----------------|---------------------|---------------------|---------------------|---------------------|
| 1.    | C              | 1.028 ± 0.14   | 1.086 ± 0.24        | 1.194 ± 0.26        | 1.380 ± 0.22        | 1.403 ± 0.22        |
| 2.    | T <sub>1</sub> | 1.304 ± 0.34   | 1.438 ± 0.37        | 1.512 ± 0.53        | 1.670 ± 0.48        | 1.840 ± 0.37        |
| 3.    | T <sub>2</sub> | 1.305 ± 0.38   | 1.473 ± 0.42        | 1.665 ± 0.31        | 1.726 ± 0.37        | 1.900 ± 0.40        |
| 4.    | T <sub>3</sub> | 1.388 ± 0.37   | 1.646 ± 0.37        | 1.694 ± 0.36        | 1.918 ± 0.33        | 2.000 ± 0.40        |
| 5.    | T <sub>4</sub> | 1.477 ± 0.34   | 1.667 ± 0.31        | 1.866 ± 0.42        | 1.932 ± 0.34        | 2.164 ± 0.31        |
| 6.    | T <sub>5</sub> | 1.543 ± 0.48   | 1.789 ± 0.36        | 1.899 ± 0.47        | 2.088 ± 0.34        | 2.470 ± 0.42        |

N – 14/Treatment



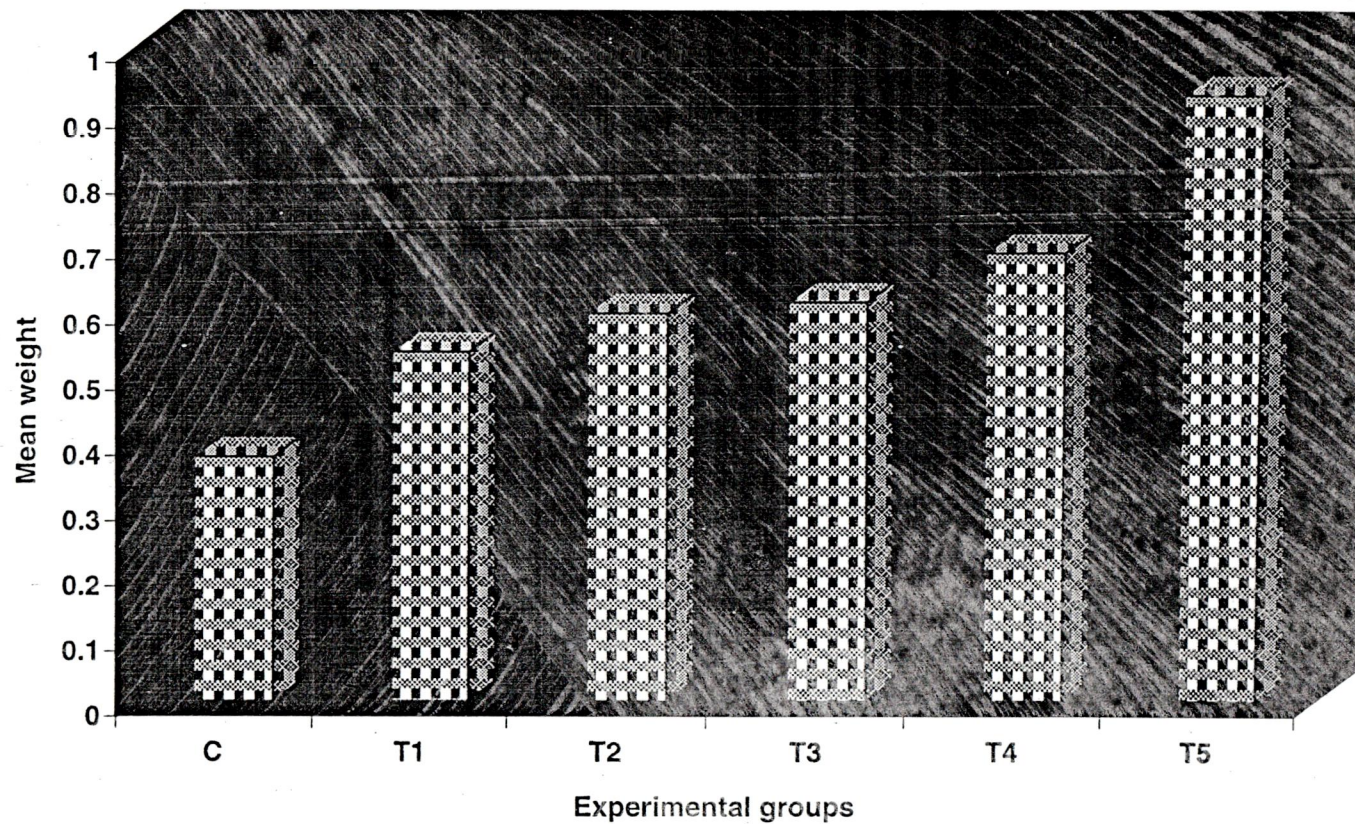
C = Control  
 T1 = 0.5 ml of MC  
 T2 = 1.0 ml of MC  
 T3 = 1.5 ml of MC  
 T4 = 2.0 ml of MC  
 T5 = Fortified feed with MC

**Fig.4. Mean weight gain by different groups 3 during experimental period**

It is noteworthy that T5 has obtained the highest increase in weight

**Table – V****Growth characteristics based on mean weight.**

| S.No. | Groups         | Mean weight gain (W2 – W1) | Growth rate(W2-W1)/No.of days | Percentage weight gain (W2 –W1)*100/W1 | Specific growth rate(SGR) $100[\log_e \text{ final wt} - \log_e \text{ initial wt}] / \text{No. of days}$ |
|-------|----------------|----------------------------|-------------------------------|--|---|
| 1.    | C              | 0.375                      | 0.006                         | 36.47                                  | .50   |
| 2.    | T <sub>1</sub> | 0.536                      | 0.008                         | 41.10                                  | .56   |
| 3.    | T <sub>2</sub> | 0.595                      | 0.009                         | 45.58                                  | .63   |
| 4.    | T <sub>3</sub> | 0.612                      | 0.10                          | 44.08                                  | .61   |
| 5.    | T <sub>4</sub> | 0.687                      | 0.011                         | 46.50                                  | .63   |
| 6.    | T <sub>5</sub> | 0.927                      | 0.015                         | 60.06                                  | .78   |



**C = Control**  
**T1 = 0.5 ml of MC**  
**T2 = 1.0 ml of MC**  
**T3 = 1.5 ml of MC**  
**T4 = 2.0 ml of MC**  
**T5 = Fortified feed with MC**

**Fig.5. Mean weight gain by different groups at the end of the experiment.**  
The mean weight gain by the T5 group is the maximum

### **GROWTH RATE (GR).**

Growth rate indicated the growth rate of fingerlings during every day of the experimental period. Fig.6 explains the GR of the experimental groups and control group where experimental groups showed a steady increase in all the groups as compared to control group. In GR also T<sub>5</sub> group scores a maximum over the other groups as also to that of the control indicating the efficacy of fortified fish feed.

### **% WEIGHT GAIN**

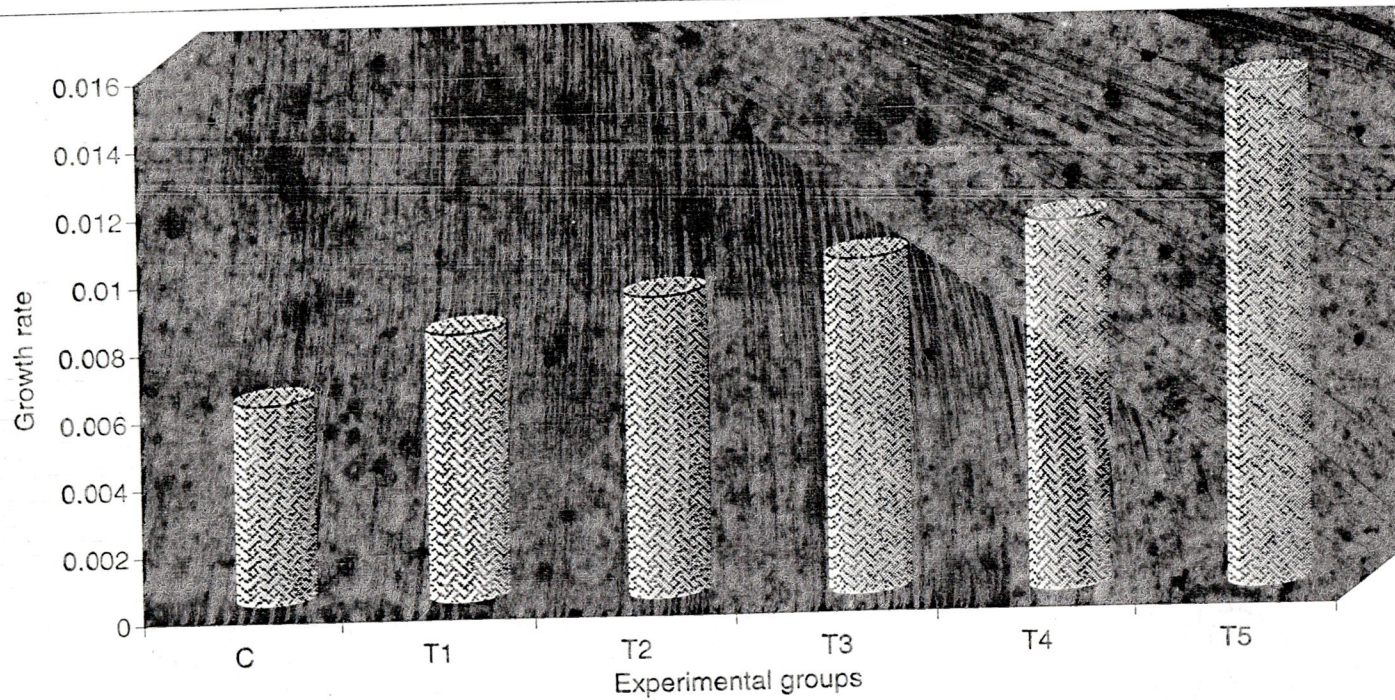
Table - V lists the % weight gain in the following manners 36.47, 41.10, 45.54, 44.08, 46.50 and 60.06 respectively for C, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>. Though there is an increase in % weight gain according to the order of group the T<sub>3</sub> group shows a little less % weight gain comparing to T<sub>2</sub> and T<sub>4</sub>. This may be attributed to the fact that the % weight gain depends on the initial weight of the fish.

Fig:7 indicates the % weight gain by different groups and the highest % weight gained is by T<sub>5</sub> group and the lowest by control group.

### **SPECIFIC GROWTH RATE (SGR).**

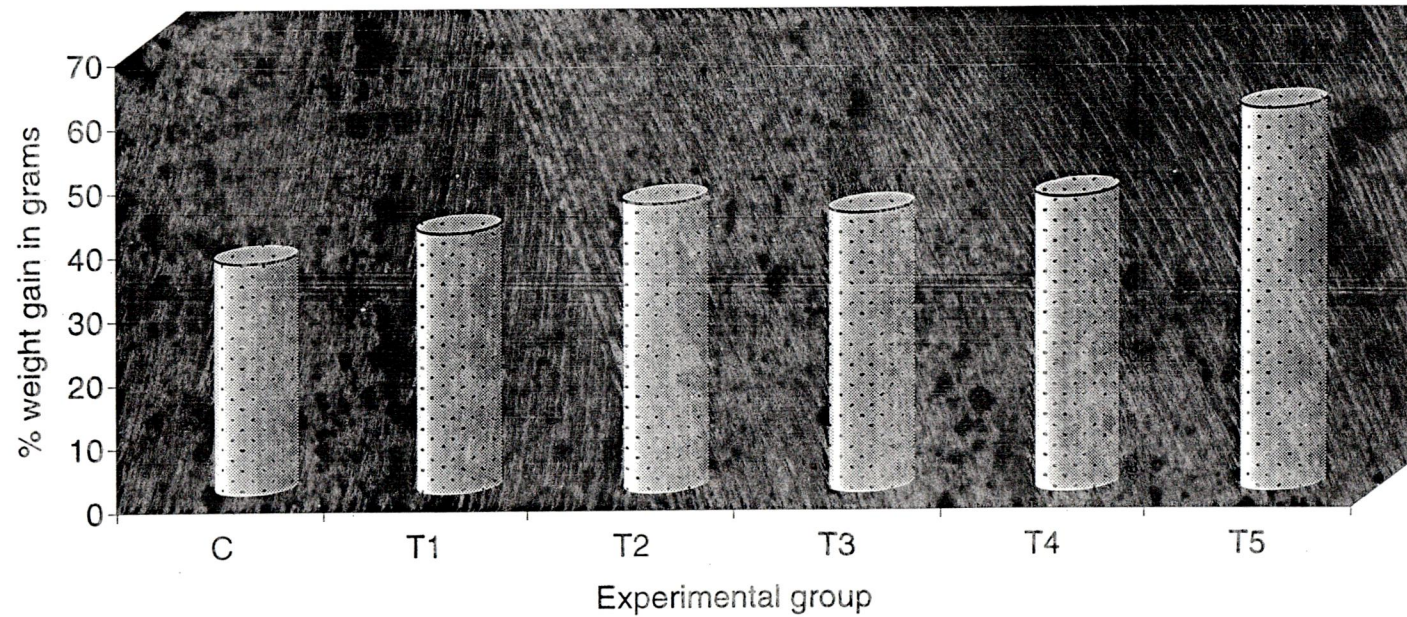
SGR is recorded in Table V. All the groups show a higher SGR over the control. Among the experimental groups the T<sub>1</sub> records 0.56, T<sub>2</sub> 0.63, T<sub>3</sub> shows 0.61, T<sub>4</sub> shows 0.63 and the highest SGR recorded by T<sub>5</sub> is of 0.78. Fig : 8 illustrates SGR recorded by different groups.

Fig : 9 illustrates the 100% survival by T<sub>4</sub> and T<sub>5</sub> groups. The MC added to T<sub>4</sub> group was 2.0 ml which helps in developing disease resistance and also facilitates growth. More than that is the incorporating MC with fish feed which gives highest result and of enhancing growth and cutting down mortality. The results recorded in tables and illustrations given in figures indicates that :



C = Control  
T1 = 0.5 ml of MC  
T2 = 1.0 ml of MC  
T3 = 1.5 ml of MC  
T4 = 2.0 ml of MC  
T5 = Fortified feed with MC

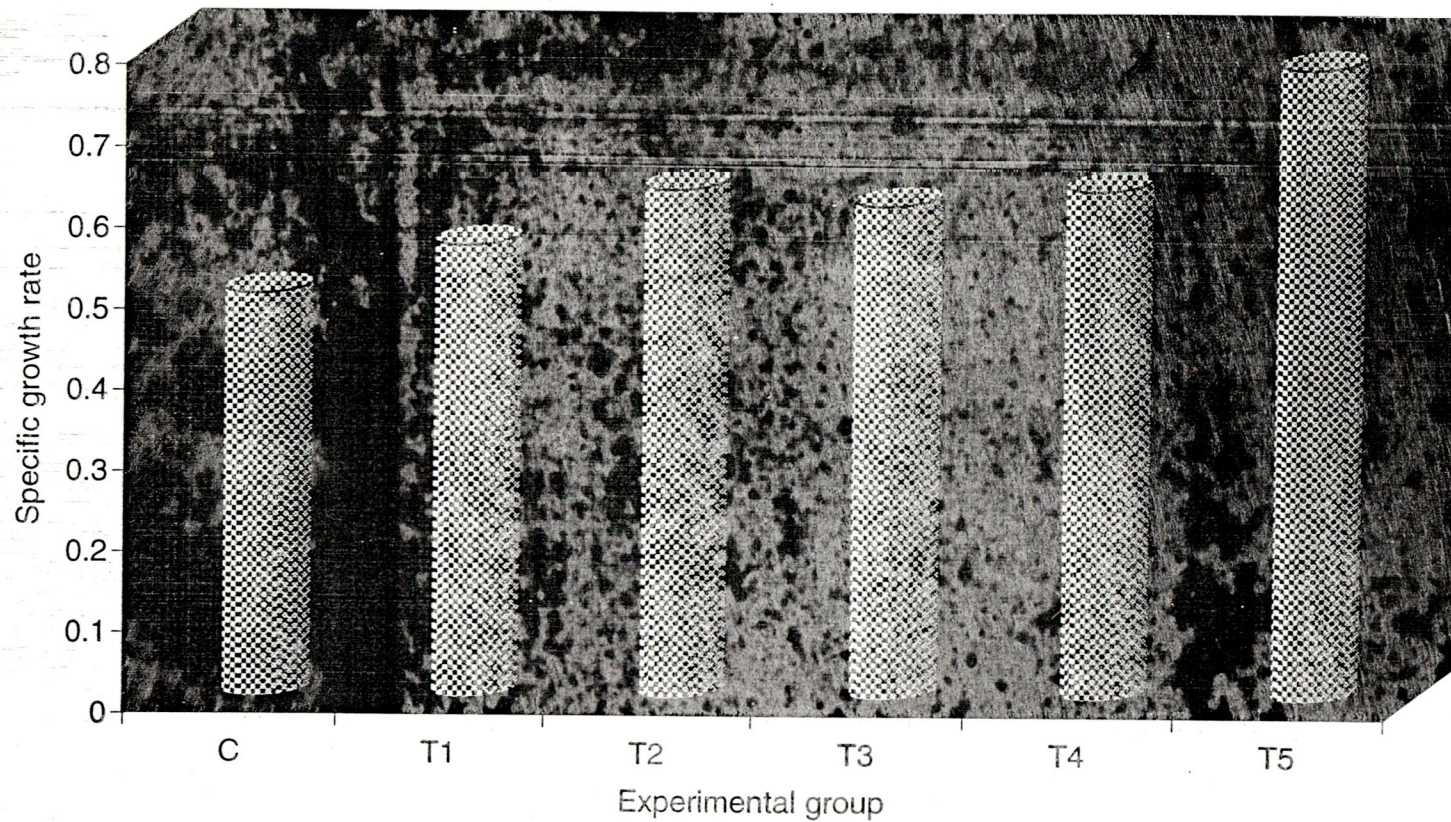
**Fig. 6. Growth rate of different groups**  
Note the highest growth rate recorded in T5



C = Control  
T1 = 0.5 ml of MC  
T2 = 1.0 ml of MC  
T3 = 1.5 ml of MC  
T4 = 2.0 ml of MC  
T5 = Fortified feed with MC

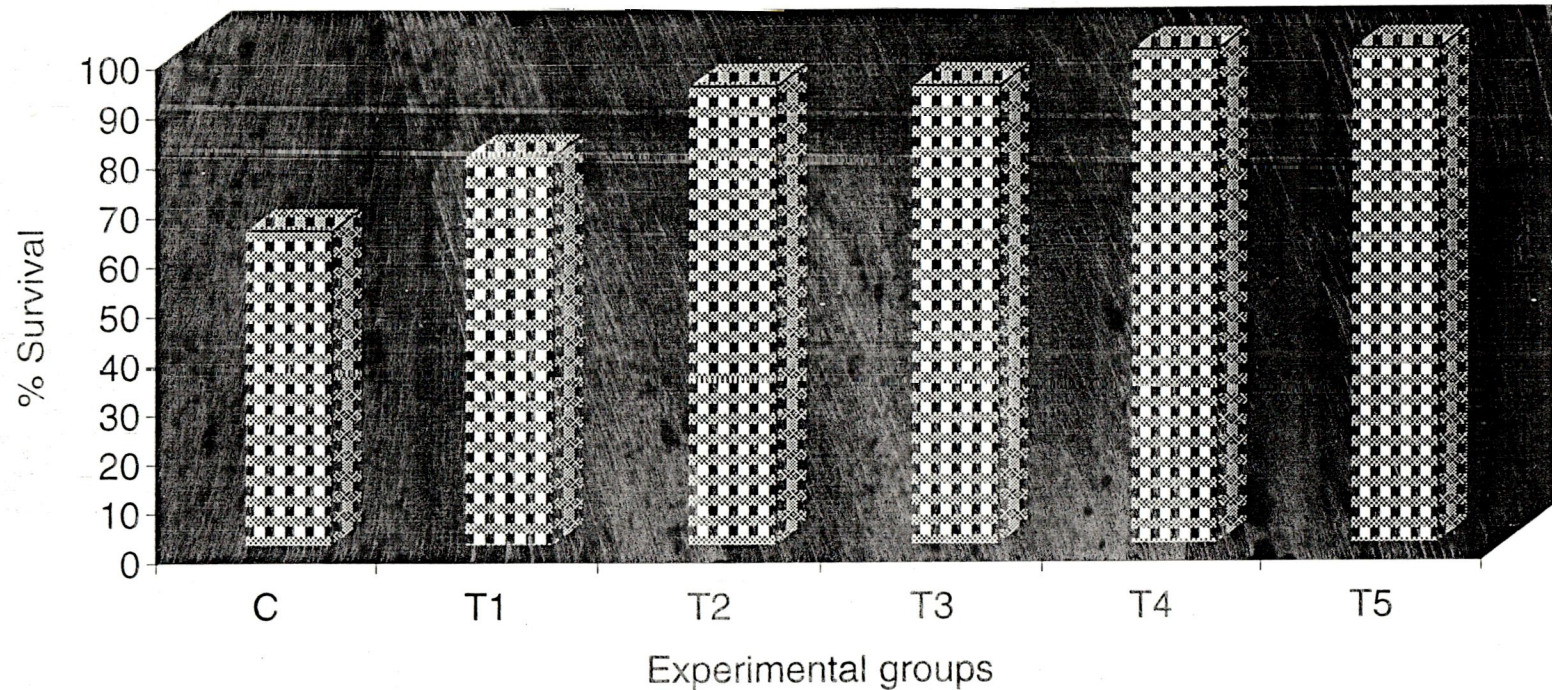
**Fig. 7. % weight gain by different groups**

Note the highest % weight gain recorded  
in T5 when compared to C, T1, T2, T3 & T4



**C = Control**  
**T1 = 0.5 ml of MC**  
**T2 = 1.0 ml of MC**  
**T3 = 1.5 ml of MC**  
**T4 = 2.0 ml of MC**  
**T5 = Fortified feed with MC**

**Fig. 8. Specific growth rate in different groups**  
Note the striking increase of specific growth rate in T5.



**C = Control**  
**T1 = 0.5 ml of MC**  
**T2 = 1.0 ml of MC**  
**T3 = 1.5 ml of MC**  
**T4 = 2.0 ml of MC**  
**T5 = Fortified feed with MC**

**Fig.9. % survival in experimental groups**

**Observe the 100% survival recorded  
in T4 & T5 experimental groups**

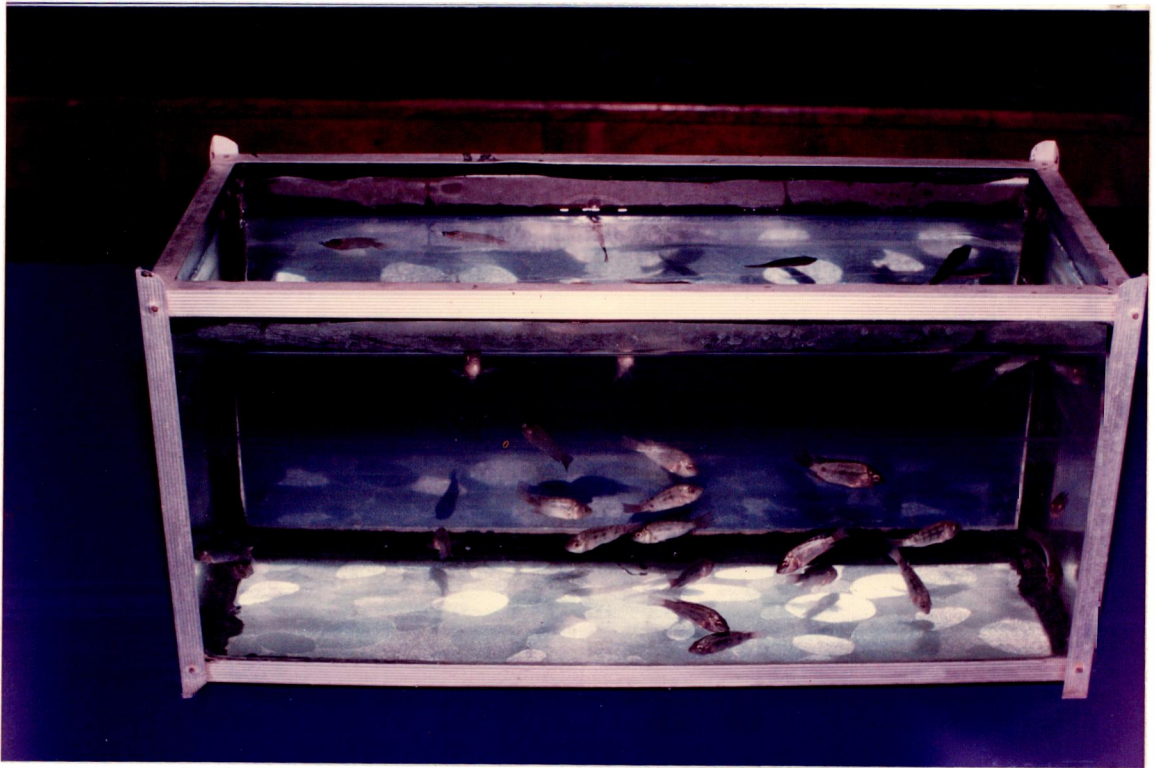
Addition of MC to the medium ranging from 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> group show that higher is the dose in the medium, mortality and enhancing growth in the form of weight gain and other growth parameters.

From the results obtained it can be noted that addition of 2 ml of MC into the water body (T<sub>4</sub>) and fortified food with MC (T<sub>5</sub>) can afford 100% survival to the fishes (Fig:9) and can produce healthy fishes. But high production can be obtained when fish feed is incorporated with MC (Plate IV).

The proven effect of microbial consortium can be summarized as below.

| S.No. | Organism                                       | Type     | Effect/Benefit                                 |
|-------|--|----------|--|
| 1.    | Lactic acid bacteria                           | Bacteria | Suppresses pathogens                           |
| 2.    | Yeast  | Yeast    | Rapid growth of fishes through growth hormones |
| 3.    | Actinomycetes                                  | Bacteria | Antibiotic effect                              |
| 4.    | Filamentous fungi (Aspergillus and Pencillium) | Fungi    | Removes off-odour from the water body.         |

From the above table it can be observed and through recorded data it is proved that MC helps in suppressing pathogens, helps in growth of fishes and also helps in removing off-odour from the water body.



**Plate IV. Fishes grown with feed fortified with MC**

# **DISCUSSION**

## V DISCUSSION

The results of this study show that fish feed incorporated with MC resulted in maximum growth and 100% survival. Yeast present in MC may help in synthesizing antimicrobial substances and growth hormones. These promote rapid growth of fishes. Similar work was carried out by Johnson *et al.* (1977) who has reported that the red yeast, *Phaffia rhodozyma* is a promising dietary supplement for shell fish.

The increased weight gain of fingerlings with MC supplemented fish feed is recorded in Table III. This weight gain may be due to the effectiveness of the food and also of the MC. The findings of Scott – Jackson *et al.* (1996) who studied the effect of microbial phytase supplemented fish feed which showed an increase in weight gain confirms the present study.

The fingerlings treated with MC did not show any disease like skin eruption or deformities in the fin. This may be due to the presence of Lactic acid bacteria in MC which may pick up disease causing pathogens or regulate the digestive tract or even immobilise the disease causing germs. This agrees with the work done by Jankauskiene *et al.* (1996) who has reported that the development of lactoflora in the intestinal tract would improve the immunological state of carps, thus increasing their resistance to various disease and infections in particular.

The mortality and the % survival as given in Table II represents the efficacy of the use of MC in growing healthy fishes and in controlling mortality. Similar work was carried out by Keong and Wee (1989) by using Cassava leaf meal as fish feed which has had similar effect. This effect might have been due to some medicinal properties of cassava leaf.

Maximum weight gain of 12.972 g was seen in T<sub>5</sub> group (Fish feed fortified with MC). Yeasts and actinomycetes present in MC would have helped in promoting rapid growth of fish. This view is supported by Petit *et al.* (1997) who proved that the carotenoid astaxanthin formed from yeast, *Phaffia rhodozyma* when supplemented along with diet helped in enhanced growth of prawn. Higher weight gain is reported when diets contained 16-18% corn gluten meal as reported by Wu, *et al.* (1995).

Growth rate observed in T<sub>5</sub> group (Fish feed fortified with MC) is maximum. Microbial cells in the food supply proteins, vitamins and various minerals along with carotenoids. The work of Torrison (1984) in Atlantic Salmon has been reported that diets supplemented with astaxanthin and canthaxanthin promoted higher growth rates.

Work carried out with feed supplemented with *Spirulina* on *Tilapia mossambica* (Raja, 1997; Rajammal, 1997) showed better performance of growth rate and specific growth rate because *Spirulina* as a blue green algae contains more protein and  $\beta$  – carotenoids. This confirms the current study. Filamentous fungi such as *Aspergillus* and *Pencillium* present in MC helps in preventing off-odour from the water and maximum of 2 ml of MC added in the medium helps in developing disease resistance and facilitates growth and also provides 100% survival more than that is the MC incorporated fish feed which helps in production of healthy fish, with higher growth rate and 100% survival.

**SUMMARY  
AND  
CONCLUSION**

## VI SUMMARY AND CONCLUSION

The present study "Use of Microbial consortium (MC) for producing healthy fishes" was based on the following premises:

To emphasize the use of Microbial consortium (MC) in enhancing the growth of fish.

Fingerlings were collected and acclimatized to laboratory condition.

Normal pelleted fish feed and fortified fish feed were prepared.

Normal pelleted fish feed consisted of bengal gram flour mixed with water and sun dried.

Fortified fish feed was prepared by mixing 250g of bengal gram flour and 100 ml of MC, pelleted and sun dried.

Six plastic tubs of seven litres capacity were taken and one served as control and the others as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>.

14 fingerlings were introduced into each tub. Group labelled as C served as control and was fed with 500 mg of normal fish feed (pelleted bengal gram flour) and other experimental groups are named as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>.

To the T<sub>1</sub> group 500mg of normal fish feed and 0.5 ml MC was added to the water body.

T<sub>2</sub> group was fed with 500 mg of normal fish feed and 1.0 ml of MC was added to the water body.

T<sub>3</sub> group was fed with 500 mg of normal fish feed and 1.5 ml of MC was added to the water body.

500 mg of normal pelleted fish feed and 2.0 ml of MC was added to T<sub>4</sub> group.

T<sub>5</sub> group was fed with 500 mg of fish feed fortified with MC.

On every alternate day, water in the tubs were changed and MC was added to the respected groups. Food was given at a particular time of a day.

Observations were made everyday to record the onslaught of diseases and mortality.

Weight of the fishes were recorded initially and after every fortnight for 60 days.

The selected growth parameters namely mean weight, growth rate, % weight gain and specific growth rate were calculated.

In different experimental groups the % mortality and % survival were calculated.

In the four concentrations where MC was added in 0.5 ml (T<sub>1</sub>), 1.0 ml (T<sub>2</sub>), 1.5 ml (T<sub>3</sub>), 2.0 ml (T<sub>4</sub>), T<sub>4</sub> scored the highest.

In all the parameters studied T<sub>5</sub> group (fish feed fortified with MC) ranked the highest.

Maximum of 2.0 ml of MC helps in developing disease resistance and also enhances growth.

Fish feed incorporated with MC helps in producing healthy fishes and highest growth with 100% survival.

From the present study, it is concluded that the addition of 2 ml of MC for 5 litres of water and also when MC incorporated along with fish feed helps in enhancing growth and 100% survival of fishes.

## **RECOMMENDATIONS / SUGGESTIONS**

The study on the effect of MC can be taken up for detoxification and pollution abatement process.

The growth promoting effect of MC can be studied in other groups of fishes.

# **BIBLIOGRAPHY**

- Ackefors, H. 1996. European aquaculture industry towards the year 2000. Copenhagen – Denmark ICES 36 pp.
- Aliah, R.S. 1996. Effect of coconut oil addition in artificial feeds on growth and body composition of carp (Cyprinus carpio.L.). Majalah BPPT (Indonesia). 71: 1 – 4.
- Almazon, G.J., Pullin, R.S.V., Angeles, A.F., Manalo, T.A., Agbayani, R.A. and Trono, M.T.B. (1986). Azolla pinnata as a dietary component for the Nile Tilapia, Oreochromis niloticus. In J.L.MacCLEAN, L.B. Dizon and L.V. Hosillos (Eds). The First Asian Fisheries Forum. Asian Fisheries Society. Manila, Philippines. 523 – 528.
- Anderson, J.S., Richardson, N.L., Higgs, D.A., Dosanjh, B.S. 1997. The evaluation of air – dried krill meal as a dietary protein supplement for juvenile chinook salmon (Oncorhynchus tshawytscha). Can. Tech. Rep. Fish. Aquat. Sci. 1 – 17.
- Appler, A.N. and Jauncey, K. 1983. The utilization of a filamentous green alga (Cladophora globerata (L) kutzin) for Sarotherodon (Tilapia) niloticus fingerlings. Aquaculture. 30: 21 – 30.
- Basudha, C.H., Vishwanath, W. 1997. Formulated feed based on aquatic weed Azolla and fish meal for rearing medium carp, Osteobrama belangeri (valenciennes). J.Aqua. Trop. 12 (3): 155 – 164.
- Bhosale, P. B. 1997. Microorganisms as fish feed in fish industries. Curr.Sci. 73(7,10): 562 – 563.
- Bisht, G.S., Bisht, D., Joshi, C. and Khulbe, R. D. 1996. Potential threat to reservoir fishery by fungi in Kumaun Himalaya, India. Curr.Sci. 71 (9, 10) : 720 – 723.

- Chakrabarthy, M.1996. Efficacy of fish feed formulated with fish and poultry offals. Environ. Ecol. 14(4): 791 – 793.
- Das, D., Sikdar,K. and Chatterjee, A.K. 1994. Potential of Azolla pinnata as biogas generator and as a fish feed. Indian. J.Environ.Hlth. 36(3): 186 – 191.
- Degani, G.,Ben-zvi, Y. and Levanan, D. 1989. The effect of different protein levels and temperatures on feed utilization , growth and body composition of Clarias gariepinus (Burchell 1822). Aquaculture. 76: 293 – 301.
- De Silva, S.S. and Anderson, T.A.1995. Fish nutrition in Aquaculture, Chapman and Hall, series 1, London.
- Elankumaran, S., Baskaran, P.and Palanichamy, S. 1992. The dietary influence on food utilization in the fresh water fish Oreochromis mossambicus and Cyprinus carpio var communis. J.Ecobiol. 4(4) :271 – 275.
- FAO, 1990. Aquaculture production. 1985 – 1988. FAO fisheries circular. 815. Revision 2, FAO, Rome.
- Fukusho, K. 1977. Nutritional effects of the rotifer, Brachionus plicatilis, raised by baking yeast on larval fish of Opleganthus fasciatus by enrichment with Chlorella spp. before feeding. Bull. Nagasaki. Pret. Inst. Fish. 3:151 – 154.
- Gokulakrishnan, P.and Bandopadhyay, S. 1995. Formulation and characterisation of some pelleted feeds for Penaeus monodon. Fishery Technology. 32 (1) : 19 – 24.

- Gopal, V., Prabakaran, S., Balasubramanian, P.R. 1996. Effect of a biogas plant effluent based pelleted diet on the growth of Oreochromis mossambicus fingerlings. Bioresour. Technol. 58(3): 315 – 317.
- Harsojo., Andini, L.S., Suwirna, S. 1992. The influence of irradiated sludge as a herbivore red niloticus fish feed. Isotope application and irradiation in agriculture , livestock and biology. Proceedings of a scientific meeting. Sundardi, T.(Eds). Badan Tenaga Atom National, Jakarta (Indonesia). 463 – 474.
- Hilge, V., Steffens, W. 1996. Aquaculture of fry and fingerlings of pike-perch (Stizostedion lucioperca L.) J.Appl. Ichthyol. 12 (3-4): 167 – 170.
- Imada, O., Kageyama, y., Watanabe.T., Kitazima.C., Fujita, S. and Yone, Y. 1979. Development of a new yeast as a culture medium for living feeds used in the production of fish feed. Bull. Jpn. Soc. Sci. Fish. 45: 955 – 959.
- Islam, S.Q.and Hossain, M.A.1994. Growth and feed efficiency of Oreochromis mossambicus fed on diets containing different levels of protein. Indian. J.Fisheries. 41 (1) : 15 – 19.
- Jankauskiele, R., Lenzer, H.and Lenzer, A. 1996. The frequency of Lacto bacillus occurrence in the content and the wall of intestinal tract of carps. Fishery and Aquaculture in Lithuania. Lithuanian society of hydrobiologists. 317 – 322.
- Jena, J.K., Mukhopadhyay, P.K., Sarkar, S., Aravindakshan, P.K., Muduli, H.K.1996.Evaluation of a formulated diet for nursery rearing of Indian major carp under field condition. J. Aquacult. Trop. 11(4): 299 – 305.

- Johnson, E.A., Conklin, D.E. and Lewis, M.J. 1977. J.Fish. Res. Board Can. 34: 2417 – 2421.
- Joseph, A.P.M., Sherief and James, T. 1994. Effect of different dietary inclusion levels of Azolla pinnata on the growth, food conversion and muscle composition of Etroplus suratensis (Bloch). J.Aqua.Trop. 9: 87 – 94.
- Juario, J.V. and Storch, V. 1984. Biological evaluation of phytoplankton (Chlorella spp., Tetraselmis spp. and Isochrysis galbana) as food for milk fish (Chanos chanos) fry. Aquaculture. 40: 193 – 198.
- Karunasagar, I. and Karunasagar, I. 1999. Diagnosis, treatment and prevention of microbial diseases of fish and shell fish. Curr. Sci. 76(3): 387 – 397.
- Keong, and Wee, K.L. 1989. The nutritive value of cassava leaf meal in pelleted feed for Nile Tilapia. Aquaculture. 83: 45 – 48.
- Krueger, A., Okoniewska, G., Pochitonow, Z., Krol, Z., Garnett, R.P. 1996. Glyphosate as a management tool in carp fisheries. Hydrobiologia. 340(1-3): 253-257.
- Kutty, M.N. 1999. Aquaculture development in India from a global perspective. Curr. Sci. 76(3): 333- 341.
- Li-Meng, H., Robinson, E.H. 1997. Microbial phytase can replace inorganic phosphorous supplements in channel catfish Ictalurus punctatus diets. J.World. Aquacult. Soc. 28(4): 402 – 406.

- Mohanty, S.N., Swain, S.K., Tripathi, S.D. 1996. Rearing of catla (Catla catla Ham.) spawn on formulated diets. J.Aquacult. Trop. 11(4): 253 – 258.
- Ota, S.K., Karunasagar, I., Tauro, P. and Karunasagar, I. 1998. Microbial diseases of shrimp. Indian. J. Microbiology. 38: 113 - 125.
- Paloheimo, J.E. and Dickie, L.M. 1969. Food and growth of fishes, effects of food and temperature on the relation between metabolism and body weight. J. Fish. Res. Bd. Canada. 23: 869 – 900.
- Park, D. 1997. Search for the cause of fish off flavour. Fish. Farm. Int. 24(3): 6.
- Park, H., Flores, R.A. and Johnson, L.A. 1997. Preparation of fish feed ingredients : Reduction of carotenoids in corn gluten meal. J. Agric. Food. Chem. 45: 2088 – 2092.
- Petit, H., Negre-Sadargues, G., Castillo, R. and Trilles, P. 1997. The effects of dietary astaxanthin on growth and moulting cycle of post larval stages of the prawn, Penaeus japonicus (Crustacea, Decapoda). Comp. Biochem. Physiol. 117A(4): 539 – 544.
- Raja, G. 1979. Studies in Pisciculture in monograph series on engineering of photosynthetic system (MCRC). 14: 1 – 60.
- Rajammal, K. 1997. Efficacy of microorganisms in the promotion of fish growth and in the reduction of selected pollutional parameters related to tannery effluent. Ph.D thesis submitted to Avinashilingam Deemed University, Coimbatore. 56 – 75.

- Scott-Jackson, L., Li-Meng, H., Robinson, E.H. 1996. Use of microbial phytase in channel catfish Ictalurus punctatus diets to improve utilization of phytate phosphorous. J. World. Aquacult. Soc. 27(3): 309 – 313.
- Sidhu, R.K. and Khulbe, R.D. 1998. A survey of impact and evaluation of water pollution on reservoir fisheries in Kumaun Himalayas, India. with special reference to microorganisms. Curr. Sci. 75(12,25): 1303 – 1308.
- Thomas., John. 1989. Assessment of the use of rice bran as a fish feed in four areas of Udonthani province in the North East Thailand. Asian. Inst. Tech. Bangkok (Thailand). 1 – 70.
- Torrissen, O.J. 1984. Pigmentation of salmonids-effects of carotenoids in eggs and start- feeding diet on survival and growth rate. Aquaculture. 43: 185 – 193.
- Tripathi, S.D., Sharma, B.K. 1992. Plant sources of feed for fish. Farmer proven integrated agriculture aquaculture : a technology information kit. Capistrano Doren L; Luna, N. Eds. Silang, Cavite Philippines IIRR pp 3.
- Venkataraman, L. V., Nigam, B. P. and Ramanathan, P. K. 1980. Rural oriented fresh water cultivation and production of algae in India. Eds: Shelef. G. Soeder. Algae biomass. Elsevier. Amsterdam. 81-85.
- Vonder, D. A., Lied, E. 1993. Metabolic effects on growth and muscle of soya bean protien feeding in cod (Gadus morhua). Br. J. Nutr. 69(3): 689-697.

Wu, Y.V., Rosati, R.R., Sessa, D.J., Brown, P.B. 1995. Evaluation of corn gluten meal as a protein source in Tilapia diets. J. Agric. Food. Chem. 43: 1585 – 1588.

Yogarajah, V., Jasothini. 1989. Fungal protein enrichment of cassava by solid state fermentation and it's use as fish feed. Asian Inst. Tech. Bangkok (Thailand). 1 – 64.