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Advances in Animal Science

Volume II

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PREFACE

We are delighted to publish our book entitled ***"Advances in Animal Science Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied animal science.*

This book is published in the hopes of sharing the excitement found in the study of animal science. Animal science can help us unlock the mysteries of our universe, but beyond that, conquering it can be personally satisfying. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, lndia for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editors

Advances in Animal Science Volume II

CONTENTS

FORMULATION OF FISH FEED USING TRADITIONAL MEDICINAL PLANT *VETIVERIA ZIZANIOIDES* FOR TREAT EPIZOOTIC ULCERATIVE SYNDROME INFECTED ORNAMENTAL FISH KOI CARP - *CYPRINUS CARPIO*

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Abstract:

Nowadays, our focus completely towards herbs and their medicinal usages, reasons are low cast easily available low side effects and environmentally friendly he world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. The medicinal plants can be used not only against diseases but even more so, as growth promoters, stress resistance boosters and preventatives of infections. Additionally, phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents without causing toxicity. The herbs can also act as immunostimulants, conferring the non-specific defense mechanisms of fish and elevating the specific immune response. In the present article, traditional medicinal plant *Vetiveria zizanioides* added as a major ingredient in fish feed formulation whichacting against EUS fish diseases, the root powder of *V. zizanioides* containing feedwas fed to experimentally infected EUS (2.7×10^4CFU) Ornamental Koi fish *Cyprinus carpio* maintained in laboratory cement tanks where, 98±2.35% were found to be recovered at 20th day. Tetracycline powder added feed used as a control in this study showed medium to weak effect recovering the EUS infected fish.

Key words: *Vetiveria zizanioides*, Epizootic ulcerative syndrome, Ornamental Koi, *Cyprinus carpio*, Herbal feed, Tetracycline feed, Treatment

Introduction:

Epizootic Ulcerative Syndrome (EUS) is one of the most destructive diseases among fresh and brackish water fish in the Asia-Pacific region and it is very common in northern and southern India, causing considerable loss to fish farmers (Chinabut *et al*., 1995). The emergence

and spread of aquatic freshwater diseases are a major conservation concern. EUS is caused by the fungus-like oomycete *Aphanomyces invadans* and *Aeromonas hydrophila* can cause significant ulceration of the skin, necrosis of muscle with extension to subjacent structures including abdominal cavity and cranium, and leading to mortality in many cases.India witnessed the first major outbreak of EUS in 1988 in the states of Tripura, Assam, Meghalaya and West Bengal. It gradually spread until 1992 in the states of Orissa, Bihar, Uttar Pradesh, Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Haryana (Das, 1997). In India, Central Inland Fisheries Research Institute (CIFRI) has been monitoring the disease since early 1988. Over the past two decades, EUS has had a serious impact on tropical fisheries, resulting in heavy economic loss. It is one of the most destructive diseases in fresh and brackish water fish in the Asian Pacific region. It is very common in India and has spread through rivers, reservoirs and paddy fields to neighboring states, causing considerable loss to fish farmers (Muthukrishnan *et al.*, 2008). Vetiver known as Khus grass is a perennial grass of Indian origin. In India it is mainly used in ayurvedic medicine. The plant has This property of Vetiver Essential Oil helps heal wounds by promoting growth of newtissues at the wounded place and also by keeping it safe from infections by inhibiting growth of microbes and promoting crowding of leucocytes and platelets at the place.Vetiver helps in the formation of new tissue is used so as to accelerate the healing and recovery of skin wounds as well to remove stains, marks on the skin and the scars themselves. Also, we used to repair the cracks and grooves in the skin caused by different circumstances such as pregnancy, diets, allergies, burns.

Many fish farmers and ornamental fish hobbyists buy the bulk of their feed from commercial manufacturers. Small ornamental fish farms with an assortment of fish require small amounts of various diets with particular ingredients. However, the small quantities of fishfeeds can be made quite easily in the laboratory, or at home, with common herbal ingredients and with simple kitchen or laboratory equipment. Henceforth, this chapter will provide the knowledge of herbal feed formulation, which are beneficial for fish health and may act against EUS disease causing pathogens *A. invadans* and *A. hydrophila.* From our earlier study it was found that EUS causing microbes*A. invadans* and *A. hydrophila* 3.7×10^4 CFU completely ward off by the herbal extract of *V. zizanioides in vitro* study Based on the findings, the present study was designed to evaluate the efficacy of *V. zizanioides* in healing the lesion caused by EUS disease causing pathogens *A. invadans* and *A. hydrophila.*

Materials and method:

Plant material:

The medicinal plant was selected for the study from the local area based on their basic

information in the available. The medicinal plant *Vetiveria zizanioides* were collected from follow land in and around Orathanadu, Thanjavur brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study. *V. zizanioides* are widely distributed in tropical and warm temperate regions. These plants are commonly used in folk medicine to treat dermatitis, gastric ulcer, abrasion, lesions and inflammation (Vaidyarathnam, 1995).

Preparation of plant powder:

The fresh rhizome of *V. zizanioides* was collected and washed under running tap water for 5 minutes; the small hairs of the rhizome were removed and the parts of the plants were chopped and shade dried for a week to achieve weight constancy. The dried parts were finely powdered in an electric blender and stored in airtight bottles

Experimental animal:

The Koi carp, *Cyprinus carpio* (27.6±4.2 g) obtained from local ornamental fish farm in Kadachanendhal, Madurai, Tamil Nadu, were used in the experiments. The fish were transported to the laboratory in plastic bags (5L) filled with oxygenated water. They were acclimatized in cement aquaria (capacity 2000L; 6×4×4 m) for 3 weeks to laboratory conditions (10 hrs. dark: 14 hrs. light). The observed water quality parameters are: dissolved oxygen.

Pathogen: *Aeromonas hydrophila:*

The reference strain *A. hydrophila* (MTCC code no-646) was purchased from the Institute of Microbial Technology (Government of India), Chandigarh and maintained in the laboratory under standard conditions. Subcultures were maintained on Tryptone soya agar slopes at 30˚ C (Hi-Media, Mumbai) (Joseph and Carnahan, 1994).

Fungi isolation and identification:

Fungi species were isolated from the water samples by baiting method and using sterilized maize, and hemp seed as described by Stevens (1974). To induce sporulation, place an agar plug (3-4 mm in diameter) of actively growing mycelium in a Petri dish containing GPY broth and incubate for 4 days at approximately 20°C, after about 12 hours, the formation of achlyoid clusters of primary cysts and the release of motile secondary zoospores should be apparent under the microscope.

Infective experiment:

Fish can be experimentally infected by intramuscularly injecting a 0.1 ml suspension of 100+ motile zoospores into fish susceptible to infection with *A. invadans* and with *A. hydrophila* at the concentrations of 10^4CFU/ml at 28°C, and demonstrating histological growth of aseptate hyphae, with red spot at 14mm in diameter, in the muscle of fish sampled after 3rd days. Each

experiment was carried out in triplicate with 10 fishes in each to quantify survival rate and ID_{50} values. The experiment lasted for 45 days. Every day the size of the induced lesion was measured with vernier caliper.

Intramuscularly injective experimental design:

Appropriate volume (0.025ml/100g body weight) of the selected serial dilution (10^1,10^2,10^3,10^4,10^5or 10^6CFU/fish) was injected intramuscularly beneath the dorsal fin of the fish using a tuberculin syringe using 20-gauge needle size (Scharperculeus, 1991). For each dilution a triplicate with ten fishes were kept and the fishes (n=10) were kept in plastic aquaria (capacity 10L). Mortality was monitored continuously on each days and dead fish, if any, removed with a forceps. The fish was considered dead when gill movement ceased and there was no response to gentle prodding (Sprague, 1973). The medium was not changed or aerated during this period (Peer Mohamed, 2000).

Confirmation of pathogenesis:

The dose which afforded at least 20% survival of the infected fish beyond 12 days after infection selected to observe to pathological signs. The following clinical signs were used to confirm the induction of pathogenesis: a) discoloration of the body and the gills, b) abnormal swimming-rushing around, whirling, reeling, rotating or swimming upside down, c) refusal of food, d) retarded movement to the water surface or bottom, e) loss of equilibrium, f) closed fin-dorsal fin, g) swelling of abdomen, h) erection of scales, and i) appearance of external bumps, sores or lesions (Balasubramanian, 2005). The cause of the disease in the fishes, which survived the inoculation, was confirmed by isolation of bacteria from the outer edge of the skin lesions. Sterile nutrient broth was inoculated with the swabs from the skin lesions. The bacterial cells were harvested and their characteristics compared for their identity to *A. hydrophila* (Selvaraj *et al*., 2004).

Feed preparation:

The fish feed was prepared by mixing the selected feed ingredients. The mixture of the feed ingredient was moistened with water and steam cooked in batches and cooled. To this feed, vitamin mix and cod liver oil capsule were also added, and then mixed thoroughly to make Dough. This feed mixture was pelletized through hand pelletized until a size of 1.0 to 4mm and dried in shade to reduce the moisture content of the feed below 10% (Vijayakumari and Sukumaran, 2014).

Experimental feed preparation:

The medicated feed was prepared by using the *V. zizanioides*40gm herbal plant powder, 15gm groundnut oil cake, 15gm fishmeal, vitamin c capsule 2mg, rice bran 5gm, tapioca powder 5 gm, cod liver oil capsule 2mg. The control feed was prepared by using the tetracycline40gm

herbal plant powder, 15gm groundnut oil cake, 15gm fishmeal, vitamin c capsule 2mg, rice bran 5gm, tapioca powder 5 gm, cod liver oil capsule 2mg. The normal diet was prepared by using without plant powder, 15gm groundnut oil cake, 15gm fishmeal, vitamin c capsule 2mg, rice bran 5gm, tapioca powder 5 gm, cod liver oil capsule 2mg.

Results:

Infective dosage:

In *C. carpio, A. hydrophila* with *A. invadans* infection produced mean percentage mortality 87, 73, 50, 44, 40 and 20 at (Group I) 3.9×10^{1}, (Group II) 3.5×10^{2}, (Group III)3.1×10^{3}, (Group IV) 2.7×10^{4}, (Group V) 2.3×10^{5}, and (Group VI) 1.9×10^{6} CFU/ml concentrations respectively. After 15 days infection produced 50% mortality with 2.7×10^{4}CFU/ml (Table 1). Since 50% mortality was observed at 2.7×10^{4}CFU /ml concentration. This dose was chosen for all the experiments.

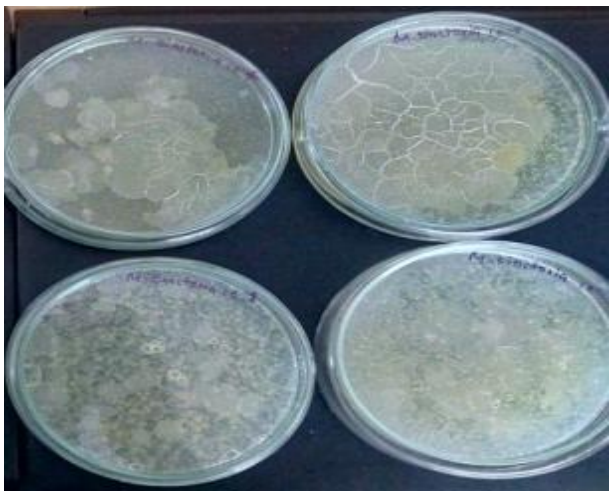

Plate I: Pathogens culture

Table 1: Decrease in the lesion size (14mm) of infected *C. carpio* treated with*V. zizanioides* powder added /not added during 15 days

Group	3rd day	5th day	10th day	15th day	20th day
Infected untreated	1.42±0.59 (14mm)	1.72±0.2 (17mm)	2.4±0.07 (24mm)	3.9±0.03 (39mm)	4.6±0.05
Infected fed with *V. zizanioides* powder added feed	1.42±0.59 (14mm)	0.53±0.33 (05mm)	0.39±0.31 (03.1mm)	0.22±0.00 (02.2mm)	0.00±0.00
Infected fed with normal feed	1.42±0.59 (14mm)	1.73±0.3 (17mm)	2.38±0.17 (23mm)	3.92±0.06 (039mm)	4.1±0.03 (41mm)
Infected treated with Terramycin	1.42±0.59 (14mm)	1.62±3.3 (16.2mm)	1.53±0.29 (15.3mm)	1.51±0.07 (15.1mm)	1.31±0.07 (13.1mm)

Clinical Sign and Gross Pathology:

In general, lesions on EUS-affected fish can be separated into three groups on the basis of gross appearance. Clinical signs in the early stage of the disease are similar. Appetite is reduced or absent and fish become lethargic, either floating just beneath the surface or swimming with the head out of the water Pinhead-sized, red spots develop on the body surface, head and fins, caudal peduncle, dorsum or operculum with no noticeable hemorrhages or ulcers. The intermediate stage lesions are represented by small (1.42 cm) dermal ulcers, with associated loss of scales, hemorrhages and oedema.

Table 2: Clinical sign and gross pathological funding in *C. carpio* (Ornamental Koi carp) infected with *A. invadans* and *A. hydrophila* at the concentrations of 2.7 x 10^4CFU/ml

Clinical sign and pathological gross		
Duration	12 hrs.	Abnormal swimming
	24 hrs.	Abnormal swimming-rushing around, whirling, reeling, rotating or swimming upside down
	2nd Day	Refusal of food, loss of equilibrium
	3rd Day	Pinhead-sized, red spots develop on the body surface
	5th Day	Noticeable hemorrhages or ulcers
	7th Day	Dermal ulcers, with associated loss of scales,
	9th Day	Closed fin-dorsal fin, resulting eventually in death
	11th Day	large necrotic open ulcers; resulting eventually in death
	13th Day	large necrotic open ulcers; resulting eventually in death

The advancedstage lesions appear on other parts of thefish's body and expand into large necrotic open ulcers; resulting eventually in death. Some affected species, such as striped snakehead, can survive with much more severe, chronic lesions that may have completely destroyed the caudal peduncle or eroded into the cranium or abdominal cavity, sometimes exposing the swim bladder (Table 2).

Size of the lesion:

The size (mm) of the lesion increased as a function of infection period (25days). The mean size of lesion on 3rd day was 1.02±0.01 mm and on the 15th day increased into 9.29±0.10mm on the in the ideal dosage 2.7×10^4CFU/ml (Table 1). In I group high concentration of infected with *A. invadans* and *A. hydrophila* 3.7×10^1CFU the mean size of lesion increased from 3.2±0.02 mm on the 3rd day to 9.88±0.06mm on the 20th day and 90% of mortality occurred on the 20th day in this concentration. Approximately the pathogens are present in the

concentration was 8.15 million /ml and in the ID_{50} concentration 6.45 million /ml. Infected fishes the mean size of lesion at the least concentration of 2.06×10^6CFU/ml caused from 1.50 ± 0.0 mm on the 3rd day to 4.62±3.17mm on the 15th day. Infected fed with *V. zizanioides* powderadded feed showed complete healing on 20th day (Plate-I)

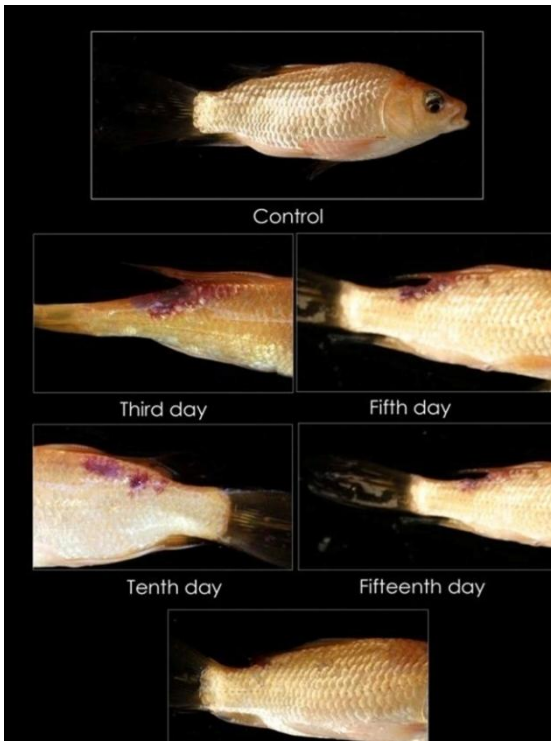

Plate II: Infected *Cyprinus carpio* treated with *Vetiveria zizanioides* powder added feed

Mortality:

On 30th day of infection 90 % of mortality occurred at the high concentration of *A. hydrophila* 3.7×10^4CFU/ml. The lesion 9.29±0.10mm on 15th day in the ideal dosage 2.7×10^4CFU/ml while on 30th day fish suffered 50 % mortality. In the infected fishes, the mean mortality value 20 % on 30th day at the least concentration of 2.06×10^6CFU/ml. But mortality rate completely nil in infected fish fed and treated with *V. zizanioides* added feed.

Preparation of plant powder:

The dried *V. zizanioides* were grinded into powdered form mechanically and sieved with a hand sieve to obtain 500 g of each of the powders which were then stored in airtight containers till formulation and preparation of experimental diets. Fresh weight was 500gm, dried weight was 462.7gm.

Plate III: Dried *V. zizanioides*

Feed formulation:

Experimental diet was formulated using the Pearson's square method and distributed into treatments. Feed ingredients used for the experimental diets include*V. zizanioides* 40gm herbal plant powder,15gm groundnut oil cake, 15gm fishmeal, sodium chloride 1mg,rice bran 5gm, tapioca powder 5 gm, cod liver oil capsule 2mg.The formulation was based on the percentage composition of the ingredient (Table 3). To prepare the diets, the dried and grinded ingredients of each diet were weighed and mixed thoroughly in a bowl, vegetable oil and warm water were then added slowly along to the mixture and mixed manually for about 30 minutes to achieve a proper consistency. The resulting mixture was pelletized (2 mm) using a traditional pelletizerand allowed to dry for 24 hrs, by air circulation before being packed into airtight containers and stored at room temperature to be crumbled before use. Formulated diet samples (10 g) were analyzed following the procedures of AOAC. Moisture was analyzed by drying the sample in an air convection oven at 105^{o}C overnight.

Table 3: Formulation and proximate composition of experimental diets (40g/100 g dry weight)

Ingredients	Experimental diet	Normal diet	Tetracycline diet
V. zizanioides	40 gm	Nil	Nil
Fish Meal	15 gm	15 gm	15 gm
Soybean cake	10 gm	10 gm	10 gm
groundnut oil cake	15 gm	15 gm	15 gm
Rice bran	5 gm	5 gm	5 gm
Tapioca flour	5 gm	5 gm	5 gm
Veg.oil	3 ml	3 ml	3 ml
Sodium chloride	1 mg	1 mg	1 mg
Cod liver oil capsule	1 mg	1 mg	1 mg
Tetracycline	Nil	Nil	40 gm

Plate IV: Herbal feed

Discussion:

The LD_{50} value of *Clarias batracus* (ip) injected with *A. hydrophila* was 3.79 x 10^{10}CFU/ml at 96 hrs (Supap, 1985). Experimentally infected *Oreochromis* with *A. hydrophila* showed LD_{50} value of 1.5 10^6 at 96 hrs (Yambot, 1998). The isolates of *A. hydrophila* and *A. sobria* showed LD_{50} value of 10^3 and 10^4 respectively indicating their virulence in native fish (Shariff and Subasinghe, 1994). In rainbow trout (*Salmo gairnern*) when 5×10^6CFU/ml were injected intramuscularly; the bacterial cells were completely cleared from the site of the injection and the organs; within 7 days the lesion was appeared. 60-g rainbow trout for held at 10°C, the 50% lethal dose of Pd-10 was $>10^7$ CFU, compared with 8.1×10^5CFU/ml for the parent strain (Leung *et al*., 1995). After 15 days infection produced 50% mortality with 2.7×10^4CFU/ml (Table 1). Since 50% mortality was observed at 2.7×10^4CFU /ml concentration. This dose was chosen for all the experiments.

To preserve and protect the environment as well as human health as a best alternative, different parts of *Azadirachta indica* (Neem) tree have been studied by Chitmanat *et al.* (2005) Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) have been said as an alternative to chemicals to treat fish ectoparasites, *Trichodina* sp. infections in tilapia (*O. niloticus*) fingerlings. Both Indian almond and garlic had low acute toxicity to tilapia fingerlings, treating the Trichodiniasis caused by Trichodina. The immunostimulant effects of the dietary intake of 3 plants (*viz*., *Viscum album*, *Urtica dioica* and *Zingiber officinale*)-extracts on rainbow trout (*Oncorhynchus mykiss*) have also been narrated by the authors. Christybapita*etal.*(2007) observed the immunostimulatory effect of aqueous extract (AqE) of *Eclipta alba* (Bhangra) leaf (oral administration as feed supplement) in tilapia fish, *Oreochromis mossambicus*. It was noted that the *E. alba* extract enhances non-specific immune responses and disease resistance of O.

mossambicus against *A. hydrophila* infection. According to Winkaler *et al.* (2007), *A. indica* extract can be used successfully in aquaculture to control fish predators. Ravikumar *et al.* (2010) observed that the chloroform extract of *Datura metel* plant has wide range of antimicrobial activity against many fish pathogens. *D. metel* which collected from the Kanyakumari coast can be used as a putative antimicrobial drug in the aquaculture maintenance. The chloroform extract of *D. metel* can be effectively used as a potential antimicrobial agent to overcome the problem of mass mortality of ornamental fish in aquarium so as to enable to enhance the market revenue throughout the world. These authors also told the antimicrobial activity of 5 Chinese herb extracts against 13 bacterial and 2 viral fish pathogens. Sharma *et al.* (2010) observed the stimulatory effect of dietary doses of *Withania somnifera* (Ashwagandha) root on immunity and disease resistance against A. hydrophila infection in Indian major carp, *L. rohita* fingerlings. Abdul Kader Mydeen and Haniffa (2011) cited that *A. indica* leaf AqE could effectively control the *A. hydrophila* infection in common carp (a fresh-water fish usually bred in ponds), Cyprinus carpio. Further, *Enterobacter* sp. and *Escherichia coli* bacteria, isolated from marine fish (*Amphiprion sebae*) showed 15 mm zone of inhibition against neem extract. The herb-supplements/additives included in the fish feeds usually maintain and improve the physiological functions. Ahilan*et al.* (2010) reported that the herbs have significant role in aquaculture. The herbal growth promoters in the carp fish feeds showed beneficial effects. There was a significant difference between different herbal additives on the effect of growth rate in goldfish. The synergistic effects of herbs have been found in *Clarias gariepinus* (Turan and Akyurt, 2005) and *Japanese flounder* (Ji *et al*., 2007).

Conclusion:

Medicinal plants are important elements of traditional medicine in the virtually all cultures and promise a cheaper source for therapeutics. Herb powder added fish feed are cheap and having lesion healing potency. The present study revealed that *V. zizanioides* traditional medicinal plant possesses potential antimicrobial activity against fish pathogenic *A. hydrophila* with *A. invadans*.

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POTENTIAL OF WHEATGRASS BASED FORMULATED FEED IN FRESH WATER FISH CULTURE

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Background:

The severe world population explosion is presenting complex challenges, being the most important one is malnutrition and shortage of food in terms of quantity and quality. Milk, meat and eggs are the important animal source of protein. Animal production is a long term project to produce adequate amount of quality protein to meet the national demand. Meat production in terms of demanding protein requirement is improving with the establishment of poultry industry and fish farming. The production of quality protein is associated with the development of fisheries on commercial basis. Fish production sector is very important not only as a main source of animal protein to ensure food security but also to improve employment and income for poverty elimination in developing countries like India (Bhosale *et al.*, 2010).

Fish is very important and common dietary animal protein source in human nutrition in Indian subcontinent. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged in developed or developing countries but in under-developed countries, it is declining. According to nutritionists, fish is an excellent substitute for red meat and an excellent source of protein. Fish flesh contains all the essential amino acid and minerals like iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations. It serves as valuable ingredient to a healthy diet because of its low carbohydrate and unsaturated fat contents. It is often recommended by doctors to heart patients since it is an excellent source of Omega 3. So the inclusion of fish in our diet can make a valuable contribution to any diet that contain mainly of cereals, starchy roots and sugar for the healthy growth (Corto-Martinez *et al.*, 2007)

The fresh water fish is highly demanded as a food in India and adjacent countries. To satisfy the increasing demand of fish as food, it is important to adopt modern practices of aquaculture. As part of these practices, supplementation of nutrients to the fish is very necessary. Good nutrition in animal production systems is essential to economically produce a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish products (Li *et al.,* 2013).

Now a day, varieties of fish meals are easily available in market. Fishmeal is a commercial product mostly made from fish that are not generally used for human consumption; a small portion is made from the bones and left over from processing of fish used for human consumption, while the larger percentage is manufactured from wild-caught, small marine fish; either unmanaged by-catch or sometimes sustainable fish stocks. It is powder or cake obtained by drying the fish or fish trimmings, often after cooking, and then grinding it. If the fish used is a fatty fish it is first pressed to extract most of the fish oil. Fish meal production is a significant contributor of over-fishing, and risks pushing fisheries beyond their replacement rate (Sahu *et al.,* (2007).

The use of feedadditives is beneficial in increasing fish production. The feedadditives are also the supplements for farm animals that cannot get enough nutrients from regular meals that the farmers provide and it includes vitamins, amino acids, fatty acids, and minerals. In some cases, if an animal does not have some specific nutrition in its diet then it may not grow properly. The nutritional values of animal feeds are influenced not only by their nutrient content, but also by many other factors. These include the feed presentation, hygiene, digestibility, and effect on intestinal health. Even with all of the benefits of higher quality feed, most of a farm animal's diet still consists of maize, wheat and soybean meal because of the higher costs of quality feed. Hence feed and feeding are among the most important factors influencing growth, feed utilization and tissue composition of the fish in intensive culture (Okumus and Mazlum, 2002).

In intensive culture systems fish production per unit of surface can be increased at well, as long as sufficient oxygen, fresh water and food are provided. Because of the requirement of sufficient fresh water, a massive water purification system must be integrated in the fish farm. One way to achieve this is to combine hydroponic horticulture and water treatment, see below. The exception to this rule is cages which are placed in a river, which supplements the fish crop

with sufficient oxygenated water. Some environmentalists object to this practice (Aly *et al.,* 2008).

The cost of inputs per unit of fish weight is higher than in extensive farming, especially because of the high cost of fish feed. It must contain a much higher level of protein. These higher protein-level requirements are a consequence of the higher feed efficiency of aquatic animals. Fish such as salmon have Fish do not use energy to keep warm, eliminating some carbohydrates and fats in the diet, required to provide this energy. This may be offset, though, by the lower land costs and the higher production which can be obtained due to the high level of input control. Aeration of the water is essential, as fish need a sufficient oxygen level for growth. This is achieved by bubbling, cascade flow, or aqueous oxygen (Butle *et al.,* 2018).

In fish culture, the risk of infections by parasites such as fish lice, intestinal worms, bacteria, and protozoa is similar to that in animal husbandry, especially at high population densities. However, animal husbandry is a larger and more technologically mature area of human agriculture and has developed better solutions to pathogen problems. Intensive aquaculture has to provide adequate water quality levels to minimize stress on the fish. This requirement makes control of the pathogen problem more difficult. Intensive aquaculture requires tight monitoring and a high level of expertise of the fish farmer (Metwally, 2009 a, b).

The formulated diet can also prove to be effectives in increasing fish production. The main issue in formulating feed is to meet the protein and essential amino acids requirements of the species. Fishmeal is generally the preferred protein source because of the high quality of the protein and its essential amino acids profile. However, fishmeal is generally expensive and is not always available. It is economically judicious to replace fishmeal with alternative protein sources including animal by-products, oilseed meal and cakes, legumes and cereal by-products and aquatic plants. Most of these ingredients are deficient in some essential amino acids and hence require supplementation or be compensated with other feedstuffs. Although most of the oilseed cakes or by-products are generally deficient in lysine and methionine, blending of different oilseed cakes often provides balanced amino acid profile. However they contain many anti-nutritional factors such as gossypol, glucosinolates, saponins, trypsin inhibitors etc. which limit their use in compound feeds or require removal or inactivation through specific processing such as heating, cooking etc. Hence, the formulation of diet is necessary with sophisticated method help to preserve the nutrients for rapid growth of fish (Farahi *et al.,* 2010).

The protein is the most expensive part of fish feed; hence it is important to accurately determine the protein requirements for each species and size of cultured fish. Proteins are formed

by linkages of individual amino acids. Fish feeds prepared with plant like soybean meal protein typically are low in methionine; therefore, extra methionine must be added to soybean-meal based diets in order to promote optimal growth and health. Lipids are high-energy nutrients that can be utilized to partially spare protein in aquaculture feeds. A recent trend in fish feeds is to use higher levels of lipids in the diet. Although increasing dietary lipids can help reduce the high costs of diets by partially sparing protein in the feed, problems such as excessive fat deposition in the liver can decrease the health and market quality of fish (Diegane and Fall, 2011).

Carbohydrates are the most economical and inexpensive sources of energy for fish diets. Although not essential, carbohydrates are included in aquaculture diets to reduce feed costs and for their binding activity during feed manufacturing. Dietary starches are useful in the extrusion manufacture of floating feeds. Cooking starch during the extrusion process makes it more biologically available to fish. Vitamins are organic compounds necessary in the diet for normal fish growth and health. They often are not synthesized by fish, and must be supplied in the diet. Minerals are inorganic elements necessary in the diet for normal body functions. They can be divided into two groups (macro-minerals and micro-minerals) based on the quantity required in the diet and the amount present in fish. Common macro-minerals are sodium, chloride, potassium and phosphorous. These minerals regulate osmotic balance and aid in bone formation and integrity (Lee *et al.,* 2012).

Micro-minerals (trace minerals) are required in small amounts as components in enzyme and hormone systems. Common trace minerals are copper, chromium, iodine, zinc and selenium. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet. Dietary nutrients are essential for the construction of living tissues. They also are a source of stored energy for fish digestion, absorption, growth, reproduction and the other life processes. The nutritional value of a dietary ingredient is in part dependant on its ability to supply energy (Agatha, 2012).

Feeding rates and frequencies are in part a function of fish size. Small larval fish and fry need to be fed a high protein diet frequently and usually in excess. Small fish have a high energy demand and must eat nearly continuously and be fed almost hourly. Feeding small fish in excess is not as much of a problem as overfeeding larger fish because small fish require only a small amount of feed relative to the volume of water in the culture system. As fish grow, feeding rates and frequencies should be lowered, and protein content reduced. However, rather than switching to a lower protein diet, feeding less allows the grower to use the same feed (protein level) throughout the grow-out period, thereby simplifying feed inventory and storage (Megbowon *et al.,* 2013).

Feeding fish is labor-intensive and expensive. Feeding frequency is dependent on labor availability, farm size, and the fish species and sizes grown. Large catfish farms with many ponds usually feed only once per day because of time and labor limitations, while smaller farms may feed twice per day. Generally, growth and feed conversion increase with feeding frequency. In indoor, intensive fish culture systems, fish may be fed as many as 5 times per day in order to maximize growth at optimum temperatures.

Many factors affect the feeding rates of fish. These include time of day, season, water temperature, dissolved oxygen levels, and other water quality variables. For example, feeding fish grown in ponds early in the morning when the lowest dissolved oxygen levels occur is not advisable. In contrast, in recirculating aquaculture systems where oxygen is continuously supplied, fish can be fed at nearly any time. During the winter and at low water temperatures, feeding rates of warm-water fishes in ponds decline and feeding rates should decrease proportionally. Feed acceptability, palatability and digestibility vary with the ingredients and feed quality. Fish farmers pay careful attention to feeding activity in order to help determine feed acceptance, calculate feed conversion ratios and feed efficiencies, monitor feed costs, and track feed demand throughout the year. Published feeding rate tables are available for most commonly cultured fish species. Farmers can calculate optimum feeding rates based on the average size in length or weight and the number of fish in the tank, raceway, or pond (Lee *et al.,* 2014).

The hormones, antibiotics and several other chemicals have been tested as growth promoters, antibacterial and for other purposes in aquatic animals, but their use in aquatic animal production cannot be recommended due to the residual effects in the muscle of fish as well as prawns. Plants are natural sources of safer and cheaper chemicals. The beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, growth promotion, the improvement of endogenous digestive enzyme secretion, and activation of immunostimulation and antibacterial, antiviral and antioxidant actions in aquaculture practices. But in recent years, the concern about bacterial resistance to antibiotics in livestock industry has led to legislation minimizing or eliminating the use of such compounds (Lee *et al.*, 2012).

Wheatgrass *Triticum aestivum* refers to young grass of the common wheat plant, which belongs to Poaceae family. This is the most commonly found herb in India which is freshly juiced or dried into powder for animal and human consumption. Wheatgrass' culms are simple, hollow or pithy, glabrous, and the leaves are approximately 1.2 m tall, flat, narrow, 20-38 cm long and 1.3 cm broad. The spikes are long, slender, dorsally compressed and somewhat

flattened (Mujoriya and Bodla, 2011; Sareen *et al.*, 2014). The phytochemical and pharmacological potential of wheat grass is well reviewed by Durairaj *et al.* (2014); Suriyavathana *et al.* (2016). Wheatgrass is a source of potassium, dietary fiber, vitamin A, vitamin C, vitamin E, vitamin K, thiamin, riboflavin, niacin, vitamin B6, pantothenic acid, iron, zinc, copper, manganese and selenium. Wheatgrass is also a source of protein. Kumar *et al.* (2016) quoted that wheat grass is a nature's wonderful medicine that helpful in treatment of various diseases and infections. Plant has been shown to have anti-inflammatory, antioxidant, anticarcinogenic, immunomodulatory, laxative, astringent, diuretic, antibacterial, antihemolytic and anti-aging properties as well improve reproductive health. Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be beneficial (Sharma *et al.,* 2016; Johri and Khan, 2017, Uraiwan *et al.,* 2019).

As well, this herb is useful for the animals in variety of ways. Aabdullah*et al.* (2014) mentioned that the wheat flour and bran is common ingredient in animal feed. The wheat bran mixed with fish meal improved the growth performance and meat quality in cattle. Emmanuelle *et al.* (2014) quoted that wheat gluten can be used as an alternative to protein in feed for carnivorous fish. Nath *et al.* (2014) perform the successful rearing of Asian catfish fry (*Clarias batrachus*) with wheatgrass powder mixed formulated feed in plastic half drum. Islam *et al.* (2017) observed that potential of wheatgrass based feed for stinging catfish fry nursing in laboratory condition. Johri and Khan (2017) had given the phytochemical characterization of wheat grass and observed antioxidant and anti-haemolytic potential. Gasco *et al.* (2018) suggested that addition of easily available phytochemically effective herbs into the fish diets help to improve the feed conversion efficiency or elevate general conditions for fish growth and maintenance (Makode *et al.,* 2018; Uraiwan *et al.,* 2019).

Review of Earlier studies

The herbs and herbal products extract contains various bioactive compounds which potential source of natural antioxidants with medicinal properties. Their addition to the feed cures many diseases, promote growth, reduce stress, improve immunity and prevent infections in fish under culture. According to Cristea *et al.* (2012), the supplementation of herbs and herbal products in fish diet is cheaper and environmental friendly with no side effects to the fish and consumers. Hence their use may lead to achieve an augmented aquaculture production inan eco-friendly, cost effective and sustainablemanner is gaining popular. In recent years, many studies are being carried to study the effectiveness of herbal supplementation in fish feed to manage fish growth and produce healthy fish. The outcomes of these studies suggested that the herbal feed

supplements promote growth, minimizes stress, improves immunity and prevents various infections in fishes that will help to produce healthy fishes for humanconsumption (Shakya, 2017). The present review highlights the importance of few effective herbs and herbal products supplementation in fish feed for better fish production.

Omoregie (2001) studied the utilization and nutrient digestibility of mango seeds *Mangifera indica* and palm Kernel *Elaeis guineensis* meal by juvenile *Labeo senegalenis.* Citarasu *et al.* (2002) developed *Andrographis paniculata, Eclipta erecta, Hygrophila spinosa, Ocimum basilium, Phyllanthus niruri, Picrorhiza kurooa, Psoralea corylifolia, Solanum trilobatum, Tinospora cordifoliaare* and *Zingiber officinalis* enriched herbal diet for producing quality larvae in *Penaeus monodon.* According to Brannas *et al.* (2003) and Elkayam *et al.* (2003), the use of such natural supplements in formulated diet, increased their behavioural and growth performances. Skidmore-Roth (2003) mentioned that *Acalypha indica, Aegle marmelos, Chrysopogon zizanioides, Cymbopogon citratus, Hordeum vulgare, Medicago sativa, Senna auriculata, Sorghum bicolor* and *Urtica dioica* are some of the most useful medicinal herbs that can be used as natural supplements. Shalaby *et al.* (2004) observed the response of Nile tilapia, *Oreochromis niloticus*, fingerlings to diets supplemented with different levels of fenugreek seeds.

Chitmanat *et al.* (2005) isolated antiparasitic, antibacterial, and antifungal solution from *Terminalia catappa* against some tilapia *Oreochromis niloticus* pathogens. Micol *et al.* (2005) observed that olive leaf extract exhibits antiviral activity against viral haemorrhagic *Septicaemia rhabdo* virus. Turan (2006) improved the growth performance in tilapia *Oreochromis aureus* by supplementation of red clover *Trifolium pratense* in diets. Yin *et al.* (2006) noticed the effectively of two Chinese herbs *Astragalus radix* and *Scutellaria radix* on non-specific immune response of tilapia *Oreochromis niloticus* (Zakes *et al.*, 2008). John *et al.* (2007) found the effectiveness of *Eichinacea purpurea, Nigella sativa* and *Origanum marjoranaas* as feed additives on the survival, growth performance and immune response of Nile tilapia *Oreochromis niloticus*.

Ardo *et al.* (2008) noticed that Chinese herbs *Astragalus membranaceus* and *Lonicera japonica* with boron enhance the non-specific immune response of Nile tilapia *Oreochromis niloticus* and resistance against *Aeromonas hydrophila*. Ashraf and Goda (2008) mentioned that dietary Ginseng herb *Eleutherococcus senticosus* supplementation improved growth, feed utilization, and hematological indices of Nile Tilapia, *Oreochromis niloticus* fingerlings.

Pachanawan *et al.* (2008) observed the potential of *Psidium guajava* supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia *Oreochromis niloticus*.

Thy *et al.* (2008) studied the effect of water spinach *Spinacia oleracea* and duckweed Lemna on fish growth performance in poly-culture ponds. Won *et al.* (2008) found that residum extract of Siberian ginseng *Eleutherococcus senticosus* increased non-specific immunity in olive flounder *Paralichthys olivaceu.* Yuan *et al.* (2008) found the appreciable effects of *Astragalus membranaceus* extracts on the expression of immuneresponse genes in head kidney, gill and spleen of the common carp, *Cyprinus carpio.* Zakes *et al.* (2008) studied the effects of *Lonicera japonica* on the growth performance and body composition of juvenile pike perch (*Sander lucioperca*).

Ergun *et al.,* (2009) proved the influence of *Ulva lactuca* meal on growth, feed utilization, and body composition of juvenile Nile tilapia *Oreochromis niloticus* at two levels of dietary lipid. Jeney *et al.* (2009) mentioned that addition of different single herbal extracts of the herbs *Artemisia capillaries, Cnidium officinale, Crataegi fructus, Glycyrrhiza glabra, Isatis tinctoria, Massa medicate* and *Polygonum multiflorum* promoted the growth and enhanced some non-specific immunity indicators of fish. Omoregie *et al.,* (2009) studied effect of varying levels of sweet potato *Ipomea batatas* peels on growth, feed utilization and some biochemical responses of the Cichlid *Oreochromis niloticus* (Faramarzi *et al.,* 2012).

Citarasu (2010) noticed that Ricebran *Oryza sativa* improved the survival and reproductive performance in fish *Artemia parthenogenetica.* Sharma *et al.* (2010) observed the efficacy of *Withania somnifera* root as a feed additive on immunological parameters and disease resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Ahmad and Abdel-Tawwab (2011) used the caraway *Carum carvi* seed meal as a feed additive in fish diets to improve growth performance, feed utilization, and whole-body composition of Nile tilapia *Oreochromis niloticus*. Chakraborty and Hancz (2011) studied the application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture.

Coutteau *et al.* (2011) mentioned that extracts of *Allium tuberosum, Aniba rosaeodora, Capsicum annum longum, Cinnamomum zeylanicum, Elettaria caramomum, Mentha piperita, Myristica flagrans, Piper nigrum, Salvia apiana* and *Syzygium aromaticum* improves the productivity and economics in aquaculture.Joseph *et al.* (2011) observed the influence of *Crossandra infundibuliformiss, Hibiscus rosasinensis, Ixora coccinea* and *Rosa indica* flowers on the growth and colouration of orange sword tail Chicilidae fish *Xiphophorus hellerei.* Kaleeswaran *et al.* (2011) observed the growth response, feed conversion ratio and antiprotease activity in *Catla catla* fed on *Cynodon dactylon* mixed diet. Obaroh and Achionye-Nzeh (2011)

studied the effects of crude extract of *Azadirachtaindica* leaves at controlling prolific breeding in *Oreochromis niloticus.*

Arumugam *et al.* (2012) observed the effect of dietary *Nelumbo nucifera* in growth and haematology of *Cirrhinus mrigala* challenged with *Pseudomonas aeruginosa*. Benny *et al.* (2012) investigated the immunostimulatory behaviour of *Musa acuminate* peel extract in *Clarias batrachus.* Falaye *et al.* (2012) replaced the maize *Zea mays* using cowpea *Vigna unguiculata* hull meal in practical feeds of African catfish *Clarias gariepinus*. Jha *et al.* (2012) studied the effects of marigold flower *Beta vulgaris* and beetroot *Calendula officinalis* meals on growth performance, carcass composition, and total carotenoids of snow trout *Schizothorax richardsonii.* Sivagurunathan *et al.* (2012) studied the immunostimulatory potential of dietary amla *Phyllanthus emblica* in growth and hematology of *Tilapia mossambicus* challenged with *Pseudomonas aeruginosa.* Yilmaz *et al.* (2012) observed the effects of herbal supplements on growth performance, change in body composition and some blood parameters of sea bass *Dicentrarchus labrax*.

Hwang *et al.* (2013) observed that dietary green tea extract improves the growth performance, body composition, and stress recovery in juvenile black rockfish, *Sebastes schlegeli*. Mamman *et al.* (2013) studied the hematological indices of *Clarias griepinus* fingerlings fed diet containing graded levels of calabash *Lagenaria vulgaries* seedmeal. Borkar *et al.* (2014) evaluated the impact of shatavari *Asparagus racemosus* and ashwagandha *Withanias ominifera*on average body weight of freshwater fish *Channa punctatus.* Gaber *et al.*, (2014) observed the mentioned that dietary *Alterna storini, Hippophaer hamnoides Phoenix dactylifera* and *Thymus vulgaris* improves the growth performance in Nile tilapia *Oreochromis niloticus* fingerlings. Karpagam and Krishnaveni (2014) studied the effect of Supplementation of *Moringa oleifera, Ocimum sanctum, Sesbania grandiflora* and *Solanum verbascifolium* leaves as Growth Promoters of Tilapia Fish *Oreochromis mossambicus.*

Adel *et al.* (2015) observed the effects of dietary peppermint *Mentha piperita* on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish. Labh and Shakya (2016) studied the effects of dietary *Choerospondias axillaris* on survival, growth and protein profile of common carp *Cyprinus carpio* fingerlings. Kaur and Shah (2017) mentioned the efficacy of Vegetable Colour from Red Sandal *Pterocarpus santalinus* on acceptability, colour development and growth of Tilapia *Tilapia mossambica*. Makode (2017) studied the effects of dietary onion on growth performance in the fresh water fish *Clarias batrachus.*

Butle *et al.* (2018) studied the dietary garlic *Allium sativum* induced effects on behaviour responses, growth performance and feed utilization in *Clarias batrachus.* Plaza *et al.* (2018) observed the effect ofspirulina *Arthrospira platensis* supplementation on tilapia *Oreochromis niloticus* growth and stress responsiveness under hypoxia. Rodge *et al.* (2018) observed the effects of dietary garlic *Allium sativum* on hematology and biochemistry of *Clarias batrachus.*

Gabriel *et al.,* (2019) studied the effect of dietary *Aloe vera* on growth performance, feed utilization, hemato-biochemical parameters, and survival at low pH in African catfish *Clarias gariepinus.* Roghieh *et al.,* (2019) assessed the effects of *Coriandrum sativum* as feed additive on mucosal immune parameters, antioxidant defense and, immune-related genes expression in Zebrafish *Danio rerio*. Like all these above herbs, wheatgrass is well known for its medicinal properties. Butle *et al.* (2019) evaluated the phytochemical and antioxidant potential of aqueous wheatgrass *Triticum aestivum* extract.

Abdus *et al.* (2020) conducted an experiment to produce hydroponic wheatgrass and feeding trials with stinging catfish, rohu and grass carp. Abdus *et al.* (2020) observed the growth response of juvenile rohu (*Labeo rohita*) to wheatgrass powder supplemented diet. They observed the effects of wheat sprout improved the growth, survival and production of experimental fish. Rana *et al.* (2020) studied the dietary supplementation of wheatgrass powder to assess somatic response of juvenile grass carp (*Ctenopharyngodon idella*).The overall somatic performance of grass carp fed wheatgrass supplemented test diets was satisfactory compared to control diet without wheatgrass. Notably, fish survival was substantially improved. The feed conversion ratio value and feed formulation cost of the respective diets were reduced in a good amount.

The phytochemical screening of the aqueous extract of wheatgrass showed the presence of various secondary metabolites. As well wheatgrass was proved to be an effective in different antioxidant assays. These potential varieties of wheatgrass suggested the effective utility in fresh water fish culture as dietary supplementation.It will beprove to be beneficial for the successful improvement of fisheries by achieving maximum yields with using safer and cheaper formulated feed to induce developmental performance of fresh water fishes.

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PRODUCTION OF VACCINE AGAINST GASTROINTESTINAL NEMATODE *HELIGMOSOMOIDES POLYGYRUS*

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Introduction:

Heligmosomoides polygyrus is a natural intestinal parasite of mice. Chronic helminth infections remain a huge global health problem. It causes extensive deaths in both humans and livestock. Some mouse strains evade immunity to the parasite naturally while in some it is induced through immunization. The mechanisms of protective immunity are not yet fully defined. Humans are hosts to nearly 300 species of parasitic worms and over 70 species of protozoa. There are number of misconception about parasitic infections that it occurs only in tropical areas but many people in temperate and subtropical areas also become infected. This statement was proposed by parasitologist F.E.G. Cox that some parasitic infection was derived from our primate ancestors and some acquired from our domestic animals or we can get infection from contact with these parasites during our relatively short history on Earth.

Materials and Methods:

Experimental animal:

The Swiss albino mouse, *Mus musculus albinus* of either sex were selected as an experimental animal for the present investigation. The mice were obtained from the Institute of Nutrition (NIN) Hyderabad, India and were kept in the animal house under local conditions of light, temperature ventilation and food. Food and water were provided *ad libitum*. Male and female healthy mice of 6-8 weeks old and 15-20 gms in weight were used according to the need of the experimental design. Animal experimentations were conducted according to INSA ethical guidelines for the use of animals for scientific research purpose, after permission from the ethical committee.

The Parasite: *Heligmosomoides polygyrus*

Experimental studies on *Heligmosomoides polygyrus* are few. *H. polygyrus* is a trichostrongyloid nematode of family Heligmosomidae, first reported from *Mus musculus* by

Spurlock (1943) and from *Peromyscus maniculatus* by Ehrenford (1954). *H. polygyrus* is a long-lived intestinal nematode of mice. Rats are known to be naturally resistant to it (Cross, 1960). Development of its free living stage has been reported by Fahmy (1956) and its complete life cycle in mice by Spurlock (1943); Baker (1954); Ehrenford (1954); Dobson (1960); Callizo (1962) and Bryant (1973). Adults of *H. polygyrus* are spirally coiled (females 18-21 mm and males 8-10 mm long) and are found in first 2 cm. attached to duodenal mucosa. The eggs of *H. polygyrus* are fully emryonated when evacuated with feces and hatch at 20-23° C. Life cycle is direct and non- migratory, infection to host is through ingestion of infective filariform 3rd stage larvae which penetrate intestinal mucosa maturing in 8-9 days after infection and females lay eggs up to 9 months producing 1280 eggs per day per female (Scott, *et al.,* 1959). Infection can easily be maintained in the laboratory and the larvae culture well within limits and thus, forms an excellent parasitological tool for experimental investigations.

Experimental protocol:

The mice were divided into following six groups –

1) Non Infected Non Vaccinated Control – 1
2) Infected Non –Vaccinated Control –2
3) Infected Vaccinated with Larval Somatic Antigens
4) Infected Vaccinated with Adult Somatic Antigens
5) Infected Vaccinated with Larval ES Antigens
6) Infected Vaccinated with Adult ES Antigens

Preparation of inoculums for infection:

A larval suspension of about 100 ml was prepared in a glass stoppered measuring cylinder of 100 ml capacity. The numbers of actively motile larvae were counted by the dilution method of Scott (1928). After vigorous shaking, 1 ml of the suspension was pipette out, transferred onto several glass slides with squares already made on their reverse with a glass marking pencil and the larvae in all squares were carefully counted under a suitable dissecting microscope. Three such counts were repeated and the average count in 1 ml was multiplied by the total volume to get the total number of the larvae. An inoculum containing the desired number of actively motile larvae was adjusted in 0.2-0.3 ml to be given to each mouse. Each mouse was orally inoculated directly into stomach with the desired number of larvae (300) by 1 ml syringe having a blunt 18 gauge-feeding needle. After inoculation, mice were kept in cages in groups of five and labeled according to the design of experiments and were fed routinely with the same standard diet.

Worm recovery:

1. L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of larval somatic antigens:

Each mouse was infected with 300 larvae and after 1, 5 and 13 days of infection worm recovery was seen. In infected non-vaccinated mice 29.01% recovery was observed after 5 days and out of them 26.26% adults were recovered after 13 days.

While in mice which were vaccinated by larval somatic antigens, 9.41%, 6.20% and 5.22% recovery was recorded after 1, 5 and 13 days of vaccination [Table-4.1, Fig. 4.1].

2. L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of adult somatic antigens:

In infected non-vaccinated mice 29.01% recovery was observed after 5 days and out of them 26.26% adults were recovered after 13 days.

While in mice which were vaccinated by adult somatic antigens, 9.66%, 7.40% and 6.07% recovery was recorded after 1, 5 and 13 days of vaccination [Table-4.2, Fig. 4.2].

3. L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of larval ES antigens:

In infected non-vaccinated mice 29.01% recovery was observed after 5 days and out of them 26.66% adults were recovered after 13 days. While in mice which were vaccinated by larval ES antigens, 6.03%, 5.26% and 3.95% recovery was recorded after 1, 5, and 13 days of vaccination [Table-4.3, Fig. 4.3].

4. L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of adult ES antigens:

In infected non-vaccinated mice 29.01% recovery was observed after 5 days and out of them 26.66 % adults were recovered after 13 days. While in mice which were vaccinated by adult ES antigens 8.21%, 6.85% and 5.36% recovery was recorded after 1, 5, and 13 days of vaccination.

Observations and Results:

Heligmosomoides polygyrus is a natural intestinal helminth of mice. It inhibits in the duodenal region of small intestine. In the present investigation, albino mice were colonized with 300 larvae each time by placing larvae directly in the stomach through gastric lavage. The larvae migrated to duodenum, to house in the submucosa where they matured and then emerged as adult worms migrating in the intestinal lumen by 13 days of time.

The present study was carried out to investigate the immunological responses and possibility of vaccine development.

Summary and Conclusion:

The % protection after administration of larval ES antigen was the highest followed by vaccination with somatic antigens, then adult ES antigen and least after vaccinated with adult somatic antigens. Larval ES > Larval somatic antigens > adult ES antigens > adult somatic antigens.

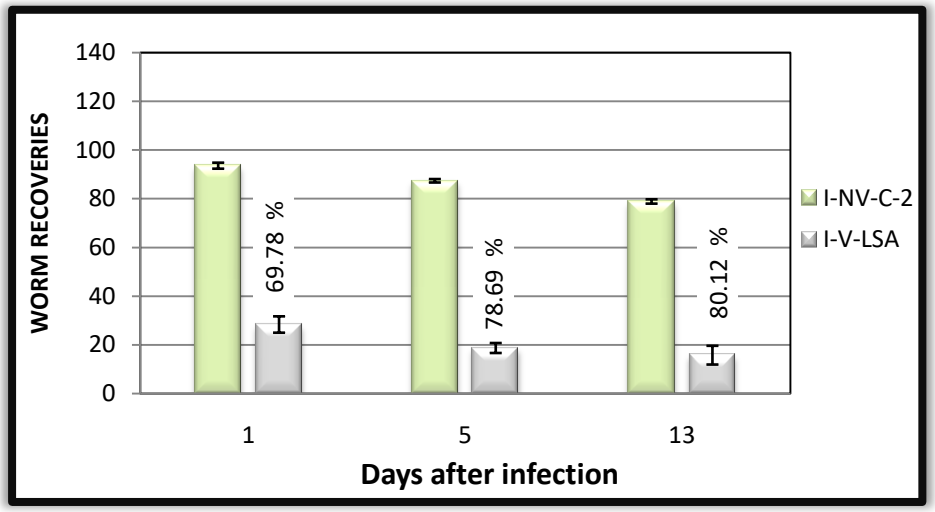


Figure 1: L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 days of administration of larval somatic antigens

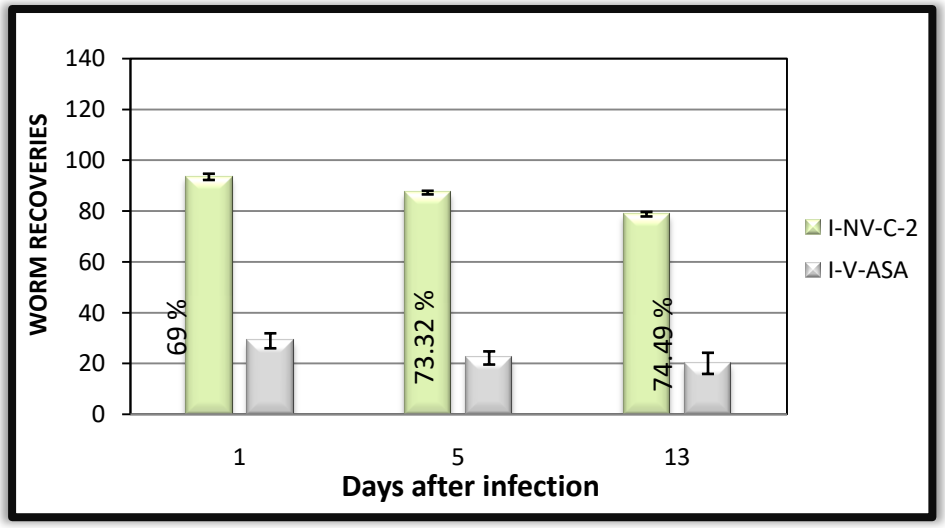


Figure 2: L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 day of administration of adult somatic antigens

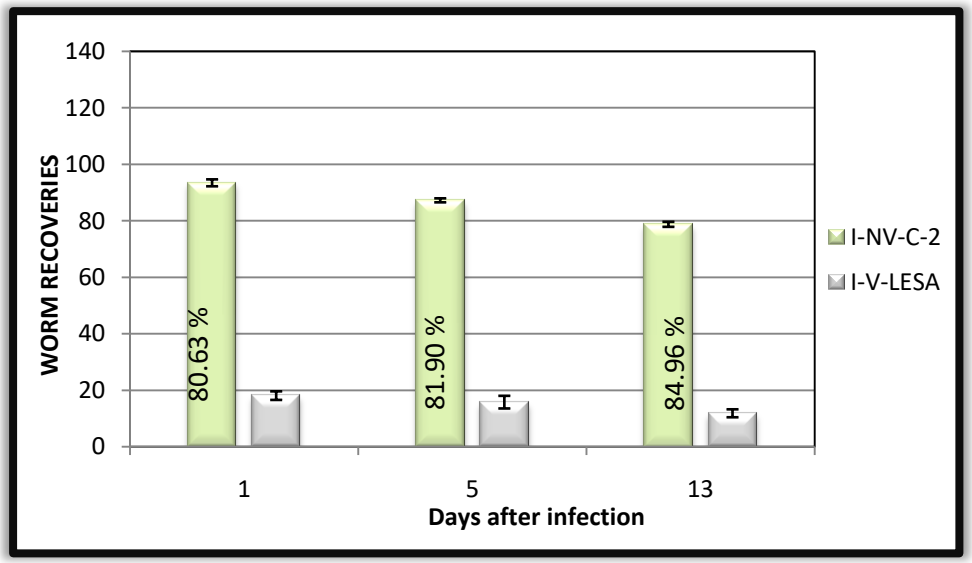
I-NV-C-2 Infected Non Vaccinated Control-2

I-V-ASA Infected and Vaccinated with adult Somatic Antigens

I-V-LSA Infected and Vaccinated with L-3 larval Somatic Antigens

Values on I-V-LSA bar denote % protection

Values on I-V-ASA bar denote % protection



Figuure 3: L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 day of administration of larval ES antigens

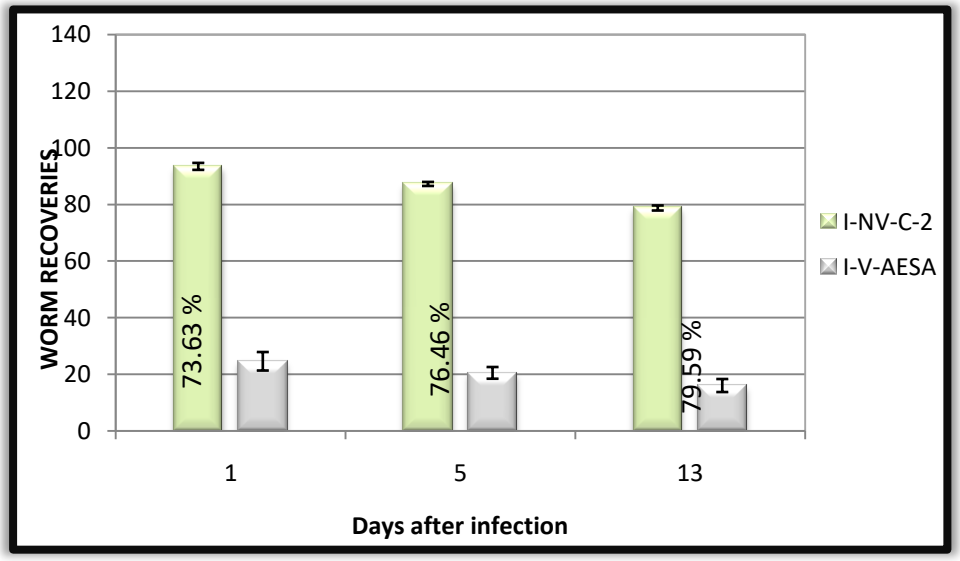


Figure 4: L-3 larval and adult worm recoveries from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13 day of administration of adult ES antigens

I-NV-C-2 Infected Non Vaccinated Control-2

I-V-LES Infected and Vaccinated with L-3 larval Somatic Antigens

I-V-AES Infected and Vaccinated with adult ES Antigens

Values on the I-V-LESA bar denote percent recovery

Values on the I-V-AESA bar denote percent recovery

After 1, 5 and 13 days of administration of larval ES antigens, the protection achieved was 80.63%, 81.90% and 84.96% respectively which was quiet better than the rest of the antigens tried during the present investigation. Even after the 1st day of administration of larval ES antigens, the larval recovery was 80.63%, while in rest of the vaccinated conditions the protection was below 75%. Vaccination with larval antigens, both somatic and ES antigens, led to less recovery of mature worms, than vaccination after adult somatic and ES antigens and similarly ES antigens were more potent than somatic antigens. These differences may be due to developmental modifications in larvae to become adult for continuation of race.

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TECHNOLOGICAL ADVANCES AND ANIMAL HEALTH

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Abstract:

In this rapidly growing world of technology new techniques are being used for increasing animal production, efficiency and profitability as well as to provide more comfort to farmers. Due to increasing market pressure dairy industry is moving towards more intensive, profit driven enterprise which in turn demands use of modern technologies that are cost effective and provide maximum benefits to the industry. As animal diseases cost the livestock industry billions of dollars each year new vaccines, disease monitoring systems and diagnostic kits can help to decrease both morbidity and mortality levels in livestock.Application of biotechnology to the livestock industry has generated a number of products for improving production, animal health, and food processing.The aim of this review is to focus on the modern technologies that are being currently used for improving animal health and increasing national economy through livestock sector.

Keywords: Advances, Animal health, Technologies

Introduction:

Animal health is important to the farmers as well as to the livestock and dairy industry of the country for gaining maximum benefits as livestock sector is the main component of India's agrarian economy providing food and nutritional security and livelihood. Over the past few years advanced digital technologies have improved every sector of the economy, including animal production, health and welfare. These include wireless and mobile technologies for animal health monitoring, disease surveillance, reporting and information sharing; advanced data processing technologies such as big data and data analytics used to uncover hidden patterns, predictions, correlation and other information; and promising technologies such as blockchain applications used for effective and efficient management of various input supply chains.In order to meet the food production needs due to increasing world population,livestock sector has to satisfy the increasing world consumption of animal-source food through sustainable animal production in

ways that promote food security, poverty reduction, public health and food safety (FAO, 2009 and Tedeschi *et al.*, 2015).

In addition to the increased world population and its demand for more reliable quality livestock products the livestock production problems are also increasing (Thornton, 2010). As the number of animals increase error burden and work load also increases. Hence for better production successful livestock farmers rapidly adapt their infrastructures with changes in technology. As such, automation systems provide options in front of the user in intense competition for convenience. Manual observation is gradually beingreplaced by many milking systems by automated recording (milk yield, milk conductivityand other measurements) leading to better quantity and quality of data.

Electronic recording, milking, heat detection auto-weighing,auto-drafting, genetic improvement, feeding, barn optimization, and health monitoring, livestockhousing and equipment designs are the technologies that provide dairyman many opportunitiesto make easier and more convenient decisions about dairy future plans.

Modern technologies in animal science use:

Biotechnology:

It involves techniquessuch as genetic engineering, cell culture, and monoclinal antibody methods that use living organisms or their partsto make or modify products, to improve plants or animals,or to develop microorganisms for specific uses. It is specifically used to produce productsthat promote growth, increase feed efficiency,carcass leanness in growing animals, and significantlyincrease milk production in lactating animals.Transgenic animals producedgrow faster, have greater disease resistance, and produce high-value pharmaceutical products. Also, newvaccines and diagnostic kits are being developed to improvelivestock health. Biotechnology is also being usedto process meat and dairy products and to detect foodcontaminants that might be present in those products.

Anabolic steroids and antimicrobial compounds:

These are currently being used in the livestock sector topromote growth and increase feed efficiency. However, new products are also being produced like protein hormones (somatotropins) and catecholamine compounds (beta-adrenergicagents) that increase growth rates inyoung animals, improves FCE (food conversion efficiency) and significantly reduce carcassfat so that meat products are leaner. Somatotropins also increase milk production in lactating dairy cows.

Reproduction technologies:

Like estrous cycle regulation, embryo cloning, superovulation, embryo and sperm sexing, ETT (embryo transfer technology) and production of transgenic animalsare being used to improve the quality of livestock herds more rapidly than could be achieved with traditional breeding.

Animal health technologies:

These are being used to create new vaccines which include deleting or inactivating the genes in a pathogen that cause disease,and inserting into a vector gene that cause an immuneresponse to a pathogen. Synthetic peptides are also beingproduced that stimulate the immune response like genetically engineered protein lysostaphin kills *Staphylococcus aureus* and achieves cure rates as high as 80 percent for mastitis in animals.Some synthetic peptides have been used to inhibit critical functionsof lentiviruses in sheep. Techniques for advanced laboratory diagnosis include enzyme immunoassays (ELISA), monoclonal antibodies, recombinant antigens, immunoblotting, nucleic acid hybridization, PCRand real time PCR techniques and nucleic acid analysis.Advances in vaccine technology aims to improve the immunogenicity and safety of vaccines, their shelf life and their cost of production. These techniques include recombinant DNA technology, modified live virus vaccines, synthetic peptides as vaccines, sub-unit vaccines, vectored vaccines, chimeric live vaccines and DNA vaccines. Immunomodulators like lymphokines (interleukins and interferons) and cytokines are hormone-like molecules that are being used for coordinating immune defences to infectious agents, cancer, and autoimmune diseases.

Digital technologies:

It includes new methods of data collection and management using advanced information and communication technologies (ICTs) andinnovations. Recent developments in ICTs and innovations lead to newopportunities for improving veterinary practice (Bellet, 2019 and EBVS, 2019), timeliness and accuracy of datacollection and reporting for diseasesurveillance and animal health monitoring (Holmstrom and Beckham, 2017). The use ofnew ICTs also facilitate mapping and monitoring the spread of infectious diseases and their coordination across sectors, as well as tracking supplies of drugs and vaccines (WHO, 2018). These developments lead to better, more efficient, and timely decisions that affect the performance and quality of veterinary services meeting standards of animal health and welfare practices (Liu et al, 2019). Mobile phones are being used for data collection onanimal diseases surveillance in animal health (Robertson *et al.,* 2010 and Madder *et al.,* 2012). In veterinary epidemiology, big data analysisresults in better

understanding of animal diseases and health related risks and improvements in the fields of bioinformatics which facilitate the understanding of host-pathogen interactions towards the development of new diagnostics, therapeutics and vaccines (Deblais *et al.,* 2019). In case of livestock and veterinary sectors, blockchain-based systems are potentially applied for efficient management of various input supply chains such as animal feed, veterinary drugs, diagnostic kits and vaccines (Makkar and Costa, 2020).

Sensor technology:

It is an effective method for health monitoring which uses a sensor for measuring physiological or behavioural parameter of an individual cow and enables automated, on-farm detection of changes in this condition that is related to a health event like a disease and requires action on the part of the farmer such as treatment. Sensors are of two types: Attached and Non-attached and currently the sensor-based data acquisition systems are classified into two categories: Non-invasive (Immobile sensors located in the barn and Mobile sensor boxes attached to the cow called external sensors) and Invasive.

Examples of non-invasive type of sensors include temperature measurements of the udder or of the face in an automatic milking station and measurement of breath composition. Surveillance cameras are another kind of immobile sensors that can continuously provide information for the cows in the herd (Poursaberi *et al.,* 2010). The most reliable way to monitor cows throughout a day is to attach sensors at individual cow by a neck collar or an ankle ribbon.

Typical sensors of this type are accelerometers, pedometers, vibration sensors, thermometers for temperature measurements, humidity sensors etc. Pedometers are cheap and simple sensors that give insight in the activity status of a cow like oestrus behaviour with good prediction capabilities (Løvendahl and Chagunda, 2010).Recently, low-cost and infrastructure-less GPS positioning sensorshave been used to identify different motionstates of cows (Godsk and Kjærgaard, 2011). The GPS sensors attached to the animal's collars identify the following activities: eating, seeking, walking, lying and standing.

Invasive sensors include mobile sensor boxes swallowed or implanted to cow called internal sensors.Typical sensors of this kind are thermometers for measuring the core body temperature or vaginal pressure during birth, sensors for measuring electrical conductivity and pH value of rumen fluid.

Advanced Animal Breeding and Genetics:

Innovations in animal breeding and genetics result in improving food quality. Genome editing techniques in animals has led to greater meat and milk production. Various approaches to retrieve superior qualities in animal breeds include artificial insemination, somatic cell nuclear

transfer, in vitro production of embryos, gene transfer, nuclear transfer and aquaculture.New animal breeds have been developed in animal husbandrywith the help of breeding and gene technology. Till 1980s livestock products demands have been met by breed substitution, cross-breeding, and within-breed selection. But these demands in future are to be met using new techniques such as artificial insemination and more specific selection techniques.

Conclusion:

Recent advances in animal health are a result of the application of new technologies in the field of animal husbandry and veterinary sciences. New technological interventions and application of digitalization in animal breeding and genetics, biotechnology, animal disease monitoring and survillence, vaccine production and other prophylactic and therapeutic approaches for improvement of animal health which in turn has increased the productivity and economic status of the country to a great extent. However, more is to be done to improve the health, productivity and efficiency of the livestock for gaining maximum benefits.

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CLONING AND GENETIC ENGINEERING

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Abstract:

The technique of creating a genetically identical replica of a cell or an organism is known as cloning. Naturally cloning occurs when a cell copies itself asexually i.e. without undergoing any genetic alterations or recombination. Bacteria and other prokaryotic organisms (those without a cell nucleus) use binary fission or budding to generate genetically identical replicas of them. With the exception of gametes (eggs and sperm), which undergo meiosis and genetic recombination, all cells that undergo mitosis, such as skin cells and cells lining the gastrointestinal system, are clones in eukaryotic beings (organisms with a cell nucleus).

Cloning is a wide term in biomedical research that refers to the duplication of any type of biological material for scientific study, such as a bit of DNA or a single cell. Segments of DNA, for example, are reproduced exponentially using a technique known as polymerase chain reaction, or PCR, which is extensively employed in fundamental scientific research. The development of cloned embryos, particularly human embryos, that are genetically identical to the species from which they are derived, and their subsequent usage for scientific, medicinal, or reproductive purposes is the topic of heated discussion.

Reproductive cloning:

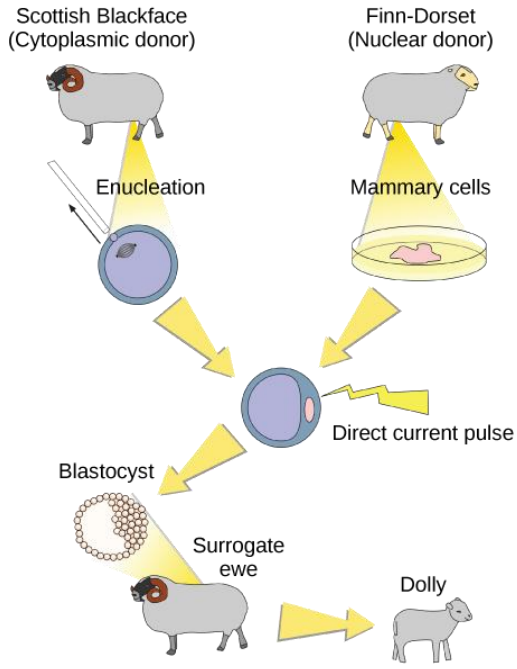
The purposeful generation of genetically identical people is known as reproductive cloning. Each new individual is exactly same as the original. Natural clones are monozygotic (identical) twins. Hence the genes and the DNA present in the nucleus of cells from two clones are identical. A method for creating a clone or an exact replica of a whole multicellular organism is reproductive cloning. The majority of multicellular creatures reproduce sexually, which entails the genetic hybridization of two individuals (parents), making it difficult to produce an exact duplicate or clone of either parent. Asexual reproduction of animals may now be intentionally induced in the laboratory thanks to recent breakthroughs in biotechnology.

Parthenogenesis, or "virgin birth," is a type of asexual reproduction in which an embryo grows and develops without the egg being fertilised. In animals where the female deposits an egg, parthenogenesis is an example. If the egg is fertilised, it becomes a diploid egg and the individual becomes a female; if the egg is not fertilised, it becomes a haploid egg and the

individual becomes a male. A parthenogenic, or virgin, egg is one that has not been fertilised. Parthenogenic eggs, which can grow into adults, are laid by some insects and reptiles.

For sexual reproduction, it is necessary to have two cells. When the haploid egg and sperm cells unite it forms a diploid zygote. The genetic information that is required to create a new human is stored in the nucleus of zygote. Early embryonic development, on the other hand, needs the cytoplasmic material present in the egg cell. Reproductive cloning is based on this concept. When the haploid nucleus of an egg cell is replaced with a diploid nucleus from any member of the same species (donor), the result is a zygote with the same genetic makeup as the donor. The procedure of putting a diploid nucleus into an enucleated egg is known as somatic cell nuclear transfer. It's suitable for both reproductive and therapeutic cloning.

Dolly, a sheep born in 1996, was the first cloned mammal. At the time, reproductive cloning had an extremely low success rate. Dolly survived for seven years before succumbing to respiratory problems. It's possible that because the cell DNA belongs to an older person, the age of the DNA will impact the lifespan of a cloned person. Several animals (including horses, bulls, and goats) have been successfully cloned since Dolly, albeit these creatures frequently have facial, limb, and cardiac defects. Attempts to produce cloned human embryos as sources of embryonic stem cells have been made. The procedure, which is sometimes referred to as therapeutic cloning, creates stem cells that are used to treat illnesses or abnormalities (unlike reproductive cloning, which aims to reproduce an organism). Despite this, therapeutic cloning initiatives have been faced with opposition due to bioethical concerns.



Molecular cloning:

Molecular cloning is a collection of molecular biology experimental methods for assembling recombinant DNA molecules and directing their replication in host organisms. Cloning refers to a technique that involves the reproduction of a single molecule to generate a population of cells with identical DNA molecules. In most cases, DNA sequences from two separate animals are used in molecular cloning: the source of the cloned DNA and the species that will act as the live host for recombinant DNA replication. Many contemporary fields of modern biology and medicine rely on molecular cloning procedures.

In a traditional molecular cloning procedure, the DNA to be cloned is acquired from a target organism and then processed with enzymes in the test tube to produce smaller DNA fragments. After that, these pieces are mixed with vector DNA to make recombinant DNA molecules. After that, the recombinant DNA is implanted into a host organism (typically an easy-to-grow, benign, laboratory strain of E. coli bacteria). This will result in a colony of creatures that reproduce recombinant DNA molecules alongside host DNA. These are transgenic or genetically engineered bacteria because they contain foreign DNA pieces (GMO). The fact that a single bacterial cell may be encouraged to take in and reproduce a single recombinant DNA molecule is used in this technique. This single cell may then be multiplied indefinitely to produce a vast number of bacteria, each containing a copy of the original recombinant molecule. As a result, both the recombinant DNA molecule and the ensuing bacterial population are usually referred to as "clones." Recombinant DNA, strictly speaking, relates to DNA molecules, whereas molecular cloning refers to the experimental procedures used to put them together. Different DNA sequences may be put into a plasmid, and these alien sequences would be taken into bacteria and digested as part of the plasmid, according to the theory. In other words, these plasmids might be employed as cloning vectors for carrying genes.

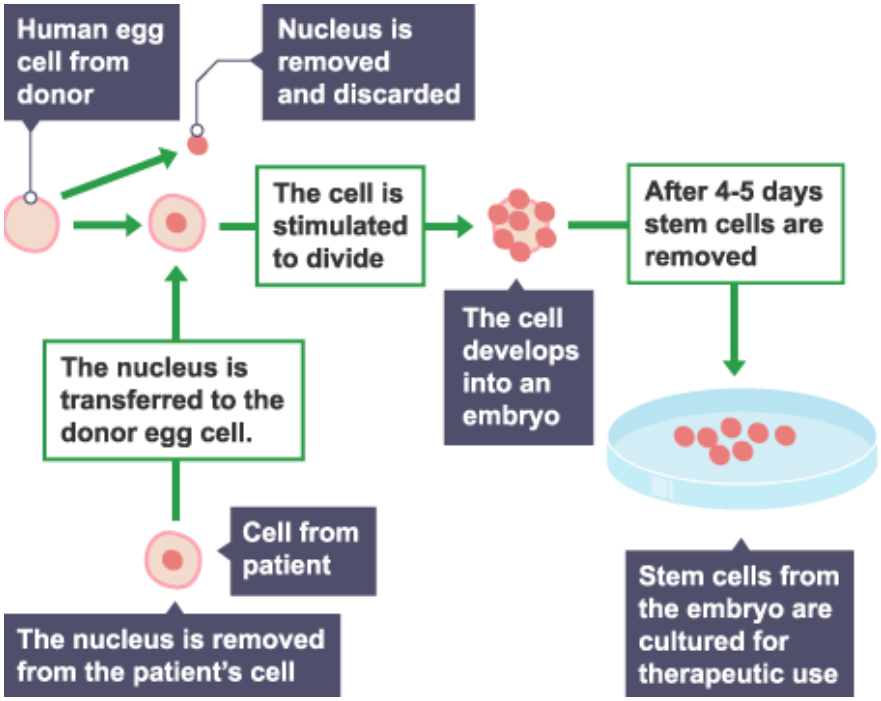
Although virtually any DNA sequence may be cloned and amplified, various variables may restrict the procedure' effectiveness. Inverted repeats, replication origins, centromeres, and telomeres are examples of difficult-to-clone DNA sequences. When inserting large-sized DNA sequences, there is also a decreased possibility of success. Insertions bigger than 10 kbp have a low success rate, yet bacteriophages like bacteriophage can be engineered to insert sequences up to 40 kbp effectively.

Therapeutic cloning:

Therapeutic cloning refers to the cloning with the purpose of treating a sickness. The nucleus of a cell, usually a skin cell, is put into a fertilised egg whose nucleus has been removed

in therapeutic cloning. A blastocyst is formed when a nucleated egg divides repeatedly. The blastocyst's stem cells are then extracted and used to generate cells that are genetically identical to the patient. Therapeutic cloning cells can then be put into a patient to address a condition that they are suffering from. In contrast to therapeutic cloning, the purpose of reproductive cloning is to generate a new individual, a proposition that has sparked widespread debate and widespread opposition.

Therapeutic cloning might result in stem cells that are genetically identical to the patient. The procedure entails transferring the nucleus from a patient's cell to an egg cell that has had its nucleus removed.



Benefits and risks associated with the use of stem cells in medicine. Stem cells show a lot of promise in terms of treating patients with diseases that are now incurable, generating organs for transplantation, and research. However, its usage raises clinical, ethical, and societal concerns. These challenges will be different for adult, embryonic, and therapeutically-cloned stem cell development and transplantation. They will also be determined by whether the stem cells will be employed for therapeutic or research purposes.

It's critical to get a balanced perspective. There are no right or wrong solutions or even answers at all, in certain cases. When talking about stem cells, there are a few things to think about:

Clinical problems:

- There is no assurance that these therapies, such as the use of stem cells to replace nerve cells lost in Parkinson's disease patients, would be successful.
- The challenge in identifying acceptable stem cell donors at the moment.
- The difficulty of acquiring and preserving embryonic stem cells from a patient. These would have to be obtained before to delivery; some clinics will retain blood from a newborn's umbilical cord.
- In stem cells cultivated for several generations, mutations have been discovered, and some altered stem cells have been seen to behave like cancer cells.
- Viruses might infect cultured stem cells, which could then be transmitted to a patient.

Ethical concerns:

- Is it ethical to make embryos for therapeutic cloning and then kill them?
- Are unused embryos created by in vitro fertilization (IVF) a source of embryonic stem cells?
- Embryos might be considered as a commodity rather than an embryo that has the potential to develop into a person.
- At what point in its development should an embryo be considered a person and treated as such?

Social Concerns:

- It is critical to educate the public on what stem cells can and cannot do.
- Whether the advantages of stem cell therapy outweigh the drawbacks.
- Because a lot of the research is done by for-profit clinics, the results aren't subjected to peer review. Because stem cell therapies are still in their early phases of research, patients may be abused by paying for expensive procedures and being offered false hope of a cure.

Conclusion:

The same scientific procedures that allow us to clone animals may be used to duplicate particular cells throughout the body. The techniques established in this sector might eventually be used to create new tissues or organs as needed. It would not endanger the animal's life, and the knowledge gathered from these procedures might lead to new advances in human medical research. We might analyze the cloned cells to see how effective each procedure is in determining how these fields can advance. Cloning animals might provide better comfort to pet owners.

Losing a pet is especially painful since these creatures are generally considered family members. Our everyday routines are made more structured by the presence of cats, dogs, and other animals. These partners keep us busy and might even encourage us in our attempts to conquer life's obstacles.

Pets provide us with a sense of purpose. Cloning animals would allow individuals to safeguard their memories of a valued friend by having a scientist make an identical animal. This endeavour would still result in a one-of-a-kind animal for the species, but it would also provide some continuity and support, potentially reducing emotional reactions. Animal cloning does not result in precise duplication.

When we clone animals, we do not achieve precise replication:

The genetic material is implanted into an embryo, which is made up of distinct cells. The clone can then create children later in life as a result of these procedures. Because Dolly the Sheep was coupled with a Welsh Mountain ram, scientists were able to produce six lambs in all. Bonnie was the first, and she was born in 1998.

Then Dolly gave birth to twins named Rosie and Sally by her carers. Cotton, Darcy, and Lucy were the names of her triplets, who were born in the fall of 2001. Some experts believed that the children would be infertile, but they were not, demonstrating the full potential of this scientific method.

Animal cloning helps us to save endangered species:

Only because 13 horses were caught from a wild herd and maintained in a zoo in the 1940s were researchers able to conserve the Przewalski's horse. Two of the horses were hybrids even back then. Standard breeding procedures aided in the survival of the species, which currently numbers in the thousands.

With the Northern White Rhino, we won't have the same luck. In March 2018, the species' only known male died. Because there are only two females of the same subspecies left alive, cloning is the only method to rescue them. This work allows us to save endangered creatures and maybe even bring extinct species back into the world.

Cloning animals allows us to develop the most desirable characteristics:

Through cloning scientists can do what other scientists have done for more than 1000 years through selective breeding. The results are comparable to those obtained through natural reproductive processes involving human intervention. This approach offers the opportunity to develop exact, desirable features in animals.

Animal cloning might be used to create dairy cows that generate more milk. Researchers may investigate the possibility of cloning certain chickens to boost commercial egg output. This

method might be used to breed livestock animals to generate more meat per corpse. When we consider the whole potential of this technology, the possibilities are practically endless

Human diseases could be reduces with the help of animal cloning:

Influenza is one of the most troublesome infections that humans face each season. Millions of people have died as a result of flu epidemics in the past, especially when a new strain of the virus spreads across the population. Even though yearly immunizations are available to prevent it, about one in every five people will contract it each year.

The influenza virus is spread via birds, pigs, and other animals. Our animal cloning techniques may be able to halt its development by increasing resistance to its activities as it develops. It's an opportunity to avert disease's negative consequences before it even begins.

Quality of food supply would not be altered by cloning:

People can consume animal products from cloned animals, according to 2008 rulings based on science at the time. Any animal species can enter the commercial food chain, according to the FDA. That means scientists may seek for methods to improve the nutrient profile of the proteins we eat, giving everyone the opportunity to eat better without having to change their eating habits. Consider this benefit to be the beef equivalent of working with enriched flour.

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EMERGING TOOLS FOR CONSERVATION AND MONITORING OF BIODIVERSITY

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Introduction:

The continual loss of biodiversity on Earth is a serious concern and challenge for the twenty-first century, and there is worldwidepolitically aware agreement to limit or stop this loss. The task is hampered in large part by a dearth of understanding about the status and biodiversitydistribution– specifically because science has yet to define the vast majority of species on the planet. To get credible estimates of population numbers and dispersal trends, all conservation initiatives to conserve biodiversity rely on species and population monitoring. Traditionally, visual surveys and individual counts have been used to physically identify species for monitoring purposes.

Traditional monitoring procedures, on the other hand, continue to be troublesome due to challenges in correctly identifying hidden species or life stages of juvenile, a steady reduction in taxonomic knowledge, non-standardized sample and the intrusivenessof certain survey practices. As a result, innovative and effective methodologies in order to keep track of biodiversity on a wide scaleare urgently needed. DNA barcoding, environmental DNA (eDNA), community DNA, invertebrate ingested DNA (iDNA) metabarcoding, and other developing conservation technologies for monitoring biodiversity include DNA barcoding, environmental DNA (eDNA), community DNA, and invertebrate ingested DNA (iDNA) metabarcoding.

DNA Barcoding:

DNA barcoding is a beneficial method foridentifying organisms at the molecular level. This method entails amplifying a segment, the cytochrome oxidase enzyme I (COI) gene with PCR, sequence analysis and matching it to a databank of already identified species. Various genes, like as rbcL and matK, are frequently used in plants for barcoding identification. The aim of this study was to find primers and reagents for polymerase chain reaction (PCR) DNA barcoding on a wide range of taxonomic groups.

The published literature was evaluated for DNA barcoding methodologies and processes for invertebrates, fish, mammals, and plants, and the PCR primers for these taxonomic groupings were discovered and arranged. DNA barcoding methods were used to evaluate by researchers

provided samples from a wide spectrum of species. Following the completion of the PCR process, the PCR result was uploaded to the BOLD and NBCI databases for DNA sequencing and analysis.DNA samples from fish, birds, animals, plants, and invertebrates might be analysed.The findings of the analysis of samples supplied by Reclamation researchers were given to the researchers. During the course of this assignment, we learnt a few things about DNA barcoding. A literature review of DNA barcoding methods is included in this paper, as well as an overview of the research and a discussion of the findings.

Taxonomic specialists have long used specialised language and literature to describe and identify creatures; DNA-based identification methods, on the other hand, employ standardised molecular biology procedures (DNA extraction, PCR, and DNA sequencing) to speed up the identification of unknown organisms (Seifert *et al.,* 2007). The objective of DNA barcoding scientists is to develop a library of every life on the planet (Stoeckle *et al.,* 2004).

Environmental DNA (eDNA):

Extracting DNA from environmental samples (environmental DNA - eDNA) to get information on species, populations, and communities offers the potential to alleviate many of the difficulties facing biodiversity monitoring (Baird and Hajibabaei, 2012; Kelly *et al.,* 2014b). The informationof DNA from higher species can be takenand examined in the environment has been a major technical and scientific achievement in the recent decade. As creatures interact with their surroundings, they continually discharge DNA into the environment.

This DNA can be derived from expelled cells or tissue in higher species, such as urine, faeces, hairs and skin and, of course, from dead persons leaking genetic material. Although macrobial eDNA possibly will reside largely inside mitochondria or tiny cells in some systems (Turner *et al.,* 2014), extracellular DNA will be present in the surroundings due to membrane breakdown (Nielsen *et al.,* 2007).

As a result, eDNA has been utilised to answer both practical and basic research problems in a variety of fields, including ecology, molecular biology, environmental sciences and palaeontology are some of the fields of study. The identification of various eDNA from macro-organisms validated the method as actually important in a conservation context, and it has been discovered in a wide range of ancient and modern terrestrial and aquatic habitat (Thomsen and Willerslev, 2015).

Community DNA:

Since the commencement of high-throughput sequencing (HTS), the application of metabarcoding as a technique of biodiversity identification has attracted biologists' interest. (Margulies *et al.,* 2005, Hajibabaei *et al.,* 2011).Changes in sampling andvariations in research laboratory processes, might affect later bioinformatics methods used to data processing and

confound the understanding of regional and of time biodiversity trends if there is no clarity between these two source materials (Creer *et al.*, 2016).

Typically, the selected populations are assembled in quantity (e.g., soil, malaise trap, or net), and individuals are separated from other sample material and mixed together prior to bulk DNA extraction (Creer *et al.*, 2016, Lévêque *et al.*, 2008). Macro-organism eDNA, on the other hand, is extracted directly from an environmental substance (e.g., soil or water) without the need to separate individual organisms or plant material from the sample, implying that the entire organism is not there. Of course, community DNA samples may contain DNA from other creatures' tissues, cells, and organelles (e.g., gut contents, cutaneous intracellular or extracellular DNA). Similarly, macroorganism eDNA samples may catch complete tiny nontarget species accidently (e.g., protists, bacteria). As a result, the distinction can, at least in part, be blurred in reality (Deiner *et al.*, 2014).

Metabarcoding of invertebrate ingested DNA (iDNA):

Using DNA metabarcoding to target vertebrate genetic material obtained from invertebrates (such as leeches, mosquitoes, or ticks, among others). It might be considered a unique example of eDNA metabarcoding because the DNA sources include swallowed stuff or faeces.

Metabarcoding:

Metabarcoding is the process of barcoding DNA or RNA in such a way that many taxa may be identified at the same time in the identical sample. The fundamental differenceamong metabarcoding andbarcoding is, the metabarcoding is used to decide the species makeup of a sample rather than focusing on a single organism. A barcode is made up of a brief variable gene segment that is valuable for taxonomic classification and is bordered by gene with a high level of conservation that may be utilised to design suitable primer (Pierre and colleagues, 2018). Researchers at the University of Guelph came up with the concept of generic barcoding in 2003 (Hebert *et al.*, 2003)

Metabarcoding, like ordinary barcoding, follows a step-by-step process that includes DNA extraction; PCR amplification, sequencing, and data analysis are all steps in the process. Different genes are used depending on whether the objective is to barcode a single species or to metabarcode many species. In the second situation, a broader gene is used.Metabarcoding starts with DNA/RNA from numerous distinct creatures taken from a single ambient or bulk sample, rather than single species DNA/RNA.

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DIMETHOATE: KILLER PESTICIDE

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The pesticides are one of the most potentially harmful chemicals liberated in the environment in an unplanned manner. Pesticides are biologically active chemical. The misused of pesticide may be harmful to humans, animals and environment. Dimethoate is widely used as a potent pesticide and acaricide in many countries and has been shown to produce some adverse health effects. Dimethoate, (IUPAC name O, O-dimethyl SN- methyl carbamoyl methyl phosphorodithioate) is widely used against a broad range of insects and mites and is also used for indoor control of houseflies. WHO and International Program on Chemical Safety (IPCS) said that the extensive use of Dimethoate poses a health hazard to animals and humans because of its persistence in water, soil and crops. Dimethoate classified as moderately hazardous by WHO and the oral dose of dimethoate is biotransformed to its oxygen analogue Omethoate, which is the active form, by hydrolysis of the methyl ester group and removal of the methyl-amido group. Omethote is considerably more toxic than dimethoate. U. S. Environmental Protection Agency (USEPA) has registered Dimethoate a systemic organophosphate insecticide but interim in 2006 it released reregistration eligibility decision (IRED) document for dimethoate in accordance with FQPA requirements.

A great proportion of acute poisoning cases are caused by exposure to pesticides, especially organophosphate (OP) compounds. Dimethoate is rapidly absorbed from the gastrointestinal tract of the body and its poisoning is usually block neuromuscular transmission in both animals and humans. The primary mechanism of action of OP pesticides is based on inhibition of the acetylcholinesterase (ache) enzyme. Once ache has been inactivated; acetylcholine (ach) accumulates throughout the nervous system, resulting in overstimulation of muscarinic and nicotinic receptors. Signs and symptoms of op poisoning can be divided into three broad categories, as muscarinic, nicotinic, and central nervous system effects.

The toxicity of organophosphorus insecticides results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system

in many animals. Furthermore, organophosphorus insecticides exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues with simultaneous generation of reactive oxygen species. Dimethoate induces a number of morphological and functional changes in several tissues and organs of the body and it displays the ability to induce free-radical processes. Some study indicates that administration of dimethoate led to induction of oxidative stress and which was affect on increased concentration of MDA in liver. Toxicity of organophosporus insecticides used compounds against human and animals were always evaluated by assessment of such biochemical parameters alterations and histopathological changes in tissues and organs. Kidney is one of the targets organs of experimental animals attacked by organophosphorous compounds.

Management of severe poisoning is difficult, requiring intensive care and use of atropine and oxime cholinesterase reactivators. The acute and chronic toxicity of dimethoate is moderately high by ingestion, inhalation and dermal absorbtion. By considering all these problems this pesticide is banned in some countries. Though it is banned, is used on field as well as in house for controlling variety of pest. So for our safety purpose we should stop the use of this hazardous pesticide for healthy life.

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DIVERSITY OF MOTHS (LEPIDOPTERA: HETEROCERA) AND THEIR POTENTIAL ROLE AS A CONSERVATION TOOL IN PROTECTED AREAS OF KATEPURNA WILDLIFE SANCTUARY OF AKOLA (M.S.)

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Abstract:

Moths are diverse group of insects belonging to the order Lepidoptera and regarded as one of the indicators of a healthy environment. Despite a large number of studies been taken up on the documentation of various wildlife taxa found in and around Katepurna wildlife Sanctuary of Akola by the biologists, information on Moths of this region remains unknown. The present study is the first documentation on the moth species of Katepurna wildlife Sanctury of Akola. The study will be carried out throughout the year surveying areas mostly in the protected areas of Katepurna wildlife Sanctuary of Akola (M.S.) and agricultural lands. Light trapping equipped with a 18w UV-Actinic tube attached to a white sheet 6 x 4 feet joined to two poles and then the tube powered by 6v Battery, which will be used to record moths from playgrounds of college, human settlements and agricultural lands. In total, 12 moth individuals were recorded belonging to 12 species Within 12 genera falling under 8 families.. The family Crambidae -3 represented the highest number of species, followed by *Geometridae* -2 , Saturniidae -2. . The less commonly observed species belonging to families are Pyralidae-1, Uraniidae-1, Noctuidae-1, Erebidae -1 and Sphingidae-1 individuals respectively.

Keywords: Moths, Lepidoptera, Katepurna Wildlife Sanctuary

Intoduction:

Akola town of the Vidharbha State of Maharashtra situated at latitude 20.70 North and longitudinal 77.07 0 East.It is an altitude of 925ft (287m) to 1036.745ft (316m) above sea level. Akola has tropical savanna climate Annual temperature range from a high of 47.60c to a low 2.20c. Annual rain fall averages 800mm. Katepurna Sanctuary is situated in the district of Akola, which comes under the Vidarbha region. The sanctuary occupies a large part of catchment area

of the Katepurna reservoir. Due to the presence of large amount of water, birds get attracted towards the place. The period between October to June is the ideal time for visiting the place. Flora- The place is very rich in vegetation cover and mainly comprises of southern tropical deciduous forests. There are more than 115 species of plants & trees like Bahada, Aola, Tendu, Dhawada, Salai, Moha, Teude and many more. Fauna- The sanctuary is a home to a wide array of animals like Black buck, Hyena, Wolf, Nilgai, Leopard, Jungle cat, Hare, Wild boar, Monkey etc. The chief attraction of the sanctuary is the Barking deer and the Four-horned Antelope.

One can also see various species of birds like Peafowl, eagles, woodpecker etc. Katepurna Wildlife Sanctuary is spread over 73.69 sq. km. It covers a great part of the catchment area of 12 the Katepurna reservoir. It is well known for for-horned antelope and barking deer. Among the winged insects, the moths belong to the scientific order Lepidoptera including the butterflies. They can be distinguished from all other insects by the two pair of wings and the body that is scale covered. Regarded as indicators of healthy environment, inventory of Lepidoptera specially the overlooked group is the first step to know what are the species present in an area and it is essential to ensure future taxonomical and ecological studies of these taxa and implement conservation perspectives for moth individuals as well as their associated habitats.

Global estimates show that there are 1, 27,000 species of moths distributed over the world and of which, 12,000 species are reported from India. Moths are in general are least studied taxa across the globe and in India.

Despite a large number of studies been taken up on the documentation of various wildlife taxa found in and around Katepurna wildlife Sanctury of Akola by the biologists, information on Moths of this region remains unknown. The present study is the first documentation on the moth species of Katepurna wildlife Sanctury of Akola.

Materials and Method:

The study will be carried out throughout the year surveying areas mostly in the protected areas of Katepurna wildlife Sanctuary of Akola (M.S.) and agricultural lands. Light trapping equipped with a 18w UV-Actinic tube attached to a white sheet 6 x 4 feet joined to two poles and then the tube powered by 6v Battery, which will be used to record moths from playgrounds of college, human settlements and agricultural lands.

The moths will be photographed and identified and those that will be difficult to identify will be kept for proper identification. Among the literatures, Fauna of British India: Moths Volume I-V by G.F Hampson will be referred for identification along with other journals.

Results and Discussion:

A total of 12 moth individuals were recorded belonging to 12 species Within 12 genera falling under 8 families. A checklist of the moth Species is tabulated in Table 1.

Table 1: List of moth species of protected areas of Katepurna wildlife Sanctuary, Akola

Sr. No.	Name of the species	Family	Genus
1.	*Automeris bilinea*	Saturniidae	*Antheraea*
2.	*Plodia interpunctella*	Pyralidae	*Plodia*
3.	*Hymenia recurvalis*	Crambidae	*Hymenia*
4.	*Pseudopanthera macularia*	*Geometridae*	*Pseudopanthera*
5.	*Micronia aculeata*	Uraniidae	*Micronia*
6.	*Spirama retorta*	Noctuidae	*Spirama*
7.	*Diaphania indica*	Crambidae	*Diaphania*
8.	Thalassodes immasaria	*Geometridae*	Thalassodes
9.	*Antheraea mylitta*	Saturniidae	*Antheraea*
10.	*Pygospila tyres*	Crambidae	*Pygospila*
11.	*Asota planaria*	Erebidae	*Asota*
12.	*Agrius convolvuli*	Sphingidae	*Agrius*

The accumulation of moths in a light source depends on the type of light source, plant communities occurring around the study site, temperature, weather conditions, altitudinal gradient, and the type of methods implemented. The moths recorded by visiting the mentioned

Localities and sheet light trap method was a valuable source for developing a preliminary data record for moths occurring in this region. The notable species accumulated in the Katepurna wildlife habitats were *Automeris bilinea* and *Antheraea mylitta* belongs to Saturniidae family on their host plants namely Syzygium cumini (jambul), Hardwickia binata (anjan).

Plodia interpunctella belong to family Pyralidae. It feeds on a wide variety of dry vegetable matter, including grains, nuts, dry beans, dried fruits, dried flowers, bird seed, dry pet foods, processed foods such as cereals and crackers, and confections.

Hymenia recurvalis, *Diaphania indica and Pygospila tyres* belong to family Crambidae species of observed as a minor pest of potato and cucumber near the crop land area of Katepurna.

Geometridae family represents two species of moth *Pseudopanthera macularia* and Thalassodes immasaria on their host plant namely, Ricinus communis, Mangifera indica (mango).

Images of some moths of protected areas of Katepurna wildlife Sanctuary of Akola

Automeris bilinea

Plodia interpunctella **Indian meal moth**

Beet webworm moth ***Hymenia recurvalis***

Pseudopanthera macularia

Micronia aculeate

Spirama retorta

Daphnia indica

Thalassodes immissaria

Antheraea mylitta

Pygospila tyres

Asota planaria

Agrius convolvuli

Micronia aculeata belongs to family Uraniidae observed near their host plant namely syzygium jambo.

The species *Spirama retorta , Asota planaria and Agrius convolvuli* represented the family Noctuidae, Erebidae and Sphingidae respectively These three families appeared to be scarce representing single species. Near the host plant namely *Solanum lycopersicum* (Tomatoes), Ficus plant and on hibiscus flower respectively.

Conclusion:

Moths also play a vital role in telling us about the health of our environment, like the canary in the coalmine .since they are so widespread and found in so many different habitats, and are so sensitive to changes; moths are particularly useful as indicator species. Monitoring their number and ranges can give us vital clues to changes in our own environment, such as the effect of new farming practices, pesticides, air pollution and climate change.

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CLIMATE CHANGE AND ITS INTERACTIONS WITH PEOPLE AND ECOSYSTEMS

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The quick anthropogenic environmental change that is being knowledgeable about the mid twenty-first century is personally weaved with the wellbeing and working of the biosphere. Environmental change is affecting biological systems through changes in mean conditions and in environment inconstancy, combined with other related changes like expanded sea fermentation and air carbon dioxide fixations. It likewise collaborates with different tensions on environments, including debasement, defaunation and discontinuity. There is a need to comprehend the biological elements of these environment impacts, to recognize focal points of weakness and flexibility and to distinguish the board intercessions that might help biosphere strength to environmental change.

Simultaneously, biological systems can likewise aid the relief of, and transformation too, environmental change. The instruments, potential and cutoff points of such nature-based answers for environmental change should be investigated and measured. This paper acquaints a topical issue committed with the cooperation between environmental change and the biosphere. It investigates novel points of view on how biological systems react to environmental change, how biological system flexibility can be upgraded and how environments can help with tending to the challenge of an evolving environment. It draws on a Regal Society-Public Foundation of Sciences Discussion held in Washington DC in November 2018, where these subjects and issues were examined. We close by distinguishing a few needs for scholastic examination and pragmatic execution, in request to boost the potential for keeping a different, tough and well-working biosphere under the difficult states of the twenty-first century.

This article is essential for the topic issue 'Environmental change and biological systems: dangers, openings and arrangements'.

The drawn out great soundness of populaces relies upon the proceeded with security and working of the biosphere's biological and actual frameworks, frequently alluded to as life-emotionally supportive networks. We overlook this since quite a while ago settled authentic truth

at ourrisk: yet it is all around not entirely obvious this reliance, especially at a time at the point when the human species is turning out to be progressively urbanized and removed from these regular frameworks. The world's environment framework is a vital piece of this complex of life-supporting cycles, one of numerous enormous regular frameworks that are presently going under strain from the expanding weight of human numbers and monetary exercises. By coincidentally expanding the centralization of energy-catching gases in the lower environment, human activities have started to enhance Earth's regular nursery impact. The essential test confronting the world local area is to accomplish adequate decrease in ozone harming substance outflows to stay away from hazardous impedance in the environment framework. Public state run administrations, through the UN System Show on Environmental Change (UNFCC), are submitted on a basic level to looking forthis result. By and by, it is demonstrating hard to track down a politically OK strategy—frequently in view of anxieties about conceivable present moment financial outcomes.

This volume looks to depict the specific situation and interaction of worldwide environmental change, its genuine or possible effects on wellbeing, and how human social orders ought to react, by means of both variation techniques to diminish effects and aggregate activity to decrease ozone harming substance outflows. As displayed later, a significant part of the resultant danger to human populaces and the biological systems whereupon they depend comes from the extended incredibly fast pace of progress in climatic conditions. For sure, the possibilityof such change has animated a lot of new logical exploration over the past decade, quite a bit of which is clarifying the complex natural aggravations that can affect the human prosperity and wellbeing—as in the accompanying model. The US Worldwide Change Exploration Program (Gold country Territorial Evaluation Gathering) as of late archived how the different impacts of environmental change on sea-going environments can interface and echo through trophic levels in flighty ways.For instance, warming in the Cold district has diminished how much ocean ice, impeding endurance rates for walrus and seal little guys that go through piece of their time on earth cycle on the ice. With less seal little guys, ocean otters have turned into the elective food hotspot for whales. Ocean otters feed on ocean imps, and with less ocean otter’s ocean imp populaces are growing and burning-through a greater amount of the kelp that gives favorable places for fish. Less fish fuel the decreases in walrus and seal populaces. By and large, there is less food accessible for the Yupik Eskimos of the Icy who depend on these species.

Worldwide environmental change is consequently a critical expansion to the range of ecological wellbeing dangers looked by mankind. The worldwide scale makes for newness—albeit a large portion of its wellbeing impacts involve increments (or diminishes) in natural impacts of climatic minor departure from human science and wellbeing. Conventional ecological

wellbeing concerns long have been centered on toxicological or microbiological dangers to wellbeing from neighborhood natural openings. Notwithstanding, in the early long periods of the twenty-first century, as the expanding human effect on the climate keeps on adjusting the planet's land, organic and biological frameworks, a scope of bigger scope ecological risks to human wellbeing has arisen. Notwithstanding worldwide environmental change, these include: the wellbeing chances presented by stratospheric ozone consumption; loss of biodiversity; weights on earthbound what's more sea food-delivering frameworks; changes in hydrological frameworks and the provisions of freshwater; and the worldwide scattering of steady natural toxins.

Environmental change and stratospheric ozone exhaustion are the most popular of these different worldwide ecological changes. Human social orders, nonetheless, have had long experience of the changes of environment: climatic cycles have left incredible engravings what's more scars on the historical backdrop of humanity. Civilizations like those of antiquated Egypt, Mesopotamia, the Mayans, and the Vikings in Greenland and European populaces during the four centuries of Little Ice Age, all have both benefited and experienced nature's incredible climatic cycles. Recorded examinations likewise uncover broad fiascos, social interruption and infection flare-ups in light of the more intense, between yearly, semi intermittent ENSO (El Niño Southern Wavering) cycles. The exhaustion of soil fruitfulness and freshwater supplies, and the blunder of water catchment bowls by means of exorbitant deforestation, likewise has added to the decay of different local populaces throughout the long term.

Today, environment researchers foresee that mankind's expanding emanation of ozone harming substances will initiate a drawn out change on the planet's environment. These gases contain, mainly, carbon dioxide (generally from petroleum product burning also timberland consuming), in addition to different other hotness catching gases like methane (from flooded horticulture, creature cultivation and oil extraction), nitrous oxide furthermore different human-made halocarbons. Without a doubt, most environment researchers now think that the amassing of these gases in the lower environment has added to the solid ongoing upswing in world normal temperature. In its Third Appraisal Report, distributed in 2001, the Intergovernmental Board on Environmental Change (IPCC) expressed: "There is new and more grounded proof that the majority of the warming saw throughout the most recent 50 years is inferable from human exercises"

Environment and human wellbeing: an old battle:

Whoever wishes to research medication appropriately, ought to continue accordingly: in the first spot to think about the periods of the year, and what impacts every one of them

produces, for they are not all indistinguishable, but rather vary much from themselves as to their changes.

Acknowledgment that human wellbeing can be impacted by a wide scope of biological disturbances, resulting upon environmental change, is a new turn of events, reflecting the expansiveness and refinement of present day logical information. By and by, the easier thought that human wellbeing and sickness are connected to environment presumably originates before recorded history. The Greek doctor Hippocrates (around 400 BC) related pandemics to occasional climate changes, composing that doctors ought to have "due respect to the seasons of the year, and the illnesses which they produce, and to the conditions of the breeze impossible to miss to every nation and the characteristics of its waters". He admonishes themto observe "the waters which individuals use, regardless of whether they be muddy and delicate, or then again hard and running from raised and rough circumstances, and afterward if saltish and ill suited for cooking," and to notice "the areas of towns, and of the encompassing country, regardless of whether they are low or high, hot or cool, wet or dry and of thediet and routine of the occupants".

Potential wellbeing effects of environmental change:

Worldwide environmental change would influence human wellbeing through pathways of shifting intricacy, scale and unequivocal quality and with various planning. Also, effects would shift topographically as a capacity both of climate and geology and of the weakness of the nearby populace. Effects would be both positive and negative (albeit master logical surveys expect dominatingly negative). This is nothing unexpected since climatic change would disturb or in any case modify an enormous reach of regular natural and actual frameworks that are a necessary piece of Earth's life-emotionally supportive network. By means of environmental change people are adding to a change in the states of life on the planet.

Population vulnerability and adaptive responses:

Human populaces, similarly as with people, fluctuate in their weakness to specific wellbeing results. A populace's weakness is a joint capacity of, first, the degree to which a specific wellbeing result is delicate to environmental change and, second, and the populace's ability to adjust to new climatic conditions. The weakness of a populace relies upon variables, for example, populace thickness, level of financial turn of events, food accessibility, pay level and dissemination, nearby ecological conditions, previous wellbeing status and the quality and accessibility of general medical services.

Transformation alludes to activities taken to diminish the effect of (expected) environmental change. There is a progressive system of control procedures that can assist with securing populace wellbeing. These systems are classified as: regulatory or administrative, designing, individual conduct. Authoritative or administrative move can be made by government,

requiring consistence by all or assigned classes of people. Then again, versatile activity might be empowered on a willful premise, through backing, training or monetary motivating forces. The previous sort of move would ordinarily be made at a supranational, public or local area level; the last option would go from supranational to individual levels. Variation techniques will be either receptive, in light of environment impacts, or expectant, in order to reduce vulnerability.

Conclusions:

Over the ages human social orders have debased or changed nearby environments and altered provincial environments. Unprecedented, the total human effect currently has accomplished a worldwide scale, mirroring the new fast expansion in populace size and energy-serious, high-throughput, mass utilization. The total populace is experiencing new human-incited changes in the lower and center airs and overall consumption of different other regular frameworks (for example soil richness, springs, sea fisheries and biodiversity overall). Regardless of early acknowledgment that such changes would influence monetary exercises, foundation and oversaw biological systems, there has been less mindfulness that such huge scope natural change would debilitate the backings for solid life. Luckily that is presently starting to change. For sure, this volume looks to introduce a far reaching conversation of the connection between worldwide environmental change and human populace wellbeing.

Worldwide environmental change is probably going to change the recurrence of outrageous climate occasions: typhoons might increment as ocean surface waters warm; floods might increment as the hydrological cycle heightens; and heat waves might increment in mid-mainland areas. As talked about exhaustively in later parts, an adjustment of the recurrence and force of hotness waves and cold spells would influence occasional examples of dreariness and mortality. The creation of different air toxins and of allergenic spores and dusts would be impacted by hotter and wetter conditions. Environmental change likewise is relied upon to influence wellbeing by means of different roundabout pathways, including the examples of irresistible illnesses; the yield of food-delivering frameworks ashore and adrift; the accessibility of freshwater; and, by adding to biodiversity misfortune, may undermine and debilitate the biological system administrations whereupon human culture depends.

Variations to the wellbeing risk presented by worldwide environmental change can be both proactive and receptive, and can happen at the full scale, meso and small sizes; that is, at the populace, local area and individual levels. Environmental change addresses an oddball worldwide test so there will be restricted freedom to complete fundamental assessment of transformation choices. There is in this manner a solid case for reasonability, both in moderating

environmental change and in adjusting to its effects. This subject is probably going to turn into a significant topic in populace wellbeing research, social strategy improvement and support during this first decade of the twenty first century. Without a doubt, thought of worldwide climatic-ecological risks to human populace wellbeing will assume a focal part in the supportability progress banter.

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SERICULTURE BASED INTEGRATED FARMING SYSTEM FOR SUSTAINABLE AGRICULTURAL DEVELOPMENT

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Abstract:

Sericulture being an efficient agro-enterprise offers appropriate combination and utilization of wide range of farm industries including, horticulture, livestock, fishery, forestry and poultry etc. Integrated use of these sectors in a judicious manner ensures the means to raise the farmer's productivity, profitability and helps to elevate their economic status as well. Integrated farming system in combination with sericulture interacts adequately with environment without dislocating the ecological balance on one hand and attempts to meet the national goals of increased production on the other. IFS is a strategy to ensure sustainable use of the natural resources for the benefit of present era while ensuring sufficient stock for future generations. In this direction, sericulture industry offers the best suited integrated and efficient disposal of resources specifically for the rural and marginal farmers.

Keywords: Sericulture, agro-enterprise, integrated, socio-economic, sustainable, resources

Introduction:

Indian economy is predominantly constituted by rural and agriculture oriented marginal and small scale farmers constituting approximately 76.2 per cent of farming community of the country (Kumar *et al.*, 2012). In India agriculture remains the prime occupation of marginal and rural farmers and limited availability of resources with confined seasonal climates prevailing in the country have made the farmers to look for supporting secondary sectors such as sericulture (Rai, 2006). Significant use of silkworm in agriculture and industrial sector has promoted the importance of sericulture in all over the world (Ramesh- Babu *et al*., 2009) and it also contributed in strengthening the national economy by generating employment on a large scale (Chanotra *et al.*, 2019). Integrated Farming System or IFS aims at the overall development of agriculture and allied sectors by ensuring efficient, effective and judicious use of available resources following a well planned management strategy for sustainable agricultural production

and enhancing the economic development so as to meet the current desires and preserving the sufficient resources and basic stock material for future generations with main emphasis on maintaining the quality of environment (Yadav *et al*., 2019). As sericulture is a rural based cottage industry involving various labour intensive sectors like moriculture, silkworm rearing and post-cocoon sector or industrial sector, integrated farming system in combination with sericulture can be viewed as in combination with various enterprises in which the end-products and of one sector can be utilized as the raw material for the next sector. For example, the waste of silkworm rearing including excreta, urine contaminated leaves, undersized worms etc. can be used for preparation of high quality FYM. The farm yard manure obtained after recycling the sericultural waste will serve as an excellent biofertilizer especially for vegetables, flower nurseries and other agricultural crops etc. The mulberry twigs obtained from pruning or training or from shoot rearing can be used as fodder for cattles. Sericultural farmers can also adopt floriculture, horticulture and fisheries etc. as part time enterprise as mulberry field allows intercropping with other horticultural and floricultural crops. Moreover, the by-products generated in post cocoon or industrial sector can also be utilized as raw material for poultry and fisheries. For example, the left over pupae after reeling of cocoons can be easily processed to be used as potential dietary material for poultry and fisheries. Thus, different enterprises under sericulture industry shares high degree of correlation with one and other and one can yield great benefit by adopting sericulture as an integrated agro-enterprise.

Criteria for integration of various agri-enterprises (Rana and Rana, 2011):

Integration of different agri-enterprises depends on some basic and flexible criteria which include:

1. Soil health and climatic conditions prevailing in a particular area.
2. Availability of required resources including land and labour to work with.
3. Current status of available resources.
4. Expected economic output of the proposed integrated farming system.
5. Management strategy and skills of the famers involved.

Scope of Integrated Farming System:

Integrated farming system comprises of collective development of moriculture, sericulture, other agricultural crops, dairy or livestock, poultry and horticulture etc. for sustainable development and prosperity of farmers involved in their respective sectors. Considering the fact that integrated implementation of various agri-enterprises in a well planned strategy with optimum efforts yields better results than any of the enterprise alone. Therefore, sericulture is the only enterprise that can be mingled easy with any of the others and offers

tremendous scope and opportunities to the farmers to elevate their socio-economic status by generating additional income.

Sericulture and Horticulture:

In India Horticulture is a blooming industry and holds unique importance among various fields of agriculture as the fruits and vegetables cultivated under horticulture are consumed as basic commodity by all being rich in nutritive values (Kumar *et al.,* 2012). Horticulture offers excellent avenues for generating employment and contributes in national economy by earning foreign exchange. The production can be increased by increasing the area under cultivation and adopting the idea of mixed cropping. Mulberry the host plant of silkworm *Bombyx mori* can be planted as tall trees under mixed cropping system or on side bunds of the vegetable crop fields. Among various horticulture crops, mango, coconut and sapota can be easy integrated with mulberry cultivation (Kerutagi *et al*., 2019). Thus the farmers need not to engage their entire land for mulberry cultivation and can generate additional income by practising sericulture on part time basis. Moreover the concept of byproduct utilization works well in this direction as the waste generated during silkworm rearing including silkworm litter, undersized and dead worms, left over leaves and twigs etc. can be recycled to produce organic manure when mixed with residual wastes of other agricultural crop, which can serve as an excellent biofertilizer to boost the growth and nutritive value of vegetables and fruits thus minimizing the dependency on chemical fertilizers.

Sericulture and floriculture:

Flower cultivation as a supplementary activity helps to improve the economic opportunities for the rural farmers. They can adopt floriculture by starting small nurseries with limited space and time. Sericultural wastes recycled into biofertilizers can be used to promote the growth of flower crops. Moreover, cultivation of flowers as intercrop in mulberry fields can also be adopted for additional benefits (Baishya *et al.,* 2004). Cultivation of flowers like marigold and gladiolus or cut flowers can be easily incorporated in IFS (Kumar *et al.,* 2012).

Sericulture and Dairy:

Through a well planned management strategy various small enterprises like sericulture, moriculture and dairy farming can be integrated which would increases involvement of labour folk and offers opportunities to the farmers to gain additional benefits. In an experiment conducted by Nagaraju and Raghavendra (2016), it has been reported that adoption of integrated farming particularly in combination of crop, dairy, sheep and sericulture generated the most

beneficial results to the farmers in terms of both income and employment generation which was recorded as 322 man days per year.

Sericulture and Poultry:

In India sericulture is viewed as a subsidiary occupation by the rural farmers. Moreover the seasonal fluctuations compel the farmers to view other alternatives. Broiler or poultry industry provides a good source of income to the marginal farmers. Silkworm pupae are considered as an excellent source of protein, fats, essential vitamins and minerals (Longvah *et al*., 2011). Thus, mixture of silkworm larvae and pupae blended in appropriate proportions with other food materials can be served as an excellent feed to the poultry fouls. Thus sericulture in combination with poultry can be viewed as one of the promising technology in augmenting handsome income for the farmers as this combination opens avenues for small scale and commercial agriculture business and hence this type of diversification yields fruitful results (M. Prein, 2002). On the other hand waste generated in poultry can be recycled to produce organic manure that can be applied as biofertilizer to boost the growth of mulberry plants which in long run helps the worms to produce good cocoons thus boosting the farmer's resources and income (Kumar *et al.,* 2012, Aklilu Nigussie, 2018).

Sericulture and vermicomposting:

Vermicomposting is the process of conversion or breakdown of crop residues of agricultural origin into organic manure with the addition of biological agents known as vermicast or earthworms. Various end products are generated at each and every stage of sericulture which can be utilized by vermicomposting and the vermicompost thus produced can be used for cultivation of various agricultural crops. In a study conducted by Kerutagi *et al.,* 2019, it has been reported that the average income of farmers increased by 38.31 per cent by adopting integrated farming system involving vermicomposting as an integral enterprise.

Some additional integrated programmes involving sericulture and other allied agri-enterprises laid emphasis on the following aspects of IFS and sericulture (Baishya *et al.,* 2004):

1. Mushroom production by utilizing seri-wastes as straw and compost.
2. Intercultivation of non-mulberry host plants like soam and salu and promotion of Vanya sericulture.
3. Integration of sericulture and pig industry.
4. Integration of sericulture and fishery.
5. Integration of sericulture and apiculture.

Interactions and linkages:

Various agro-enterprises integrated under IFS can be viewed as an inter-related series or chain of activities as given below:

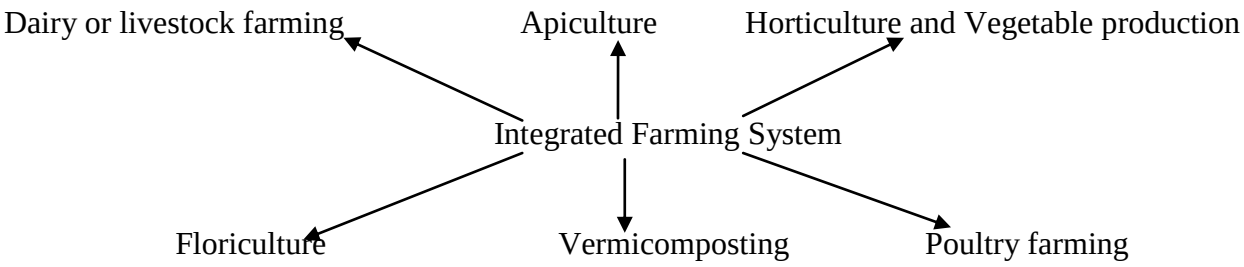

Figure 1: Various agri-enterprises involved under IFS

Advantages of Integrated Farming System:

Integrated farming system offers numerous advantages as it ensures adequate pooling and utilization of available natural resources in an efficient manner so as to provide tremendous opportunities to the farmers to gain more and more profit in terms of productivity and income generation. Some important points under this category are described below:

1. Enhanced production:

Integrated farming system if adopted and executed through a proper management strategy, results in tremendous increase in terms of production per unit area at given interval of time.

2. Increased profitability:

Integrated farming system provides great profit in terms of income generated as the integration of various enterprises ensures minimum input and maximum output returns i.e. output/input ratio remains high in IFS.

3. Sustainability:

As IFS ensures integrated and judicious use of available resources it also ensures the sustainability for future too.

4. Eco-friendly:

Integrated farming system aims at efficient utilization of natural resources while maintaining the ecological balance.

5. By-product utilization:

Integrated farming system ensures zero or minimum wastage of any material. Maximum emphasis is made on utilization of waste products and mostly the inter-related fields works in a chain system in which the waste of one field serves as the raw material for the next.

6. Introduction and adoption of new technologies:

By adoption of IFS, famers can link various agro-enterprises like dairy, poultry, horticulture, floriculture and sericulture etc. which ensures optimum income to adopt latest technologies to improve their production status.

7. Employment generation:

IFS increase the requirement of labour for performing different activities in different sectors thus reduces the problem of unemployment. IFS provide enough scope to engage family labour round the year.

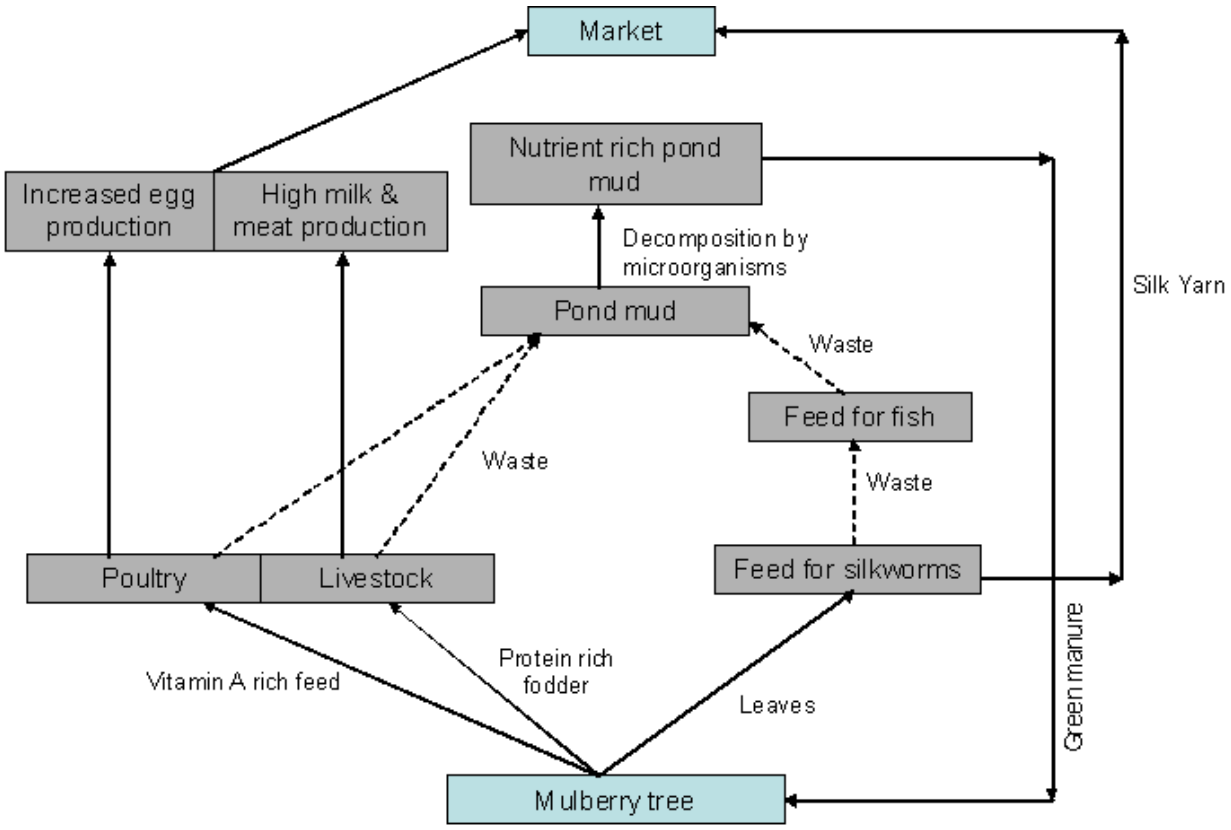

Figure 2: Generalized scheme of integrated Farming system in relation to Sericulture

Constrains in adoption of Integrated Farming System (J. Pushpa, 2010):

1. Lack of well coordinated extension services.
2. Lack of proper demonstration.
3. Lack of composite credit facilities.

4. Lack of awareness among the farmers.
5. Inadequate supply of basic raw material to start any enterprise.
6. Lack of knowledge for proper planting system.
7. Inadequate marketing facilities.
8. Poor health management system in livestock and dairy farming.
9. Lack of irrigation facilities.
10. High mortality rate.

Conclusion:

Integrated Farming in relation to sericulture offers numerous advantages over ordinary system of mulberry cultivation and silkworm rearing and aims to ensure sustainable agriculture with profitable outcomes. It extends numerous benefits to the sericulture practising farmers as they can grasp handsome income via introduction of several part time enterprises including horticulture, floriculture, fisheries and poultry etc. which enables them to elevate their soco-economic status as it has tremendous potential for employment generation in rural areas. Therefore, the concept of integrated farming or mixed farming system should be made familiar to the sericultural farmers for exponential increase in the crop productivity and elevation in the farmer's income resources with sustainable development of the agriculture.

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AN OVERVIEW OF DIFFERENT TYPES OF TOXIC ENVIRONMENTAL CHEMICALS AND THEIR CELLULAR RECEPTORS

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Abstract:

Chemicals that have ended up in the environment as a result of human activity and are harmful to human health are known as environmental pollutants. The recent increase in the presence of these compounds in the environment has raised concerns about human safety. Humans are exposed to these contaminants on a daily basis through polluted air, soil, and food. Dioxins, pesticides, plasticizers, and heavy metals are only a few of the many compounds that make up environmental contaminants. These substances have a negative impact on human health, including endocrine abnormalities, neurological system problems, infertility, cancer, immune system disorders, metabolic diseases, and respiratory disorders. The harmful effects of these chemicals are mediated at the cellular level via their interactions with cellular receptors. In light of this, the current review aims to consolidate information on the cellular interactions of environmental chemicals. Previous research has revealed that these compounds have a wide range of mechanisms of action, which vary depending on the cell model, species, chemical concentration, and exposure period. The majority of environmental hazardous substances, acted through nuclear receptors. A vast number of compounds, in particular, acted through the aryl hydrocarbon receptor. It is generally known that the arylhydrocarbon receptor plays a role in the toxicity of environmental toxins. Overall, this study focused on the critical receptors that are known to mediate chemical toxicity. This effort will help us understand the toxic mechanism of chemicals in a better way.

Keywords: Environmental chemicals, cellular receptors, dioxins, pesticides, heavy metals

Introduction:

Global concern regarding the sources, sinks, distributions, fates, and impacts of environmental toxins has grown in recent decades. Because of these pollutants' pervasive presence in the environment and their negative impacts on the environment, and human health,

understanding of their toxic mechanism is needed. Briefly, environmental pollutants are described any physical, chemical, biological, or radiological compound that negatively impact the air, water, soil quality, or living beings (Steve *et al.,* 2005). In the present review we are mainly concerned about chemical pollutants. It primarily consists of three chemical classes: industrial chemicals, agrochemicals, and heavy metals (National Research Council, 2006; Sexton *et al.,* 2011; CDC). The industrial chemicals contain dioxins, PCBs, and plasticizers whereas agrochemicals mainly consist of pesticides. The animal and human body are regularly exposing to these chemicals either directly from foodstuffs or indirectly by the environment. Though all environmental chemicals are not harmful to human, the body has effective detoxification mechanism through which it transformed the toxic compounds to less toxic form and then excrete them (Murphy, 2001). But, some of them are resistant to metabolism, and due to their stable nature, they persist in the body for a long time, usually called a persistent organic pollutant. Some of the toxic environmental chemicals are dioxins, polychlorinatedbiphenyls (PCBs), phthalates, bisphenols (BPA), organochlorine and organophosphate pesticides and heavy metals such as lead, cadmium and arsenic. These chemicals adversely affect the human health causing endocrine disruption, immune system disorders, cancer, nervous system damage, reproductive problems, respiratory disorders and metabolic diseases (Turusov *et al.,* 2002, Lemaire *et al.,* 2004; Jamal *et al.,* 2013; White and Birnbaum, 2009; Costa, 2008). There are several available studies described the toxic effects of these chemicals. The present review is specifically focused on the cellular mechanism of toxicity of environmentally relevant chemicals. To be more specific, this study will describe the different cellular receptors found to be involved in the toxicity of environmental chemicals. It could help to understand their toxic effects and subsequently help in improving their toxicity profiles.

Types of environmental chemicals:

Environmental chemicals are defined as toxic chemicals that are present in a higher concentration than normal concentration. It may be due to human activity and has a negative impact on the environment and living beings. In includes different classes of chemicals such as dioxins, PCBs, pesticides, plasticizers and heavy metals.

1. Dioxins

Dioxins Among them, dioxins are the family of structurally and chemically related polyhalogenated aromatic hydrocarbons consists of 75 polychlorinated dibenzo-p-dioxin congeners (PCDD) and 135 polychlorinated dibenzofurans (PCDF) congeners (COMMISSION DIRECTIVE 2006/13/EC February 2006). Among these 210 chemicals, 17 chemicals are reported for their human toxic effect. Dioxins have been released into the environment

accidentally in the course of industrial practices such as the production of pesticides and chlorine bleaching in the paper industry. The term dioxin commonly used for the most toxic representative of this class is TCDD or 2, 3, 7, 8-tetrachloro-dibenzo P-dioxin. It is a known cancer-causing agent (NIEHS (National Institute of Environmental Health Sciences) 2001).

2. Polychlorinated biphenyls (PCBs)

Another, toxic group of organic compounds is polychlorinated biphenyls (PCBs). The PCBs are long-lasting chemical compounds that were widely employed in the 1930s and 1940s. PCBs have contaminated different environmental matrices worldwide due to their extensive usage (Borja *et al.,* 2005). It comprises dioxin-like polychlorinated biphenyls (DL-PCBs) and non-dioxin-like polychlorinated biphenyls (NDL-PCBs). These compounds have long-range transport and bioaccumulation efficiency (Jing *et al.,* 2018).

3. Pesticides

Pesticides are being used to control the pests in the crops and to improve the quality of the crops all around the world. The term pesticide comprises herbicide, insecticide, nematicide, bactericide, insect repellent, and fungicide. Pesticide residues that have been reported in milk include chlorinated pesticides, organophosphates, herbicides, fungicides, and insecticides (Aytenfsu *et al.,* 2016). Among pesticides, the most predominant and toxic class is organochlorine pesticides. Organochlorine pesticides (OCPs) are organic compounds known for their toxicity, lipophilic properties, and bioaccumulation potential (Harrison, 2007). Most of the countries of the world prohibited or restricted their usage but still, their microbial degradation resistant, lipophilic and persistent nature allows them to exist in the environment (Erdoğrul *et al.,* 2004; Kaushik and Kaushik, 2007).Organochlorine is a class of pesticide widely includes harmful contaminates, sub-class like dichlorodiphenylethanes, the chlorinated cyclodienes (aldrin, dieldrin, heptachlor, etc.), and the hexachlorocyclohexanes (lindane). These compounds had been consumed in the agricultural field to control pests and insects causing tropical diseases such as malaria, Chagas disease from 1940 to 1990 (Santos *et al.,* 2015). Seeing the presence of these contaminants in the food products their use in agricultural work was restricted. Despite that, some of these insecticides are still used as a controlling agent of tropical diseases in some countries (D Amato *et al.,* 2002; Santos *et al.,* 2015). Persistent organic pollutants list prepared under Stockholm Convention contain 12 highly toxic chemicals of which 9 are organochlorine pesticides (UNEP, 2010). Some of the OCPs are known as endocrine disruptors like dieldrin and heptachlor (Nassar *et al.,* 2016) while some compounds such as dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCH), are recognized as a carcinogenic chemical to humans (The International Agency for Research on Cancer (IARC) 2006). Other than them

organophosphate are also widely used broad-spectrum pesticides. They have a very specific toxic mechanism mediated through inhibition of acetyl cholinesterase enzyme (Kazemi *et al.,* 2012).

4. Plasticizers and bisphenol

A plasticizer is a substance that is applied to another substance (typically a plastic or an elastomer) to make it softer or more malleable. Across 100 distinct plasticizers are produced around the world today, with phthalate ester plasticizers accounting for around 85 percent of them (Godwin, 2017). Another synthetic compound used as plasticizer is bisphenol (BPA). It is the most abundant synthetic compound used worldwide for the production of water and infant feeding bottles and containers for the storage of food products. It has been established that BPA can be liberated from containers and migrate into the food products kept in them (Food Standards Agency. 2001). It is known endocrine disruptor and negatively impact the human health (Rochester, 2013).

5 Heavy metals

Chemically “a metal having a density more than 5 g/cm3" is called heavy metal (Csuros and Csuros, 2002). Trace essential, valuable, radio nuclei, and very poisonous metals are among them. Copper, iron, zinc, selenium, and cobalt are essential heavy metals that play a vital part in a variety of biological processes in humans (Mertz, 1981). The phrase "heavy metal," on the other hand, refers to metals that contaminate food and cause harm even in trace amounts (Duffus, 2002). Such toxic heavy metals include lead, mercury, arsenic, and cadmium thatare highly dispersed in the environment. These metals are used in a variety of industrial processes such as metal plating, textiles, battery manufacturing, metallurgy, chemical manufacturing, automobiles, and petroleum (Arruti, 2010, Sträter *et al.,* 2010).

Route of exposure of environmental chemicals:

Environmental pollutants are widely present in the air, soil, and/or water (Maria and Mary, 2012). Some of these compounds are very stable and have a long half-life and high persistency in nature (Jorgenson, 2001). Previous study has shown that agricultural and horticultural fields’ soil is heavily contaminated with organochlorines and dioxins (Hilber *et al.,* 2008.) These persistent chemicals easily passed from the agricultural fields’ soil to the crops and subsequently to the animals. After metabolization in animals, most of the chemicals are transformed into excretory form and removed from the system. Yet some persistent contaminants are stored in the fatty tissue of the body and move to the circulation and subsequently reappear in the milk and animal products (Martínez *et al.,* 1997). Humans are directly exposed to these chemicals through contaminated air, soil, water and indirectly via contaminated animal products,

fruits, vegetables and foodstuffs (Jayaraj *et al.,* 2016; Wang and Needham, 2007;Wilkowska and Biziuk, 2011).

Mechanism of action at cellular level:

Environmental chemicals have a variety of effects on physiological cell processes, resulting in changes in normal cell function, particularly at the molecular and biochemical level (Dizer *et al.,* 2001). Humans get exposed to these chemicals through different routes and within human these compounds distribute to the whole body mainly through circulation or accumulates in fat-rich tissues. In addition, most of the pollutants can permeate into the cell by passive diffusion due to their lipophilic nature and can interact with cell receptors and subsequently alter the cellular environment.Several studies reported that xenobiotics predominately act through nuclear receptors such as aryl hydrocarbon receptor (AhR), estrogen receptor (ER), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). Nuclear receptors (NRs) are present in the cytosol in the inactive complex form and when lipophilic pollutant/ligand binds with them, NRs get activated and transferred to the nucleus. In the nucleus, these receptors bind with the xenobiotic responsive element in the genes and alter the transcription of downstream genes. In general, NRs regulate the important physiological process of the cell such as growth, development, and metabolism.

1. Arylhydrocrabon receptor

Among all NRs most prominent receptor responsive to the toxic action of environmental chemicals is the aryl hydrocarbon receptor (AhR). It controls the transcription of genes involved in xenobiotic metabolism and cellular proliferation (Lewis *et al.,* 2006). Concerning the affinity of chemicals to AhR, industrial chemicals were screened virtually using bioinformatics tools such as ligand and structure-based methods. Structurally similar small halogenated and polycyclic aromatic hydrocarbon compounds were selected from the AhR modulator database. The obtained result showed that 41 compounds among 429 selected compounds had an affinity to the AhR. Further, it was found that among 41 probable ligands, 4 compounds already confirmed their AhR affinity during *in vitro* assays in humans (Larsson *et al.,* 2018). Likewise, the affinity of non -planar PCBs such as PCBs- 99, 138, 163, 180, and 194 were checked in human liver cells and their stimulatory effect on the PXR and CAR was observed (Salman and Plant, 2012). The activity of lower chlorinated PCB congeners was also checked against various nuclear receptors. Interestingly obtained results showed the dose-dependent effect of tested PCBs, like in nanomolar concentration most of them acts as anti-androgenic compounds while in micromolar concentration acts as estrogenic compound PCBs 8, 28 and 31 inhibit the AhR while

PCB- 4, 8, and 18 were found agonist to CAR and PXR (Pencikova *et al.,* 2018). Similarly, Takeuchi and coworkers screened the 200 pesticides against AhR using DR-EcoScreen cells. Among all tested pesticides, 11 pesticides showed the AhR-agonist activity. It includes acifluorfen-methyl, bifenox, chlorpyrifos, chlorpropham, diurondiethofencarb, linuron, isoxathion, quinalphos, propanil, and prochloraz. Further, their structural similarity analysis showed that three herbicides propanil, diuron, and linuron have a strong affinity to AhR due to their similar chemical structure like AhR natural ligand. This AhR agonistic activity of selected herbicides was validated in the *in vivo* model and results showed the stimulatory effect of these herbicides on AhR markers genes CYP1A1 and CYP1A2 (Takeuchi *et al.,* 2008). In case of heavy metals their mechanism is not clear. However, apparently it is established that lead is acting through AhR. Effect of Industrial chemicals like lead and mercury were investigated at transcriptional level on various nuclear receptors. Amusingly, it was found that these chemicals increase the mRNA of AhR receptor and ARNT protein. Further, AhR responsive genes like CYP1A1 and CYP1B1 were also checked and found significantly upregulated in the test subject (Cavallini *et al.,* 2016). Moreover, two metal responsive elements (MREs) have been identified in the CYP1A1 promoter region, suggesting the involvement of this gene in lead cellular mechanism (Williams, 2000). In another study CYP1A1 expression was analyzed in response to lead and TCDD and finding shows that lead induces CYP1A1 gene expression alone or synergistically with TCDD, by forming AHR/ARNT/XRE complex (Korashy *et al.,* 2005). Overall, AhR is the primary receptor that mediates the toxicity of dioxins and some pesticides.

2. Estrogen receptor

Another important receptor involves in the toxicity of xenobiotics is the estrogen receptor (ER). It exists in two different isoforms of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Among both the isoforms, ERα is predominantly involved in the estrogenic activity of different compounds and works as a ligand-dependent transcription factor (Jordan and O'Malley, 2007). ERα affinity is not limited to only natural ligands but it also binds with estrogenic xenobiotics/endocrine disruptors (Sengupta and Jordan, 2008). This is well supported by a previous study, in which 200 pesticides and their isomers were tested for estrogenic activity against human ER isoforms (hERα and hERβ) along with human androgen receptor (hAR) in Chinese hamster ovary cells. It was found that 47 pesticides had hERα affinity and 33 showed hERβ affinities while 29 pesticides had agonist activity against both the ER isoforms. In the case of hAR, none of the tested pesticides showed hAR mediated activity Kojima *et al.,* 2004. In addition, differential interaction of BSA with ERα and ERβ was checked in Hela cells and found

that BPA has a dose-dependent estrogenic activity to ERα (Hiroi *et al.,* 1999). This is correlated with BPA's endocrine disruptor activity *(*Krishnan *et al.,* 1993).

Table 1: Environmental chemicals and their receptors

Receptors	Xenobiotics	Action	Reference
ERα	Anilofos, chlorpyrifos, bromophos ethyl & methyl, dichlorofenthion, EPN, ethion, fenitrothion, isofenphos, isoxathion, leptophos, phenothoate, prothiofos, quinalphosaldrin, dieldrin, alpha, beta & sulfate endosulfan, endrin, heptachlor epoxide, methoxychlor, methiocarb, cyfluthrin, cypermethrin, fenvalerate, flucythrinate, chloronitrofen, DDT and DDE, Cadmium and arsenic	Agonist	Kojima et al.,2004 Stoica *et al.*, 2000 Morales *et al.*, 1994 Choe *et al.*, 2003
ERβ	Leptophos, prothiofos, quinalphos, tolchlofos methyl, alpha & beta endosulfan, sulfate endosulfan, heptachlor epoxide & heptachlor	Agonist	Kojima et al.,2004
AhR	Dioxins, HCH, aciflurofen methyl, bifenfox, chlorprofos,chlorpropham, diethofencarb, isoxanthion, quinalphos, propanil, diuron, linuron, prochloraz, and lead (Pb)	Agonist	Janoseke*t al.*, 2006 Takeuchi *et al.*, 2008 Doan *et al.*, 2019
	PCB118, PCB 138, PCB 28, HCB, gamma-HCH, dieldrin and chlordane	Antagonist	
PXR	Aldrin, dieldrin, alpha, beta &sulfate endosulfan, endrin, heptachlor epoxide, methoxychlor, PCBs- 4,8 & 18, BPA, Mono-2 ethylhexyl phthalate	Agonist	Kliewer*et* al.,2002 Sui *et al.*, 2012 Hurst and Waxman, 2004
AR	Parathion, cypermethrin, aldrin, endrin, dieldrin, chlordane, endosulfan and organophosphate fenitrothion	Antagonist	Xu *et al.*, 2008 Lemaire *et al.*, 2004 Tamura *et al.*, 2003
CAR	Dieldrin, PCBs-4,8 18, 99, 138, 153 180 & 194	Agonist	Salman *et al.*, 2012, Wang et al 2020
GCR	Arsenic	Antagonist	Kaltreider et al.,2001 Sargis *et al.*, 2018
	BPA, endrin, dicylohexyl phthalate	Agonist	

3. Other nuclear receptors

Based on these studies, it can be established that xenobiotics predominately act through the aryl hydrocarbon receptor and estrogen receptor alpha. However, few xenobiotics also bind with androgen receptor (AR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). The androgen receptor (AR) is a key transcriptional regulator involved in male reproduction and development. The chemicals causing the negative effects to humans might be due to AR binding inhibition and subsequent AR-dependent transcription (Xu *et al.,* 2008). Similarly, the nuclear receptor family of ligand-activated transcription factors includes the pregnane X receptor (PXR), which is an important part of the body's defense against harmful xenobiotics. A wide range of lipophilic xenobiotics, including as prescription medicines, herbs, pesticides, endocrine disruptors, and other environmental pollutants, activate PXR (Kliewer, 2003). Another nuclear receptor CARis activated by a wide range of chemicals and it found to be involved inthe transcription of genes of xenobiotic metabolism pathway (Maglich *et al.*, 2002). One more nuclear receptor which mediate the toxic effects of theses is glucocorticoid receptor (GCR). The table given below summarized the different nuclear receptors, their xenobiotic ligands and their agonist or antagonist action (Table 1).

Conclusion:

Most of the environmental chemicals are act through nuclear receptors at the cellular level. Among all nuclear receptors, arylhydrocarbon receptor and estrogen receptor alpha mediate the toxicity of most the dioxins and pesticides respectively. Some chemicals may act through more than one receptor on the other hand two or more chemicals are act through one receptor. However, a specific mechanism can’t be delineated for a chemical as it may vary depending on cellular model, period and amount of chemical exposure. More research is needed to impart specific knowledge on the toxic mechanism of chemicals.

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HEAT STRESS AND ITS IMPACT ON HORMONAL PHYSIOLOGY OF HOMEOTHERMIC ANIMALS

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Abstract:

Stress is either a short-term or long-term unusual condition of the body produced by physiological, environmental and poor management factors which change the equilibrium between the body and its environment. An environmental stressor such as heat stress is detrimental for animal health and animal agriculture also. Health problems like respiratory infection, poor diet, endocrine disorders, immunosuppression, etc. are the manifestations of chronic heat stress. Several hormones are involved in the heat stress response like cortisol, catecholamines, thyroxine, and gonadotropins. Heat stress affects the neuroendocrine profile via the activation of the Hypothalamo-Pituitary-Adrenal (HPA) axis and the Sympathetic-Adrenal Medullary (SAM) axis. HPA axis is stimulated in heat-stressed animals to release cortisol, causing lipolysis and proteolysis for energy production. Adrenaline secretion is elevated in heat-stressed conditions to increase heart rate and blood pressure to cope up the body with this situation. Glucocorticoids act on CNS and thereby inhibit TSH secretion, and plasma T_3 & T_4 levels get decreased to reduce metabolic rate and body heat generation. These hormonal changes in the body are required for a 'fight-or-flight' response. Heat shock proteins (HSP_s) such as Hsp70 and Hsp90 play a predominant role as a biomarker of heat stress. The phytochemicals like curcumin, lycopene, and antioxidants like Vitamin C are used to treat a heat-stressed victim.

Keywords: Stress, HPA axis, Endocrine disorders, Heat shock proteins, Phytochemicals.

Introduction:

Stress as a general term is used to describe the sum of biological responses or defense mechanisms of the animal body. According to Selye (1976), "stress is the nonspecific response of the body to any demand" and a stressor is "an agent that produces stress at any time". Stress is a situation that tends to disturb the equilibrium between the organism and its environment. Heat stress results from a negative balance between the net amount of energy flowing from the

animal's body to its surroundings and the amount of heat energy generated by the animal. All animals have a particular zone of thermal comfort that varies from species to species, physiological status, the velocity of ambient air, or degree of solar radiation (NRC, 1981). When the environmental temperature exceeds the thermoneutral zone (TN), the animal lives in a warm zone where thermoregulatory reactions are very limited. But when the environmental temperature increases beyond the upper critical leval, that results in heat stress in the animal body (Fig. 1). This imbalance is caused by various factors such as environmental factors like sunlight, thermal irradiation, air movement, humidity, noise, cold, etc., and physiological factors like disease, vaccination, changes in feed, nutritional deficiency, etc. (Lara *et al*., 2013). Another factor also responsible for stress in livestock animals is a poor management system which includes improper ventilation, overcrowding of animals, improper handling, and poor transportation. Further, various agro-climatic zones have variable climates which are also influenced by seasonal variation throughout the year. Hence, the condition of stress is altered differently from place to place. Various environmental stressors such as heat stress are detrimental to animal production and growth rate acceleration in livestock farming (Ghosh *et al*., 2017).

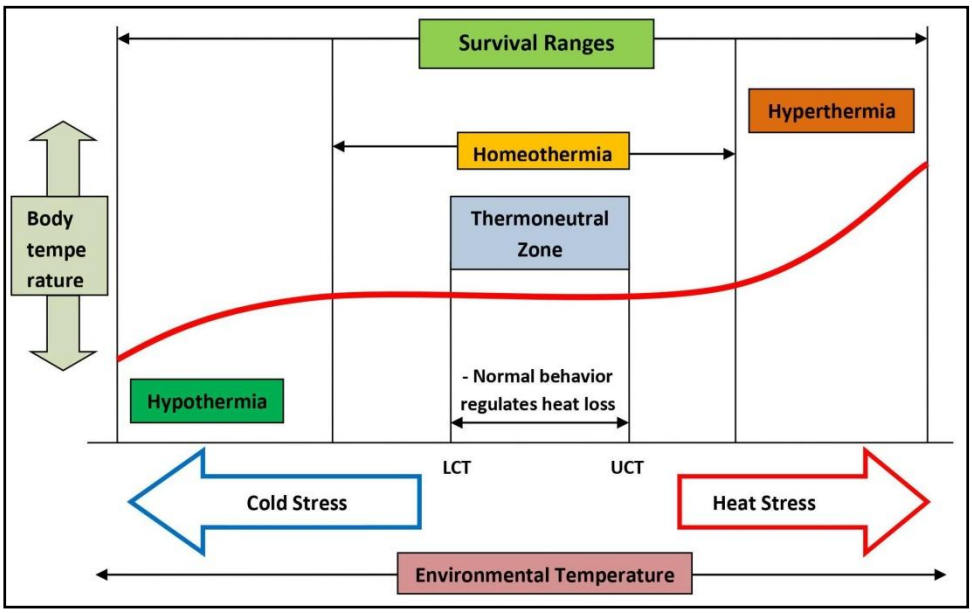

Figure 1: Effects of environmental temperature on body heat regulation (LCT: Lower Critical Temperature, UCT: Upper Critical Temperature)

Several studies have reported that heat stress affects different physiological systems in animals. Birds are mostly affected by heat stress because they have no sweat glands (Renaudeau *et al.*, 2012). When the temperature exceeds the comfort level of birds, they lose the ability to

efficiently dissipate heat to maintain their homeostasis. This leads to physiological changes that are related to alterations in hormonal status (Teetar *et al*., 1985). These physiological alterations can be summarized as an increase in body temperature, depressed immunity, impairment of endocrine function, mitochondrial dysfunction, generation of oxidative stress, increase in transcriptional activity of oxidative genes (Nrf-2 & NF-κβ), etc. Animals experiencing heat stress tend to reduce their stress by producing a special type of protein called Heat Shock Protein (HSP), which generates some antioxidant molecules at the cellular level (Akbarian *et al.*,2016) and activates Nrf-2 downstream signaling pathway (Lee *et al*.,2019). Many studies reported that using of phytochemicals are the potent regulator of transcription factors for generating antioxidant molecule as a stress response (Sahin *et al.*, 2013; Lee *et al.*, 2017).

Impacts of heat stress in the endocrine system:

Responses of stressor play a critical role in the maintenance of homeostasis in animals for their survival in that environment. Various stressors stimulate the endocrine system, and a wide variety of homeostatic conditions of the body are maintained mostly through the Hypothalamo-Pituitary–Adrenal (HPA) axis (Garriga *et al*., 2006; Star *et al*., 2008). Stimulation of the HPA axis and Sympathetic adrenal medullary (SAM) axis through the neuronal circuit enhance the secretion of Glucocorticoids and Catecholamines as long-term and short-term stress responses respectively (Fig 2).

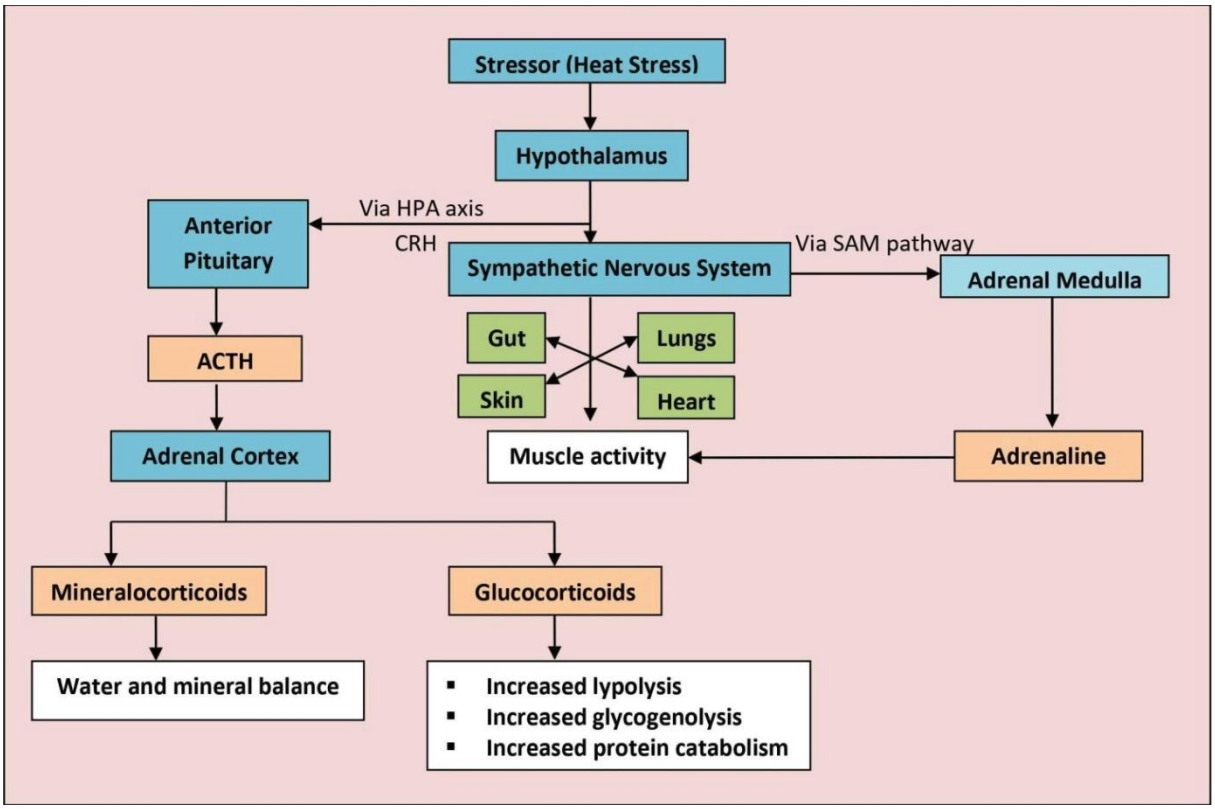

Figure 2: Schematic representation of hormonal regulation of stress response

This neuroendocrine system plays a major role as a stress response of the body including changes in the digestion of food, metabolism rate, heart rate, respiration rate, blood pressure, and immune responses. Glucocorticoids play a critical role in the regulation of the immune system by cytokine production mediated through the HPA axis (Webster *et al*., 2004). Glucocorticoids permit, stimulate or suppress ongoing stress response and organize the brain for a concomitant stressor (Sapolsky *et al*., 2000). Endocrinological changes caused by chronic heat stress in animals like in broiler, stimulate lipid accumulation through increased *de novo* lipogenesis, reduced lipolysis, and enhanced amino acid catabolism (Geraert *et al.,* 1996). Heat stress causes various hormonal changes in homeothermic animals.

Cortisol:

The actions of cortisol are firmly regulated to ascertain that the body can respond rapidly to stressful effects and bring back to a normal state just as quickly. In response to various stressors, corticotropin-releasing factor (CRF) is released from the parvocellular neuron of paraventricular nuclei (PVN) of the hypothalamus into the hypophyseal portal vein to stimulate the corticotroph cells of the anterior pituitary gland which release adrenocorticotropic hormone (ACTH) in the systemic circulation. ACTH binds to the type-2 melanocortin receptor of the adrenal cortex, thereby releasing glucocorticoids mainly cortisol in the circulation (Chida *et al*., 2007). Heat stress-induced animals show an elevated level of glucocorticoids which create different physiological variations such as increased blood glucose level, enhanced proteolysis, and glycolysis for providing more energy as a stress response.

A high amount of glucocorticoids reduce immune response as an initial inhibition of macrophage and T-cell activation. It also suppresses the cellular immunity provided by the thymus gland but lowers glucocorticoids level that may induce antibody production. This biphasic role may depend on the blood corticoids concentration (Hall and Goldstein, 1984).

Mineralocorticoids:

Chronic heat stress increases the secretion of mineralocorticoids via the HPA axis. It causes the retention of water and mineral balance in the body and helps to maintain blood volume and blood pressure (El-Nouty *et al.,* 1980).

Catecholamines:

As a short-term stress response, heat stress activates the sympathetic innervations of the adrenal medulla that constitute another neuroendocrine system. Axons, derived from the central sympathetic neuron innervate chromaffin cells of the adrenal medulla and the synapse release acetylcholine which evokes the secretion of catecholamines (epinephrine & nor-epinephrine) in

the circulation. The molecular mechanism of action of epinephrine is triggered by the G-protein coupled β-adrenergic receptor which ultimately activates the cAMP pathway for performing various functions like increasing heart rate, vasodilation of skeletal muscle and blood vessel, and breaking down of glycogen to glucose for providing more energy to the cell. These physiological changes are required for the body to adapt to unfavorable situations. This is commonly known as "fight-or-flight" action. This response is also known as the first stage of general adaptation syndrome (GAS). When the animal's body cannot maintain homeostasis, they undergo a stress condition. Then GAS helps to return the animal to its normal functioning followed by three consecutive stages namely alarm, resistance, and exhaustion.

Thyroid Hormones:

Normally body temperature and metabolic activity are regulated by thyroid hormones-triiodothyronine (T_3) and tetraiodothyroxine (T_4). Many studies revealed that in heat-stressed animals, T_3 concentration gradually decreases (Elnagar *et al*., 2010 and Mack *et al*., 2013) and T_4 concentration inconsistently decreases, increases, or has no alteration (Bobek *et al*.,1980) via Hypothalamo-pituitary-thyroid axis (HPT). TSH secretion is also inhibited by heat stress to reduce heat generation and BMR.

Gonadotropins:

Heat stress can also alter the Hypothalamo-Pituitary-Gonadal (HPG) axis and directly affects reproductive functions. The reduced eggshell quality and egg-laying capacity are commonly found in female poultry birds that occur by alteration of the normal status of reproductive hormones and disruption of ovarian function (Rozenboim *et al*., 2007). In males, semen volume and its quality get reduced in the heat-stressed animal. Spermatogenesis is sharply hampered in excessive heat-stressed birds and other animals by disruption of testicular functions (Joshi *et al*., 1980). Thermal stress can reduce the concentration of serum LH and lower the granulosa cell activity in laying hen (Novero *et al*., 1991). The male rats exposed to chronic heat stress at the onset of puberty lead to a significant increase in body weight, tail of epididymis, and prostate gland weights. The exposure of chronic heat stress at post-puberty shows a significant decrease in body weight, testis, head and tail of epididymis, prostate and seminal vesicle weights as well as a significant decrease in the level of luteinizing hormone (Ashwaq Hassan, 2019). High-temperature exposure is detrimental to women's reproductive health. High-temperature stress affects the menstrual function of career women, such as soldiers, textile workers, and steelworkers, resulting in a significant increase in the incidence of abnormal menstruation (GaiHong *et al*., 2020).

Leptin:

Leptin secretion is relevant in metabolism for energy homeostasis. During heat-stressed conditions in animals, leptin affects energy metabolism following reducing feed intake by upregulating fatty acid oxidation and downregulating lipogenesis (Allihaud, 2006 and Rabe *et al.*, 2008).

Insulin:

Heat stress alters metabolic parameters and may change glucose metabolism and insulin signaling (Ganesan *et al*., 2018). Prolonged heat stress (>24 hrs) increases basal and circulating insulin, and decreases adipose tissue mobilization in a variety of species (Sanz Fernandez *et al*., 2015). Insulin is a major anabolic hormone that controls energy functions including glucose and lipid metabolism. Insulin stimulates glucose uptake through an intracellular signaling cascade that leads to membrane translocation of Glut4 in skeletal muscle. Heat stress impairs the sarcolemma Glut4 transporter protein abundance which is consistent with reduced circulating insulin and feed intake (Ganesan *et al*., 2018).

Heat stress and cellular homeostasis:

Heat stress is a potent inducer of cellular dysfunction. Most of the cellular energy is produced by oxidative phosphorylation in mitochondria but chronic heat stress leads to mitochondrial dysfunction, downsizing of mitochondrial metabolic oxidative pathway, increase in production of reactive oxygen species (ROS), and alteration in the pattern of antioxidant enzyme activities which ultimately affect the downstream cell signaling pathway of Nrf-2 and NF-Ƙβ (Akbarian *et al*., 2016). Heat stress also causes lipid peroxidation, cytoskeleton assembly alteration, and protein unfolding in the endoplasmic reticulum.

Cells from all organisms respond to a variety of stresses by rapid synthesis of the polypeptide, known as Heat shock proteins (HSPs). HSPs play important role in normal conditions as well as in cellular stress such as Hsp70, Hsp90, etc. Many physiological factors like hyperthermia, oxidative stress, ROS, etc. modulate Hsp70 expression in the cell. Any stressor is thought to activate the heat shock factor (HSF) which remains bound to HSP in a normal condition in an inactive form. These HSFs get separated from HSPs in the cytosol and get activated during phosphorylation by protein kinase and form trimers. These phosphorylated HSF trimer complexes enter the nucleus and bind to the heat shock element (HSE) of the promoter region of Hsp70 gene. A new HSP is synthesized in the cytosol after mRNA transcription from the nucleus. This molecular mechanism revealed that Hsp70 plays a critical

role in the development of thermotolerance and protection from stress-induced cellular damage (Kevin. C. Kregel, 2002). Hence, HSPs are considered as a potent biomarker of cellular injury.

Role of phytochemicals in heat stress:

Phytochemicals are the products that are derived from plants as secondary metabolites in the form of dried plant material, extract, or essential oil. Curcumin is a potent bioactive phytochemical found in turmeric that regulates oxidative stress and anti-inflammatory activity by its antioxidant capacity (Sahin *et al.,* 2012). Curcumin can alleviate the production of SOD and GST to prevent oxidative damage of cells in a heat-stressed animal by Nrf-2 gene activation. It also triggers the production of one of the members of the vitagene family i.e. HSP which is a potent stress-reducing factor (Sahin *et al.,* 2012). Vitamin C is an antioxidant molecule that reacts with reactive oxygen, singlet oxygen, and H_2O_2 with ascorbate peroxidase to neutralize their toxic effects (Forni *et al.,* 2018). Lycopene is the intermediate substance of carotenoids and is commonly found in tomatoes. It acts as a scavenger of ROS, lipid peroxyl molecule, nitric oxide, etc. to prevent cellular damage and inflammatory actions by modulating the gene transcription of NF-Ƙβ and Nrf-2 activation (Petyaev, 2016 and Sahin *et al.,* 2011). Root ginger paste is also an effective dietary supplementation applied for reducing heat stress by its antioxidant and anti-inflammatory properties (Habibi *et al.,* 2014).

Conclusion:

It can be concluded that the hormonal changes help to cope up the animals in heat stress conditions. These changes are required for the body as a “fight-or-flight” response. These hormonal changes maintain the body's homeostatic condition by changing physiological responses such as heart rate, respiration rate, metabolism, etc. This is commonly termed as General Adaptation Syndrome (GAS). Besides, heat stress can directly hamper the cellular mechanism by generating ROS, causing oxidative stress as well as protein unfolding, and modulating different gene transcription activities like Nrf-2 and NF- Ƙβ. Chronic heat stress generates HSPs which act as a biomarker in heat stress to protect cellular toxicity and reduce inflammatory actions. One of the most effective techniques to recover the heat-stressed condition in homeothermic animals is the use of phytochemicals that are present in turmeric powder, tomato powder, lime juice, root ginger paste, etc. Due to their modulating role on anti-inflammatory genes and the presence of antioxidant enzymes, phytochemicals protect from oxidative stress.

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DETERMINATION OF MICROBIAL GUT DENSITY IN FRESH WATER FISHES

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Abstract:

Probiotic bacteria are essential for beneficial effect on particular organism's health and host nutrition for healthy gastrointestinal function. In the present study four economically important food fishes were collected from fish market of Chikkmagaluru town and microbial gut density of *Oreochromis niloticus*, *Pangasius bocourti*, *Catla catla*, *Ictalurus furcatus* were determined. The microbial gut density of fishes varies from place to place as it depends on the feeding habit and other physiological attributes of fish. Total viable count (TVC) obtained in terms of colony forming unit (cfu) from the gut of *Oreochromis niloticus* is 112×10^3 which is ~1.12×10^5 CFU/ml, *Pangasius bocourti* is 105×10^3 which is~$1.05\ 10^5$ CFU/ml, *Catla catla* is 53×10^4 which is ~5.3×10^5 CFU/ml, *Ictalurus furcatus* is 118×10^4 which is ~1.18×10^5 CFU/ml respectively and Gram's staining was carried out for the obtained colonies. Comparatively the microbiome of *catla catla* showed higher colony count (5.3×10^5 CFU/ml). The study has established the basis for future large-scale investigations of the gut microbiota of fishes. In order to make assumptions about the ecological consequences of microbiome composition, a much deep and large-scale investigation of the gut microbiota of fish in this region is also necessary.

Keywords: microbiota, *Catla catla*, Gram staining, fresh water fish, colony count

Introduction:

Fish has been one of the main foods for humans for many centuries and still constitute an important part of the diet in many countries (Leisner *et al.*, 1995). The short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level almost beyond the reach of the low income group (Ezeri *et al.*, 2001). As a result, there is a considerable increase in the demand for fish being the cheapest source of animal protein (Ladipo *et al.*, 1981). These important attributes makes the commodity readily susceptible to microbial attack particularly bacteria (Adams *et al.*, 1999). Probiotic bacteria are essential for beneficial effect on particular organism's health and host nutrition for healthy gastrointestinal function. The

action of intestinal flora results in vital benefits, including protection against pathogens and development of Immune system. Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are populated with bacteria. Being rich in nutrient, the environment of digestive tract of fish confers a favourable culture environment for the microorganisms (2). Animals harbour a complex microbial community, consisting of bacteria, yeast, viruses, archaeans and protozoans, in their gastrointestinal (GI) tract. These microbes influence various host functions including development, digestion, nutrition, disease resistance and immunity. One important aim of GI microbiota studies therefore is to give a scientific basis for developing effective strategies for manipulating GI microbial communities to promote the host health and improve productivity. The diversity generally increases as the diet of the fish changes from carnivorous to omnivorous to herbivorous.

The composition also differs due to different environmental conditions.
Acinetobacter, Aeromonas, Flavobacterium, Lactococcus, and *Pseudomonas.*

Obligate anaerobes *Bacteroides, Clostridium, and Fusobacterium,* and members of family *Enterobacteriaceae* dominate the gut of freshwater species. The guts of marine fish are dominatedby

Aeromonas, Alcaligenes, Alteromonas, Carnobacterium, Flavobacterium, Micrococcus, Moraxella, Pseudomonas and *Vibrio*.

The world's water bodies are teeming with microorganisms. It is estimated that 3.6×10^{30} microbial cells account for more than 90% of the total fresh water biomass. Fish harbors heavy loads of bacteria on their gut, skin and adhering slime. The bacterial flora of fish show considerable variation with the environmental conditions in which the fish live. Normally the pH of fish gut ranges from 7-8. The bacterial commodity in fish gut is very dense compared to surrounding water, which suggests that gastrointestinal tract (GIT) provides a favourable ecological niche for survival of microbes. Most of the bacterial species in the gut of fish are non-culturable and thus several isolation techniques introduced to explore the microbial communities in gut of fish. The feeding habit of fish strongly influences the gut microbiota. In fish, the structure, composition and ecological function of gut microbiota are greatly influenced by the factors from host such as genetics, weight, gender, immunity, This study reviews the current knowledge on the microbiota in some selected fishes, emphasizing the compilation of results reported regarding the most frequently observed bacterial genera. Also the influence of environmental and host factors on the establishment of the bacterial populations that become a part of gut of the fish and the importance of these communities on host health, development and

nutrition. An attempt is made in this study to investigate the bacterial micro flora associated with selected fresh water fishes of Chikkmagaluru town.

Materials and Methods:

Sample Collection

Four economically important food fishes were collected from fish market of Chikkmagaluru town, Karnataka, India. The samples were collected freshly and brought to the laboratory for further bacteriological analysis. The following fish species were selected for the study due to their availability in the local fish market.

1) *Oreochromis niloticus*
2) *Pangasius bocourti*
3) *Catla catla*
4) *Ictalurus furcatus*

Isolation of gut microbes

Collected fishes were washed with distilled water to remove the unwanted particles. Then the fishes were dissected to remove the digestive tract. The digestive tracts were homogenized by centrifugation. Homogenization is carried out in order to obtain uniform distribution of cells within the solution. After centrifugation the supernatant was taken and serially diluted in distilled water in the test tubes to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. The dilution is pour plated on nutrient agar (Nutrient agar media, 5g of Nacl, 5g of peptone, 1g of yeast extract/beef extract, 15g of agar per 1000ml of distilled water) and incubated for 24hour at 37^0C.After incubation the colonies that developed on the petri plates were counted. Those counts within 30-300 colony forming units (cfu) were reported as total viable count (TVC).

Comparing the microbial density among the selected fishes

From the colonies obtained, the total viable count of the bacterial isolates from the gut of selected fish such as *Oreochromis niloticus, Pangasius bocourti, Catla catla* and *Ictalurus furcatus* is calculated by using the following formula.

Microbial load, CFU/ml =Number of colonies × reciprocal of the dilution factor.

From this formula the total viable count of the gut microbial load in terms of cfu can be calculated. After having the total count for calculation the obtained colonies were subjected to Gram staining procedure for the identification of the presence of gram positive and gram negative bacteria.

Result and Discussion:

In the present study microbial load in the gut region of the fresh water fishes like *Oreochromis niloticus, Pangasius bocourti, Catla catla, Ictalurus furcatus* were determined.

The Nile tilapia (locally called as jalebi fish), is a teleost fresh water fish of class Actinopterygii, family Cichlidae, widely known for its importance in aquaculture. These are omnivorous, they feed on phytoplankton, and macrophytes, insects, detritus and zooplankton were the most important food items.

After incubating the petri plates for 24 hours at 37^{o}c, the colonies obtained were taken for further enumeration of the bacterial density by using the formula to find the total viable count of bacterial in terms of colony forming unit.

The gut bacterial count obtained from the *Oreochromis niloticus* fish by serial dilution are as follows,

330 number of colonies from 10^{-2} dilution.

122 number of colonies from 10^{-3} dilution.

14 number of colonies from 10^{-4} dilution.

According to the above mentioned formula the colony count should range from 30 to 300 so the count within this form the colony forming unit. Therefore 122 from 10^{-3} dilution is taken for consideration, which is; ~ 1.22×10^{5} CFU/ml.

Pangasius bocourti

Pangasius bocourti which belong to class Actinopterygii and family pangasiidae is locally known as Basa fish. They are fresh water residents, benthopelagic, and potadromus. They are the bottom feeders. They feed on oligochaete, shrimps, crabs, molluscs, fishes and plants.

After incubating the petri plates for 24 hours at 37^{o}c, the colonies obtained were taken for further enumeration of the bacterial density by using the formula to find the total viable count of bacterial in terms of colony forming unit.

The gut bacterial count obtained from Pangasius bocourti fish by serial dilution are as follows,

313 number of colonies from 10^{-2} dilutions.

105 number of colonies from 10^{-3} dilutions.

28 number of colonies from 10^{-4} dilutions.

According to the above mentioned formula the colony count should range from 30 to 300. So the colony counts within this form the colony forming unit. Therefore in the obtained colony counts, the count obtained from the 10^{-3} dilution will be considered as its ranges above 30

and below 300 colonies. From this 105 numbers of colonies from 10^{-3} dilution make a colony forming unit and is taken for consideration, which is applied to the formula to get the final result, that is; ~ 1.05×10^{5} CFU/ml.

Catla catla

Catla catla also known as the major south Asian carp, is an economically important south Asian freshwater fish in the carp family Cyprinidae. Catla is a surface and mid water feeder. Adults feed on zooplankton using large gill rakers, but young ones on both zooplankton and phytoplankton. Catla is comparatively large when compared to other fresh water fishes and they are Voracious feeders feed on many phytoplankton as well as some zooplanktons. Due to this property of catla there are the chances of presence of microbial flora in its gut in large quantity.
After incubating the petri plates for 24 hours at 37^{o}c, the colonies obtained were taken for further enumeration of the bacterial density by using the formula to find the total viable count of bacterial in terms of colony forming unit.

The gut bacterial count obtained from *Catla catla* fish by serial dilution are as follows,
221 number of colonies from 10^{-2} dilution.
146 number of colonies from 10^{-3} dilution.
53 number of colonies from 10^{-4} dilution

According to the above mentioned formula the colony count should range from 30 to 300 so the colony count within this form the colony forming unit. Therefore in the obtainedcolony counts, the count obtained from the 10^{-4} dilution will be considered as its ranges above 30 colonies and below 300 colonies. From this 53 numbers of colonies from 10^{-4} dilution make a colony forming unit and is taken for consideration, which is applied to the formula to get the final result, that is given as; ~ 5.3×10^{5} CFU/ml.

Ictalurus furcatus

Ictalurus furcatus commonly called as blue cat fish is the largest species of North America. catfish and is spread worldwide reaching length 165cm.Blue cat fish are opportunistic predators and eat any species of fish they can catch, along with freshwater mussels, frogs and other readily available aquatic food sources.

After incubating the petri plates for 24 hours at 37^{o}c, the colonies obtained were taken for further enumeration of the bacterial density by using the formula to find the total viable count of bacterial in terms of colony forming unit.

The gut bacterial count obtained from *Ictalurus furcatus* fish by serial dilution are as follows,
441 number of colonies from 10^{-2}dilution.

346 number of colonies from 10^{-3}dilution.

118 number of colonies from 10^{-4} dilution.

According to the above mentioned formula the colony count should range from 30 to 300 so the colony count within this form the colony forming unit. Therefore in the obtained colony counts, the count obtained from the 10^{-4} dilution will be considered as its ranges above 30 colonies and below 300 colonies. From this 118 numbers of colonies from 10^{-4} dilution make a colony forming unit and is taken for consideration, which is applied to the formula to get the final result, that is given as; ~ 1.18×10^5 CFU/ml.

Comparing the microbial density among the selected fishes.

The microbial gut density of fishes varies from place to place as it depends on the feeding habit and other physiological attributes of fish. From the colonies obtained, total viable count (TVC) obtained in terms of colony forming unit (cfu) from the gut of *Oreochromis niloticus* is 122×10^3 which is ~1.22×10^5 CFU/ml, *Pangasius bocourti* is 105×10^3 which is~$1.05\ 10^5$ CFU/ml, *Catla catla* is 53×10^4 which is ~5.3×10^5 CFU/ml, *Ictalurusfurcatus* is 118×10^4 which is ~1.18×10^5 CFU/ml (Table.2)

Table 1: Comparison of gut microbial density of selected fishes

Fish Species	**Colony Count In Each Dilution**			**Total Viable Count (Cfu)**
	10^{-2}	10^{-3}	10^{-4}	
Oreochromis niloticus	330	122	14	1.22×10^5
Pangasius bocourti	313	105	28	1.05×10^5
Catla catla	221	146	53	5.3×10^5
Ictalurus furcatus	441	346	118	1.18×10^5

The bacterial density obtained from all the selected fishes were recorded in the Table 1. By plotting the graph of selected fishes verses its gut microbial density in terms of cfu, variation in bacterial total viable count of selected fishes namely Oreochromis niloticus, Pangasius bocourti, Catla catla and Ictalurus furcatus can be concluded and is shown in fig.1. By plotting the graph for the obtained data, the comparative analysis of gut microbial density of selected fishes is made. Comparatively the microbiome of *catla catla* showed higher colony count (5.3×10^5 CFU/ml).

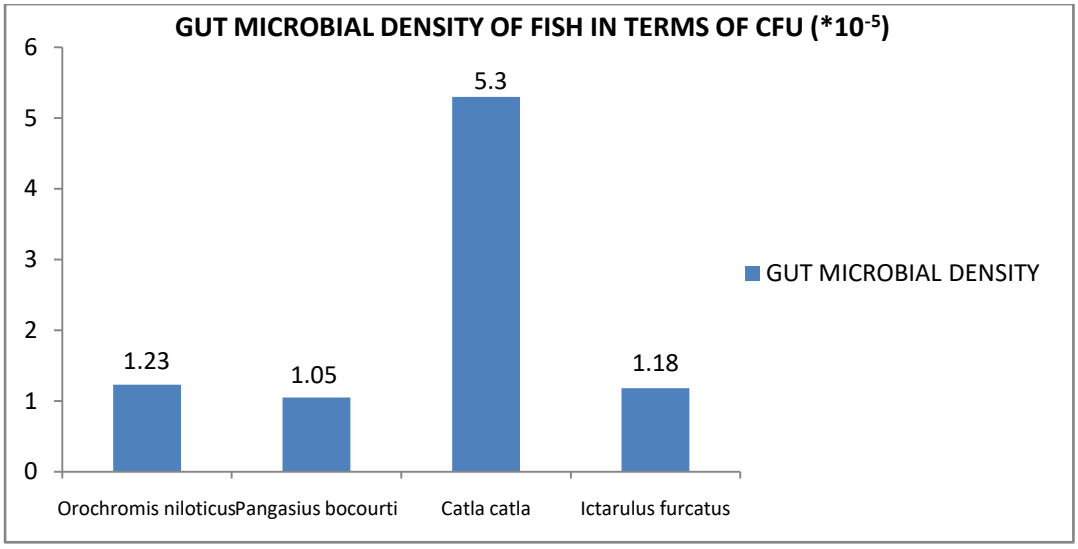

Figure 1: Graph plotted by comparing the gut microbial density of selected fishes X-axis depicts the fish species, Y-axis depicts the gut microbial density in terms of cfu.

Differentiation of gram positive and gram negative bacteria present in the obtained colonies**.** The colonies were observed with different morphology and those which showed varying outcomes in gram's staining technique. The below table depicts the result of gram's staining obtained for the selected fish species.(Table.2)

Table 2: Gram's staining carried out for the obtained colonies

Fish Species	**Possibility of Bacteria**	
	Gram Positive	**Gram Negative**
Oreochromis niloticus	Present	Present
Pangasius bocourti	Present	Absent
Catla catla	Absent	Present
Ictalurus furcatus	Present	Absent

The colonies which appear large and purple in color (Fig 2), when subjected to grams staining showed positive to the staining which illustrates the presence of gram positive bacteria. Some colonies appear translucent with discrete colonies, when subjected to gram staining showed pinkish colonies (Fig 3) which depicts the presence of gram negative bacteria.

By subjecting the obtained colonies to gram's staining technique we majorly interpret the difference between gram positive and gram negative type of bacteria. In *Oreochromis niloticus* we observed both gram positive and gram negative bacteria; in case of *Pangasius bocourti* we observed gram positive bacteria and *Catla catla* showed the presence of gram negative bacteria

and *Ictalurus furcatus* showed the presence of gram positive bacteria in its gut. The gram positive and negative bacteria have both developmental and declinable effects on the host metabolism. The negative effect involves the occurrence of necrosis of tissues of the host body not only so much it also obstruct the vessels, capillaries of the host thus inhibit the supply of sufficient blood to the host body and in turn deplete the oxygen supply to the body, thus the host will not be able to have a proper developmental aids and it leads to the death of the individual.

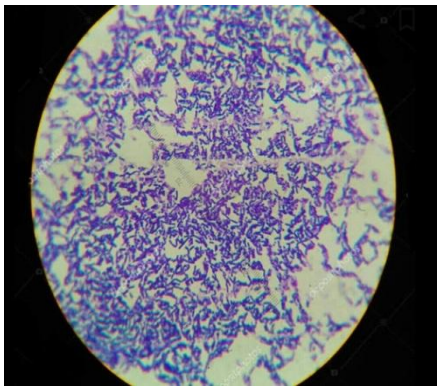

Figure 2: Gram positive bacteria

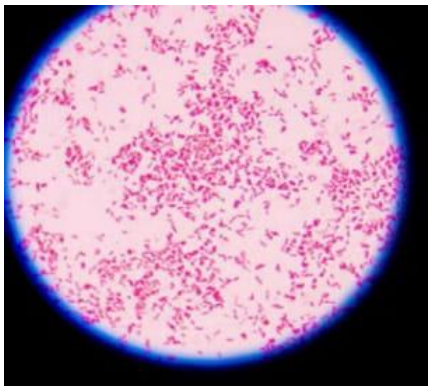

Figure 3: Gram negative bacteria

Conclusion:

The gut microbiota has a fundamental influence on host fitness by contributing to host metabolic capabilities, immunity levels and development. The gut microbial community is in turn influenced by several factors such as genetics, environment, food intake, geographical location and other individual differences of the host. This explores the metabolic potential of the gut micro flora and uncovers functional variation with diet or host associated factors.

This study provides the microbial density in gut of four economically important fishes (*Oreochromis niloticus, Pangasius bocourti, Catla catla and Ictalurus furcatus*) of Chikmagalur town available throughout the year. Comparatively the microbiome of catla showed higher colony count (5.3×10^5 CFU/ml), this depicts that the microbiome within gut of catla is more compared to other selected fishes. *Catla catla* is comparatively large when compared to other fresh water fishes and they are voracious feeders feed on many phytoplankton as well as some zooplanktons. Due to this property of catla there are chances of presence of microbial flora in its gut in large quantity.

The study has established the basis for future large-scale investigations of the gut microbiota of fishes. In order to make assumptions about the ecological consequences of

microbiome composition, a much deep and large-scale investigation of the gut microbiota of fish in this region is also necessary.

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STUDIES ON BREEDING CHARACTERISTICS OF NATURALLY AND INDUCED BRED DOTTYBACK FISH, *PSEUDOCHROMIS DILECTUS* (LUBBOCK, 1976)

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Abstract:

Present study documents the baseline data on the induced captive bred dottyback fish, *Pseudochromis dilectus* vis-a-vis natural bred one. High fecundity rate (1000 - 1200 eggs) was found in natural breeders when compared to the induced fishes (700 - 900 eggs). There was carce difference found in parental care, fanning frequency and embryonic development in natural and induced bred fishes. However, the successful hatching was occurred in natural spawning (90-95%) which was higher than the induced spawned (70 - 75%) fishes. Larvae Size and growth was high in natural bred compared with induced bred.

Keywords: *P. dilectus*; Natural spawning; Induced spawning; Fecundity

Introduction:

Marine ornamental fishes are one of the most popular attractions in worldwide due to their adaptability to live in confinement. Over the past decades, marine aquarium industry has undergone a significant transformation and this industry growing day by day due to their high commercial value (Ajith Kumar *et al.*, 2012). Marine aquarium trades are providing employment opportunity to coastal people and also serve as a source to earned foreign currencies to many developing countries (Nanthini *et al.* 2010). India is endowed with wide variety of marine ornamental fishes, while our contribution was only 2.5% (US$ 3.8 million) to the total Asian ornamental fish exports (Dey, 2010). Successful breeding of marine ornamental fishes is a rare phenomenon compared to fresh water ornamental fishes and only a few marine species have been raised on commercial scale all over the world. The ability to meet demands for marine

ornamentals, utilizing wild-caught fish is decreasing due to stringent regulations on collections that deplete wild stocks and cause damage to fragile coral reef ecosystems (Dhaneesh *et al.*, 2012). The long-term sustainable trade of marine ornamentals can be achieved only through development of culture technologies. In this circumstance, possible alternative is captive propagation of target species which ultimately resulting in decreased dependence on wild caught specimens. Hence, it would help to safeguard the coral reef, develop a new source of organisms for aquarium trade and the way to marine biodiversity conservation and also extend this technology to coastal community for their livelihood development (Setu *et al.* 2010).

Hatchery production technology of dottyback fishes were lack. The sunrise dottyback, *P. flavivertex* was bred by Olivotto *et al.* (2006) and Sayadi *et al.* (2012) studied the effect of temperature and juvenile density for the development of neon dottyback, *P. aldabraensis*. Recently, Sweet (2013) reported the hatchery production technology of sixteen species of dottybacks. Broodstock maturation and reproduction in captivity is a challenging task in aquaculture. Many works were deals with induced breeding of fishes using hormones (Leong, 1977). Hormone is used for stimulate the fish to spawn either naturally or artificially and various combinations of hormones can be used to induce ovulation of eligible female and male fishes. Few hormones are mostly used in aquaculture industry are human chorionic gonadotropin (HCG), follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), estrogen, testosterone and fish pituitary glands (Dave and Sukumaran, 1984). Peter (1980) found that HCG and FSH have induced ovulation, when used alone, while Yaron (1995) recommended HCG as the preferred hormone for *Morone* culture.

According to Lam (1982), effective dosage of HCG may vary from species to species. This hormone is currently preferred by most of the aqua-culturists, because it is effective, economically feasible and readily available in the markets (Marte *et al.*, 1987). Marine fishes are concern, some species will spawn after simple environmental changes and others required more advance methods, including administration of hormone products (Cheah and Lee, 2000). In some tropical freshwater species, sexual maturation takes longer year. In *Morulius chrysophekadion* takes 2 to 3 years (Unsrisong *et al.*, 1990), *Osphronemus gouramy* (Purakkiet *et al.*, 1990), *Probarbus jullieni* (Rodrarung and Janesirisak, 1990) and *Puntius gonionotus* takes more than 10 years (Sukumasavin and Leelapatra, 1993).

In India, studies on hatchery production technology of dottyback fishes are lacking. To evaluate these strategies, the present work has been planned to develop breeding technology of red head dottyback, *P. dilectus* and also an attempt was made with induced breeding, using Human Chorionic Gonadotropin (HCG) hormone. Furthermore, reproductive behavior, fecundity

rate, fanning frequency, parental care and embryonic development during natural and induced breeding have been documented. The ultimate goal of this study is to extend the perfected captive production technology of *P. dilectus* to the aqua farmers for their livelihood development.

Materials and Methods:

Collection and Broodstock development of *P. dilectus*

The fishes were procured from the traders of Mandapam and Tuticorin coastal areas, Southeast coast of India. They were packed in oxygenated polythene bags, one fish per pocket containing 500 ml of seawater and they were transported to marine ornamental fish hatchery located at CAS in Marine Biology (Annamalai University). Latter they were accommodated in cement tank (5000 L capacity) with contains 3000 L estuarine water. The tank was installed with biological filter, provided with dead coral pieces, PVC pipes and live rocks (Ajith Kumar *et al.*, 2012). The fishes were fed twice a day with boiled meat of oyster, shrimp, green mussel, clams and *Acetes* at the rate of 5% of their body weight. Uneaten food particles and excreta were removed by siphoning after 30 mins. of feeding. Approximately 50% of water was exchanged at three days intervals.

Breeding techniques

The protocol proposed by Olivotto etal. (2006) was adopted for natural spawning. The pair formation was observed after eight months of rearing. Before spawning, their reproductive behaviors were documented at regular intervals. After the continuous rearing over a year, natural spawning was observed. The induced breeding was carried out using human chorionic gonadotropin hormone (Rottmann *et al.,* 1991). The HCG hormone was purchased from local medical store. Before injecting the hormone, the length and weight of the male and female fishes were noted. 300 IU volume of hormone was injected (intramuscular) to male and female fishes separately using insulin syringe needle. After hormonal injection, the behavioural characters of male and female fishes were observed and documented at regular intervals.

Spawning, fecundity rate and parental care

The spawning intervals, fecundity rate and parental care were studied (Dhaneesh, 2009). The fecundity rate was calculated using the formula (%) = Number of eggs in particular area×Total number of egg clutch area×100. Parental care was mostly found to done by male than female during this time male fish didn't consume food properly. The fanning frequency of male and female fishes was determined at different time intervals. The embryonic developmental study was carried out as described by Olivotto *et al.* (2006). The size of egg clutch and

individual egg was measured using micrometer. The hatching duration was found to be varied between natural and induced spawners. The larvae were transferred to the larval rearing tank (250 L capacity) containing 200 L of filtered estuarine water. The larvae were fed with microalgae enriched rotifer and wild plankton from second day onwards. The length and mouth size of the larvae were measured.

Data analysis

The present study results were analysed using Pearson correlation coefficient statistical analysis using Origin Software. A probability of 0.05 was used for find out the statistical difference between the means. The means±S.D. of the data result has been expressed in this study.

Results:

Total 40 numbers of adult fishes were procured and conditioned in quarantine tanks. The standardized water quality parameters were followed (Salinity 26 - 28 ppt, Temperature 28 - 30ºC, pH 7.8 - 8.2, Dissolved oxygen 4.5 - 5.5 mg/l, Ammonia 0 - 0.01 ppm and photo period (12 hrs light: 12 hrs dark). Initially, the average length (3.8 - 4.2 cm) and weight (2.2 - 3 g) of the fishes were measured. After three months rearing differentiation (Length 4.5 - 5.2 cm; Weight 3.2 - 3.8 g) was observed in male and female fishes and they were kept in ten oval tanks. Among the ten pairs maintained, three fishes were dead due to bacterial infections and their partners were used for reproductive biology studies. Then the existing seven pairs were reared by feeding with polychaetes, boiled prawn, clam, oyster and green mussel. Followed by pair formation was occurred in three pairs and each pair (Length 5.5 - 5.8 cm; weight 3.8 - 4.4 g) was separated and kept in 750 L capacity spawning tanks. The fishes exhibited reproductive behavior like pairing after ten months rearing and the length and weight of the fishes were found to vary from 6.2 - 7cm and 4.5 - 5.6g. Spawning always occurred between 11 am to 1 pm.

The average length (cm) and weight (gm) of the fishes were; 6.3 - 7.2 and 4.8 - 5.6 respectively at time of HCG hormone injection. On the 4^{th} day, more reproductive behaviour was exhibited and spawning occurred between 12 pm to 1 pm. In natural spawning (NS), the spherical egg ball was around 2 cm diameter and total number of eggs in a clutch was found to vary approximately 1000 - 1200 nos. In the case of induced spawning (IS), the spherical egg ball was around 1.5 cm diameter and total number of eggs in a clutch was found to vary between approximately 700 - 900 nos. The spawning frequency was noticed between ten to fifteen days intervals in NS.

Table 1: Captive breeding technology of *Pseudochromis* species

***Pseudochromis* species**	**Spawning rate**	**Incubation period and Fecundity rate**	**Larvae Size**	**Country**	**References**
Pseudochromis flavivertex	95%	4 days and 1000 - 1200 nos.	4.1 - 4.2 mm with mouth gape 140 - 150 µm	Italy	Olivotto *et al.* (2006)
Pseudochromis flavivertex	90 - 95%	5 to 6 days and 1000 - 1200 nos.	3 - 3.5 mm with mouth gape 120 to 130 µm	Brazil	Mies *et al.* (2014)
Pseudochromis aldabraensis	80 - 85%	4 to 5 days and 900 - 1000 nos.			
Pseudochromis springeri	80 - 85%	4 to 5 days and 800 - 1000 nos.			
Pseudochromis dilectus	91 - 95%	4 days and 400 - 500 eggs	5.1 - 5.3 mm with mouth gape 150 to 160 µm	India	Madhu *et al.* (2016)
Pseudochromis fridmani	80 - 85%	4 to 5 days and 1500 - 2000 eggs	2.43±0.31 mm with mouth gape 100 to 120 µm	Portugal	Araujo *et al.* (2016)
Pseudochromis dilectus	Natural Spawning (NS): 90 - 95% Induced Spawning (IS): 70 - 75%	**NS**: 4 days and 1000 - 1200 nos. **IS**: 4 to 6 days and 700 - 900 nos.	3.5 - 3.8 mm with mouth gape 120 to 130µm	India	**Present Study***

No spawning intervals observed in induced breeding pairs, as continuous hormone injection was made at a week interval to obtain frequent spawning. During incubation, the male fan the eggs continuously, but female done it occasionally and it showed less parental care. The maximum and minimum fanning in a day made by the male was 1.62 and 0.37 minutes and in case of female it was 0.88 and 0.06 minutes. The hatching of larvae normally occurred on 4th day of incubation. The larvae hatching rate has been noticed high in natural spawning (90 - 95%), when compared to induced spawning (70 - 75%).

The newly hatched larvae were measured to be 3.5 - 3.8 mm with the mouth size of 120 to 130µm, were slender and pigmentation was very light. The eyes, mouth, gut and fin fold were developed. The larvae were fed with algal enriched rotifer (*Brachionus plicatilis*) and wild plankton (Copepod, Sagitta, Mysis, Zoea etc) at a density of 15 - 20 nos/ml. The sixth day larvae were measured about 5.2 - 5.4 mm and the mouth size was 130 - 140µm. On 11th day, the larvae were measured at 6.5 - 6.8mm and mouth size was 150 - 160µm. Juvenile stage (Length 2.8 - 3.2 cm; weight 0.8 - 1.5 g) was achieved after reared of three months. The present study result was compared with earlier reports (Table 1).

The present study has provided baseline data for the hatchery production of *P. dilectus*, particularly on broodstock development, spawning and larval rearing. In this study, totally 40 numbers of adult fishes were procured and conditioned in quarantine tanks (Fig. 1). Initially, the size of the fishes was ranged between 3.8 to 4.2 cm and weight 2.2 to 3 g. The water quality parameters were standardized and followed (Salinity 26 - 28 ppt, Temperature 28 - 30ºC, pH 7.8 - 8.2, Dissolved oxygen 4.5 - 5.5 mg/lit, Ammonia 0 - 0.01 ppm and photo period (12 hrs light: 12 hrs dark). After rearing three months, differentiation was observed in male and female and they were kept in ten oval tanks separately and during that time, the fishes attained the size ranged between 4.5 - 5.2 cm and weight 3.2 - 3.8 g. Among the ten pairs maintained, three fishes were dead due to bacterial infections and their partners were used for reproductive biology studies. The existing seven pairs were reared and these fishes were fed with polychaetes, boiled prawn, clam, oyster and green mussel. Followed, pair formation was occurred in three pairs and each pair was separated and kept in 750 L capacity spawning tanks (Fig. 2). The length and weight of the fishes ranged from 5.5 - 5.8 cm and 3.8 - 4.4 g at the time of first spawning.

Figure 1: Conditioning of *P. dilectus*

Figure 2: Experimental set-up for pair formation and spawning

The fecundity rate, spawning frequency, parental care and fanning frequency were recorded. In natural spawning (NS), the spherical egg ball was around 2 cm diameter and total number of eggs in a clutch may vary between approximately 1000 - 1200 nos. In the case of induced spawning (IS), the spherical egg ball was around 1.5 cm diameter and total number of eggs in a clutch may vary between approximately 700 - 900 nos only. The spawning frequency was noticed between ten to fifteen days intervals. No spawning intervals in induced breeding pairs, as continuous hormone injection was made at a week intervals to obtain frequent spawning. There were no changes in parental care and fanning frequency in NS and IS fishes. Mostly the male fish guards and protects the eggs until they hatch. During this period, they were exhibited two processes, fanning and mouthing. It was also noticed, during this time male was consumed small amount of food only. During incubation, the male fan the eggs continuously, but female done it occasionally and it showed less parental care. The maximum and minimum fanning in a day made by the male at one time was 1.62 and 0.37 minutes respectively and in female, it was 0.88 and 0.06 minutes respectively.

In the present study, the newly spawned eggs were milky white in color. As the embryo develops, eggs color turns to black on 2^{nd} to 3^{rd} day and later turns to silver color on 4^{th} day, which is the indication of hatching. At this stage, the glowing eyes of the developing larvae inside the egg capsule were clearly visible, when viewed from a short distance. The hatching of larvae normally occurred on the night of 4^{th} day of incubation. The successful hatching of larvae was occurred in natural spawning was more (90 - 95%), when compared with induced spawning (70 - 75%). The fishes not showed any parental care with the fry after they hatched. Newly hatched larvae were very active and swam near the water surface and the yolk sac was almost completely reabsorbed. First food was offered the day after hatching.

The newly hatched larvae were measured 3.5 - 3.8 mm and mouth size 100 to 120µm, were slender and pigmentation was very light. The eyes, mouth, gut and fin fold were developed.

The larvae were fed with algal enriched rotifer (*Brachionus plicatilis*) and wild plankton (Copepod, Sagitta, Mysis, Zoea etc) at a density of 15 - 20 nos/ml. The sixth day larvae were measured about 5.2 - 5.4 mm and the mouth size was 130 - 140µm. On 11th day, the larvae were measured at 6.5 - 6.8mm and mouth size was 150 - 160µm. The body was still transparent (Fig. 3). There was no much variation was observed in larval size, growth and feeding behaviour of the larvae between NS and IS.

Figure 3: Larval rearing system and different days of newly hatched larvae

Discussion:

In worldwide, overall 21 species of dottybacks were bred commercially (Sweet, 2013). However, in India there is lack of information on breeding of dottybacks in captivity. Totally 13 species of dottybacks are distributed in India, among them only one species (*P. dilectus*) was found in Gulf of Mannar region. The red head dottyback fish in the Southeast coast is a popular aquarium fish that inhabits exclusively in the Gulf of Mannar region. Since, the collection of reef fishes in India is entirely forbidden and the import is restricted, the *P. dilectus* is rarely encountered in aquarium shops and even if they are available, the prices are very high. Therefore, presently an attempt has been made in this study through captive breeding and rearing techniques for this important species. Induced breeding technique was also attempted. The present study results have been compared with earlier reports: spawning, incubation period, fecundity rate and larvae size was varied between species level in same genus of *Pseudochromis*. In these circumstances, red head dottyback was collected and conditioned in captivity using estuarine water and an attempt has been made to develop the hatchery production technology. Since, the time taken for natural spawning of this species was lengthy, a trial on induced breeding using HCG hormone was also attempted. Previous research findings are evidenced that, HCG is specific responsible for ovulation process. Research is currently being conducted with

different hormones, but these hormones has given promising result in maturation and enhanced the brood stock development (Harvey *et al.* 1993). Induced breeding of fishes was achieved using HCG hormone in Indian major carps (More *et al.* 2010), *Poecilia sphenops* (Sudha, 2012) and Sea bass, *Lates calcarifer* (Thirunavukarasu *et al.* 2004; Hill *et al.* 2009). Madhu *et al.* (2016) has been reported the breeding technique of *Pseudochromis dilectus* and achieved 95% of spawning and fecundity rate of 400 to 500 eggs. Compared with this present study achieved 90 - 95% of natural spawning and fecundity rate of 1000 - 1200 eggs. In case of induced spawning achieved 70 - 75% and fecundity rate was 700 - 900 eggs. Highest spawning and fecundity rates have been achieved from present study.

Finally, it is concluded that this is the first attempt on natural and induced breeding of *P. dilectus* using estuarine water. Larval rearing and juvenile production will be strengthened, which will achieve the goal of successful captive production *P. dilectus* in captivity. Moreover, this technology will be extended to coastal community as livelihood development, which can indirectly conserve this species in wild.

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UNDERSTANDING GENETIC

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Genetic is the study of transmission of hereditary characters from parents to offspring's, the physical, morphological characters (traits) observed in organisms called as phenotype. The traits are coded on genes present on DNA segments, whereas RNA fragments in lower prokaryotes called as genotypes. Different researchers have given definition for the genetics. Genetics is a field of science that includes the study of inheritance and genetic variations by investigating the DNA, genes, genome, chromosome and other components of it. The phenomenon of inheritance was first explained by Gregor Johann Mendel during the late 18's. As per Mendel's finding, "the traits inherited from parents to their offspring's." Some traits are physical while some are biological. What he called a trait is now known as genes; genes pass down to consecutive generations. The phenotypes of an individual maybe different then the genotype, expression of phenotypic characters depend upon the expression of dominant and recessive genes present in the individual. There are various branches of genetic-Human genetics, Clinical genetics, Population genetics, Molecular genetics, Cytogenetics, Preimplantation genetics, Plant genetics, Microbial genetics, archaeogenetic are some areas.

Human Genetic:

Gotra system of Hinduism to prevent inbreeding

Hereditary characters are traits expressed in the family from great ancestors, in Hinduism it called as *Gotra system ("the word gotra denotes the progeny (of a sage) beginning with the son's son). The marriages from same gotra are prohibited as they have same (mythological) ancestry*, People within the gotra are regarded as siblings and marrying such a person can lead to higher chances for the child to get genetically transferred diseases or weak genes or accumulation of recessive genes. Reason behind this practice (not marrying in same gotra) is the "Y" Chromosome which is expected to be common among all male in same gotra. So, the woman too carries similar X Chromosome and if married, their offspring may be born with birth defects, in almost all Hindu families, marriage within the same gotra is not practiced, thus to prevent inbreeding and completely eliminate all recessive defective genes from the human DNA. The ancestry is tracked from great sages, it is basically lineage of "Y" chromosome, because it is

standalone chromosome which does not have crossing over, gene exchange, thus passes the genes from father to son to grandson to great grandson and so on (so paternal lineage is considered). Gotra system also protects the Y chromosome, also diluting the frequency of defective genes or eliminating them. The gotras are named as Kashyap, Gautam, Shandilya, Angirasa, Atri, Bhrigu, Vasishta, Kutsa, Bharadwaj are some of the names. Typically, genes from the mother and father are shuffled or "cross over" to produce a genetic combination unique to each offspring. But the Y chromosome does not undergo crossing over, and, as a result, its genes tend to degenerate, while repetitive DNA sequences accumulate.Y chromosomes are highly dynamic and have mechanisms to acquire and maintain genes," says Amanda Larracuente, an assistant professor of biology at Rochester.

With a 30% difference between humans and chimpanzees, the Y chromosome is one of the fastest-evolving parts of the human genome (Wade, 2010). The Y chromosome was identified as a sex-determining chromosome by Nettie Stevens at Bryn Mawr College in 1905 during a study of the mealworm (class Insecta) *Tenebrio molitor*. Stevens proposed that chromosomes always existed in pairs and that the Y chromosome was the pair of the X chromosome discovered in 1890 by Hermann Henking.Stevens named the chromosome "Y" simply to follow on fromHenking's "X" alphabetically derived (Bainbridge, 2003; Schwartz, 2009).

The idea that the Y chromosome was named after its similarity in appearance to the letter "Y" is mistaken. All chromosomes normally appear as an amorphous blob under the microscope and only take on a well-defined shape during mitosis. This shape is vaguely X-shaped for all chromosomes. It is entirely coincidental that the Y chromosome, during mitosis, has two very short branches which can look merged under the microscope and appear as the descender of a Y-shape.

The genes on the Y chromosome cannot undergo genetic recombination, the "shuffling" of genes that occurs in each generation which helps to eliminate damaging gene mutations. Deprived of the benefits of recombination, Y chromosomal genes degenerate over time and are eventually lost from the genome. Thus few functional gene are present on y chromosome like, TDS testis determining factor, sex-determining region SRY, hypertrichosis, Y-chromosome-linked diseases are rare.

Animal behaviour to prevent inbreeding

Even the animals do not allow inbreeding between brothers and sister. In elephants the male calf is distanced away from the matriarchal herd ones he attains sexual maturity. The entire

heard is of female and males are solitary. Same is the case with the Lions pride. This means it's genetically coded to follow this phenomenon which is passed over from millions of years. This behavior brings genetic variations and saves the herd from deleterious effects, thus the survival of species is benefitted. This is subject of evolutionary genetics. Better progeny is reproduced from out breeding, also called as out breeding vigor, this brings new variations have better chances of survival, also diluting the recessive genes ,which is observed in nature, whereas, inbreeding has deleterious effects with expression of recessive and weak genes (alleles from same ancestors), in breeding also creates depression termed as 'inbreeding depression'.

Human intervened in breeding of some domesticated species.People were improving plant crops and domesticated animals by selecting desirable traits from individuals for inbreeding, selective breeding. The example milk gene trait in cattle, muscle trait for beef production, egg laying in white leghorns, fast development of broiler chicken (white leghorn variety bred for meat), which is doneby using biological skills, sheep are careful selected to produce more wool, in vitro, in vivo fertilization. Systematic inbreeding and maintenance of inbred strains of laboratory mice and rats is of great importance for biomedical research. The inbreeding guarantees a consistent and uniform animal model for experimental purposes and enables genetic studies in congenic and knock-out animals. Inbreeding is generally deleterious, even in flowering plants. Inbreeding is generally deleterious, even in flowering plants. Since inbreeding raises the risk that bad copies of a gene will be expressed, inbred progeny suffers from reduced viability.

Care taken by lower organisms to save the progeny

Several organisms perform binary fission. Bacteria, for instance, use it as a way to reproduce. Bacterial fission entails chromosomal replication, chromosomal segregation, and cell splitting**.** The protozoans like amoeba, paramecium, euglena etc., undergo asexual reproduction as well as sexual reproduction. When conditions are optimal and favorable, they use asexual mode of reproduction called binary fission it can be called as cloning. The word asexual describes a reproduction that occurs without involving sex cells (gametes). Instead, the somatic cells undergo an asexual process that will produce a clone of the parent. So that large number of progenies is produced, latter in unfavorable conditions some of them can survive. These organisms do undergo sexual reproduction, Woodruff (1907 published in 1929) claim of keeping paramecium healthy for 22,000 generations without conjugation (sexual reproduction). If binary fission continues repeatedly for a longer period of time, Paramecium loses its vigor andare physiologically depressed, reduces in size, ceases to multiply, degenerates in the organization, and eventually die, but the clone can be rejuvenated to regain its former vigor by nuclear

arrangement, this is brought about by conjugation. Thus, conjugation is essential for continued binary fission.In species of *Paramecium tetraurelia*, the asexual line of clonally aging Paramecia loses vitality and expires after about 200 fissions if the cells fail to undergo autogamy or conjugation. Experiments by Smith-Sonneborn, Holmes and Holmes and Gilley and Blackburn demonstrated that, during clonal aging, DNA damage increases dramatically. When clonally aged P. *tetraurelia* are stimulated to undergo meiosis in association with either conjugation or automixis, the genetic descendants are rejuvenated, and are able to have many more mitotic binary fission divisions.

Therefore the primitive way asexual reproduction advanced to sexual reproduction, causing the genetic material to reshuffle by mechanism of crossing over in gametogenesis in higher organisms, so that they get fair chance of survival.

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VERMICOMPOSTING OF RICE STRAW USING EARTHWORM *EUDRILUS EUGENIAE*

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Abstract:

Rice straw was collected from Veerakkal village, Athoor (T.K), Dindigul District, and Tamil Nadu and earthworm species (*Eudrilus eugeniae*) were collected from as Sakthi Vermiform, Sanampatti, and Vadipatti (T.K), Madurai District, Tamil Nadu. Rice straw are collected and shopped into small pieces, it was cured in the open to shade area for 15 days. The vermibeds were prepared by mixing the rice straw with cured cow dung in 1:1(1 kg of pre decomposed rice straw and 1 kg of cow dung) ratio. The physical parameters such as pH, electrical conductivity, temperature, nitrogen, phosphorus, potassium, organic carbon were analysed once in 15 days upto 60 days.

Keywords: Rice straw, *Eudrilus eugeniae*, vermicompost

Introduction:

Large quantities of organic wastes produced worldwide have necessitated researchers to develop appropriate waste recycling technologies in order to protect and preserve the environment. The burning of huge amounts of organic wastes is a common practice in developing countries to return complex organic resources back into the soil. These scenarios were seen especially in rice cultivation where rice straws are chopped and open burnt in the rice field after harvesting. For example, in Thailand which is a major global exporter of rice 8 to 14 million tonnes of rice straw are burnt in the rice field each year (Edwards *et al.*, 1985). In India, 170 million tonnes rice straw is open burnt each year. Open burning of rice straw is also a problem in many other countries, recycling this large amount of organic waste in an environmentally friendly manner helps and contributes significantly towards sustainable agricultural practices (Reddy and Ohkura, 2004).

Vermicomposting process shows great potential in the degradation of wastes converting some portion of wastes into earthworm biomass and respiration product and expelling in remaining wastes as earthworm cast (Reddy and Sreenivasa Raju, 1988). The excreted vermicast is reported to contain high amounts of mineral, vitamins, plant growth hormones, proteins, and enzymes (Bano *et al.,* 1987). This implies that feeding rates of different earthworm species may vary in relation to their morphological differences. The selection of earthworm species with a high rate of growth, reproduction and vermicast production is required to accelerate waste breakdown and stabilization (Bansal and Kapoor, 2000). Besides that, identifying earthworm species that produces vermicast with high plant available nutrient content is important to produces high quality vermicompost. Among the recommended epigenic earthworm species that have been widely used in vermicomposting of organic wastes in the topics are *Eudrilus eugeniae* (Tripathi and Bhardwaj, 2004).

By using a common feed substrate, the composting efficiency of earthworm species in producing vermicast with high decomposition rate and nutrient content can be performed. Therefore, the current research is carried out to evaluate the biological effectiveness (Rate of reproduction, change in total biomass and rate of decomposition) of earthworms Eudrilus eugeniae as vermicomposting agent ofagriculutural waste rice straw. Vermicomposting is the process that involves the oxidation and stabilization of organic matter involving the joint action of earthworms and microorganisms (Reinecke *et al.,* 1992; Pramanik *et al.,* 2007).) into nutrient rich plant growth media i.e, vermicast.

Materials and Methods:

Collection of rice straw and earthworms:

Rice straw was collected from Veerakkal village, Athoor (T.K), Dindigul District, and Tamil Nadu and earthworm species (*Eudrilus eugeniae*) were collected from as Sakthi Vermiform, Sanampatti, and Vadipatti (T.K), Madurai District, Tamil Nadu given in plate 1 and 2.

Predecomposition of rice straw:

Rice straw are collected and shopped into small pieces, it was cured in the open to shade area for 15 days. Water was sprinkled on the waste twice in a day in order to hasten the predecomposing process. A plastic tray of 45x15x30 cm size was used. Predecomposition of rice straw in tray was given in plate 3.

Plate 1: Collection of sample (Rice straw)

Plate 2: Collection of earthworms

Plate 3: Predecomposition of rice straw

Plate 4: Predecomposition of rice straw

Preparation of vermibed:

The vermibeds were prepared by mixing the rice straw with cured cow dung in 1:1(1 kg of pre decomposed rice straw and 1 kg of cow dung) ratio. After 15 days of predecomposition of rice straw givenin plate 4.

Inoculation of earthworm:

The 15 clitellate *Eudrilus eugeniae* adult earthworms were inoculated into each of these trays. The earthworms entered into the media immediately after the inoculation except in the control tray. These trays were kept undisturbed in shades. Watering was done regularly twice in a day in order to maintain the temperature and moisture content of the medium during the entire composting period was 60 days.

Physicochemical analysis of vermicompost:

The vermicomposting was carried out in an environmentally controlled experimental chamber were temperature of 27±1ºc and the vermibeds were maintained to contain a moisture level of 70-80%. Various physiochemical parameters such as pH, electrical conductivity, and total nitrogen (%), total phosphorus (%), total potassium (%) and organic carbon were analysed using the standard methods.

Result and Discussion:

During vermicomposting process physiochemical parameters were measured, and also the population of earthworms were counted the results are given in table 1 and 2.

Table 1: physicochemical parameters analyzed on 1st, 15th, 30th , 45th ,60th days

Sr.No.	Parameters	1st day	15th day	30th day	45th day	60th day
1.	pH	7.4	7.7	7.9	7.0	7.5
2.	Temperature	29^0C	30^0C	28^0C	28^0C	27^0C
3.	Electrical conductivity(ds\m)	1.7	1.9	1.6	2.9	2.2
4.	Organic Carbon (%)	23.02	29.03	25.09	20.22	19.10
5.	Total Nitrogen (%)	2.10	2.02	2.30	1.61	1.43
6.	Total Phosphorous (%)	0.98	0.87	1.52	1.20	1.08
7.	Total Potassium (%)	2.19	1.64	2.08	1.10	1.93

Table 2: Enumeration of earthworm's population after composting

Sr.No.	Earthworms	Tray I	Tray II	Tray III	Tray IV
1	Egg	75	60	55	62
2	Young ones	150	210	230	250
3	Adult	45	50	54	59

After vercomposting process the final compost is given in plate 5

Plate 5: fine compost

Vermicomposting utilizes earthworms as bioreactors to biodegrade organic waste to humus. Earthworms have important functions by virtue of their feeding and general behavioral activities like burrowing, digesting, excreting with decomposing, microorganisms and supporting

further decomposition of biodegradable matters. They decompose complex waste materials to similar forms. The whole process is known as vermicomposting. They modify the soil structure, fertility, improve plant growth and are important in sustaining productivity Bhiday (1994). The physicochemical parameters of rice straw during vermicompost on 1^{st} day such as the pH (7.4), temperature 29^0C, electrical conductivity 1.7 (ds/m), organic carbon (23.02%), nitrogen (2.10%), phosphorous (0.98%), potassium (2.19%). During 60^{th} day pH (7.5), temperature 27^0C, electrical conductivity 2.2 (ds/m), organic carbon (19.10%), nitrogen (1.43%), phosphorous (1.08%), potassium (1.93%). Bano *et al.* (1987) also reported the standardization of agro industrial wastes for vermicompost practices were the physico chemical parameters 30 day such as pH (7.9), electrical conductivity (1.6), and nitrogen (2.30), organic carbon (25.09), potassium (2.08), phosphorous (1.52), C; N ratio (10.91) Edwards (1998) and Reddy (2004), also reported on the vermicomposting of rice straw substrate and cow dung physico chemical parameters 45 day of such as pH (7.0), organic carbon (20.22), nitrogen (1.61), potassium (1.10), phosphorous (1.20), C;N ratio (12.55). In higher content of physico chemical characteristics such as the pH, Ec, Organic carbon, Na, Ca, K was observed when *Eudrilus eugeniae* introduced vermicompost. The vermicomposting (*Eudrilus eugeniae*) the enumeration of growth rate of worms (Egg, Young ones, Adult) from 60 days worked vermicompost. The growth rate of earth worms after 60 days in Tray I (Egg 85, young ones 150, adult 45) was analyzed. The growth rate of earth worms after 60 days in tray II (egg 60, young ones 210, adult 50) was analyzed. The growth rate of earth worms after 60 days in tray III (egg 55, young ones 230, adult 54) was analyzed. The growth rate of earth worms after 60 days in tray IV (egg 62, young ones 250, adult 59) was analyzed. In the high rate young ones has been analyzed in the tray I, II, III, and IV. In the high rate of eggs has been analyzed in the tray I. In the high rate of adult has been analyzed in the tray IV (Kale and Bano, 1988).

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DICHLORVOS MEDIATED BEHAVIOURAL RESPONSESIN FRESH WATER FISH, *CHANNA GACHUA* (F. HAMILTON)

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Abstract:

The quality of water resources are rapidly degrading due to increasing industrialization, urbanization, use of various fertilizers, insecticides and pesticides.Dichlorvos is widely used to control variety of domestic, agricultural and garden pests which was used to evaluate their toxicity in fresh waterfish, *Channa gachua*. Groups of 10 healthy, well acclimatizedfishes exposed to the different concentrations of dichlorvos for 96 hrs. As a response against toxicity of the dichlorvos fish showed remarkable behavioural changes such as rapid jerkymovement, jumping, hypersensitivity,restlessness, fast opercular and oral movement, excessive mucus secretion, discoloration of body, shading of scales,enormouslyprotruded and yellow belly, torsion, inverted hangingand frequent surfacing. So tremendous discharged of the toxic chemicals into the environment may leads detrimental physiological, biochemical and metabolic effects on non-target organisms.

Keywords: Pesticide, toxicity, behavior, restlessness, hypersensitivity

Introduction:

All types of pollution has emerged as one of themost significant environmental problem in recenttimes.Due to industrialization, discharging of effluents from various industries and pesticides caused serious problems in water bodies and consequently affecting the ichthyofauna directly or indirectly (Kumar and Pant, 1981). The pervasive use of pesticides in agriculture, public health and forestry ultimately leads to the contamination of aquatic biotopes posing a great threat to theaquatic environment (Tripathi and Yadav, 2015).

Pesticide is a biological weapon, which is discovered by the human to kill the insect pests and to fight against vectors of disease. Nowdays pesticide usages became an indispensable and integral part of world agriculture and it will not stop but will increase day by day. However, the intensive use of these chemicals in agriculture and public operations has changed the ecological balance of many non-target organisms like fishes (Todd and Leeuwen, 2000). Unsafe spraying

and improper handling of the chemical pesticides may cause high risk of the health hazards who doing the job like groundskeepers, fumigators, gardeners, pet groomers, and farmers. Aquatic ecosystem is the ultimate sink for agricultural, industrial and domestic runoff.

Dichlorvos (DDVP) is the highly potent organophosphate pesticide used worldwide to control the varieties of pests and ectoparasites. Fishes are ideal custodians for behavioural assays of various toxicants stress due to direct contact with the contaminated water and their ecological relevance in ecosystem systems.Behavioural modification is one of the most sensitive indicators of environmental stress (Olla *et al*., 1983). Behavior provides a unique perspective linking between physiology and ecology of an organism and itsenvironment (Little and Brewer, 2001). Behavior allows an organism toadjust to external and internal stimuli in order to meet the struggle for surviving in a changingenvironment. Alterations in fish behavior can also provide important indices for ecosystem assessment and indicates the deterioration of water quality. So the present research work was aimed to study the behavioural alterations in fish *C. gachua* after exposure to dichlorvos.

Material and Methods:

Material:

Experimental fish: The fish *C. gachua* was selected for experiment due to its easy availability and suitability for toxicity testing. Live specimen of *C. gachua* of size 15 ± 1 cm and weight 50 ± 5gm were obtained from Krishna River around Karad city with the help of fisher man. They were acclimatized in laboratory for 10 days.

Pesticide: Commercially available organophosphorus pesticide dichlorvos was used for present research work which was brought from Local agro chemist shop.

Methods:

Experimental set up: During experimentation, groups of 10 healthy, well acclimatized fishes were transferred to the experimental aquarium having different concentrations of dichlorvos. In each experimental group remarkable behavioural changes were observed in fish during the experimentation as a response against the toxicity of the pesticides.

Result:

Behaviour of control fish

In aquarium the fish from control group showed normal behaviour. They were active and swim calmly; they showed normal behaviour with well balancing. Their opercular and oral movement was quite normal.Secretion of mucous was normal.The scales were intact and skin

color was normal.They remained at the bottom of aquarium and occasionally they came to the surface of water, feeding habit was normal. They were active and sensitive to touch and sound.

Behavioral changes in fish exposed to pesticides

The fishes became restless and rapid, erratic swimming was observed. They showed rapid jerky movement of body and tried to jump out of water to avoid the toxic medium. Fish became hypersensitive and showed rapid opercular movement. They were hanging vertically in the aquarium frequently and become inverted because of loss of equilibrillium, they were lethargic and sluggish. Discoloration, loss of scales and excessive mucus secretion from the body surface that giving slimy appearance was noticed. Belly was slightly protruded and became yellowish.Finally the opercular activity was stopped and fish settled down at the bottom of aquarium after death. At higher concentrations of the pesticides operculum and mouth remain opened after death.

Discussion:

Animal behaviour is a response to surrounding habitat. In aquatic habitat the behavioural changes in fishes indicate the water pollution or unfavorable, undesirable change in water bodies. Contaminants affect the fish behaviour including locomotion, swimming performance, opercular and oral movement, feeding, etc. Fishes are sensitive and vulnerable to quick response to the toxicity.

In present study the control fish, *C. gachua* showed normal pattern of behaviour. But after exposed to dichlorvos fishes showed drastic and undesirable changes in their behaviour. They frequently dashes to the wall and tried to jump out from aquarium. They were restless and their opercular and oral movement was very fast at the beginning of exposure period. Excessive secretion of mucus, discoloration of body, shading of the scales was observed. Their belly was protruded enormously and became yellow and they became inverted and floated near the surface. They lost their balance and settled down to the bottom. Similar behavioural changes were observed by Deshpande (2000), when he studied the effect of two pyrethroids i.e. fenvalerate and cypermethrin at sublethal and lethal concentration against *L. rohita.* According to him these changed behaviour might be due to combination of disturbance in physiological, biochemical, enzymological and hormonal aspects in fishes. Bhilave (2001) reported similar behavioural changes in fish, *C. mrigala* exposed to cadmium chloride. According to him the changes in behaviour might be due to disturbances in biochemical and enzymological aspects in fishes. As per Halappa and David (2009) *C. carpio* exposed to chlorpyriphos exhibited disrupted behaviour. According to them pesticide inhibits the acetylcholinesterase activity which leads in to accumulation of acetylcholine in cholinergic synapses causing hyper stimulation due to that behavioural changes occurred in fish. Shrivastav *et al*. (2010) studied dimethoate induced

behavioural changes in *H. fossilis*. According to them these behavioural changes were the symptoms of stress of toxicological nature of the environment. Similar abnormality in behaviourin fish, *C. gariepinus* were observed by Lapido *et al*. (2011) after exposure to paraquat dichloride. Chavan (2014) observed changed behaviour such as increased opercular movement, increased surfacing with mouth open, loss of equilibrium, irregular swimming, vertical hanging, rapid jerks to jumps outside and finally settling down to the bottom, excessive mucus secretion and yellowness on body in *C. mrigala* after exposure to lead acetate and mercuric chloride. Dey and Saha (2016) reported excessive mucous secretion, fast swimming, hyperactivity, increasing opercular movement, pale gills, irregular, erratic swimming, loss of balance, blood patch on operculum in fish, *L. rohita* after exposed to lambda cyhalothrin 5% EC and marshal (Carbosulfan 25 % EC). *C. gachua* is an air breathing fish which visited surface of water periodically to engulf atmospheric oxygen but the surfacing behaviour was found altered by toxic medium in present study. The increasing surfacing activity observed at lethal concentration of pesticides, in the present study might be the response of fishes to meet the demand of oxygen from air because of suffocation and in oxidative or metabolic stress. Surfacing phenomenon i.e. significant preference to upper layers in exposed group might be due to elevated demands for oxygen during the exposure period (Katja *et al.*, 2005). Here it is assumed that the dullness in body color and altered metabolism might be the response by the fish towards the adaption to the toxic substances. The feeding was impaired and reduced drastically from toxic medium that may leads into lower energy production that may compel the fish to use of the stored biochemical constituents to cope up with the high energy demand. Depression in appetite is a common response that can impact on growth and reproduction of fish. In present study it is assumed that the organophosphate pesticides might inhibit the acetylcholinesterase (AchE) activity as stated earlier by Gohel and Dodia (2015) that may paralyze the sensory and neuromuscular system that leads into changes in normal behaviour and muscular function resulted into the hypersensitivity, erratic jerky movements, unbalancing, loss of equilibrium in fishes in the present study. On the exposure to pesticides, fishes showed distress, lethargy and excessive mucus secretion all over the body. There was excessive mucus secretion by fish which was the response of fish against the toxic medium that form the barrier between body and toxic medium which may reduce the contact between body of fish and toxic medium. Similar assumption was mentioned by Rao (2006) in *O. mossambicus* exposed to organophosphate insecticides (RPR-V). According to him mucus forms a barrier between the body and the toxic medium to minimize its irritating effects or to scavenge it through epidermal mucus.

So, in present work the fish showed well marked abnormal behavioural changes in response to organophosphate pesticides for the defense and adaptability against the toxicant

stressed condition. At the end due to toxic medium fish unable to survive in toxic medium which results into significant mortality.

Conclusion:

The current study evidenced that dichlorvos is highly toxic and had a detrimental impact on the behavioural responses of *C. gachua*at lethal concentrations. Due to pesticideinstinctive behavioural response has been reduced and morphological features were affected. Impairments in behavioural responses might be due to inhibition of brain AchE activity because of action of organophosphate pesticide. These behavioural responses canbe used as a tool in bio monitoringprogramme to monitor ecotoxicity risk of pesticides to the test species.

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VERMICOMPOSTING OF SUGAR CANE BAGASSE USING *EISENIA FETIDA*

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Abstract:

Sugar Cane waste was collected from surrounding area of Kodairode, Dindigul (district), Tamil Nadu. Earthworm species, (*Eisenia fedita*) were collected from SS Vermiform Pandiyarajapuram, Vadi Patti, Madurai District, Tamil Nadu India. Sugar Cane waste are collected and cut into small pieces and kept under shade condition. Water was regularly sprinkled and the substrate was regularly turned for 15 days. For the preparation the vermicompost the predecomposed sugar cane waste was directly mixedwith cow dung in 1:1 (1 kg of predecompost and 1 kg of cow dung) ratio on dry weight basis in tray (15cm x60 cm size). The substrate should hold 60-80 percent moisture content and kept for 24hrs stabilization. Fifty numbers of healthy, cilitellate *Eisenia fedita* king berg were introduced in the plastic tray. The vermicomposting trail was carried out in the rearing room. The substrates were turned once in 3 days. The vermicompost was analysed for various physico chemical parameters .such as pH, electric conductivity, total phosphous, total nitrogen and total potassium using standard procedure

Keywords: Sugar Cane bagasse, *Eisenia fedita*, vermicompost

Introduction:

In Indian economy the sugar mill has an essential place and contributes considerably to its export. India is the second longest producer of suger and its by product amongst the 83 sugar cane producing countries in the world. The industries of large to medium size as compared to 138 during 1950-1951. About 270 million tons of sugar cane per year is produced in India. During the manufacturing process large amount of by products such as bagasse press mud and sugar cane residue are produced. Bagasse is the fibrous waste produced in the sugar cane juice extraction process Ansari and Ismail (2001). It constituents cellulose (50%), hemicelluloses

(25%) and lignin (25%) bagasse is a highly homogenous materials constitute around 30-40% of pith fibre, which is obtained from the core of the sugar cane. The estimated generation is 0.25-0.30 per ton of sugar cane. Vermicomposting is a cost effective, ecofriendly and appropriate waste disposal technique for efficient recycling of animal waste, crop residues, agro industrial and sewage sludge. Sugarcane trash and press mud could be used for vermicomposting along with cow dung and other crop residues. Vermicomposting improves the nitrogen and phosphorous availability besides improving soil physical properties earthworm act as bio-concentrates of heavy metal and toxic material (Edwards and Bohlen, 1996; Edwards, 2004). Vermicompost usage improves crop production and material soil health. *E.fetida* worm are used for vermicomposting of both domestic and industrial organic waste. They are native to Europe but have been introduced to every other continent expect Antarctica (Ismail, 1997, 2005).

Materials and methods:

Collection of sample:

Sugar Cane waste was collected from surrounding area of Kodairode, Dindigul (district), Tamil Nadu, the sugar cane waste was given in plate 1.

Plate 1: Collection of sample (sugar cane waste)

Plate 2: Adult earthworm (*Eisenia fedita*)

Collection of earthworm:

Earthworm species, (*Eisenia fedita*) were collected from SS Vermiform Pandiyarajapuram, Vadi Patti, Madurai District, Tamil Nadu India. The earthworm species (Eisenia fedita) was given plate 2.

Predecomposition of sugar cane waste:

Sugar Cane waste are collected and cut into small pieces and kept under shade condition. Water was regularly sprinkled and the substrate was regularly turned for 15 days. The predecompost tray was given plate 3.

Preparation of vermibed:

For the preparation the vermicompost the predecomposed sugar cane waste was directly mixed with cow dung in 1:1 (1 kg of predecompost and 1 kg of cow dung) ratio on dry weight basis in tray (15cm x60 cm size). The substrate should hold 60-80 percent moisture content and kept for 24hrs stabilization.

Inoculation of earthworm:

Fifty numbers of healthy, cilitellate *Eisenia fedita* king berg were introduced in the plastic tray. The vermicomposting trail was carried out in the rearing room. The substrates were turned once in 3days.

Physico Chemical analysis of vermicompost:

The vermicompost was analysed for various physico chemical parameters .such as pH, electric conductivity, total phosphous, total nitrogen and total potassium using standard procedure (Mane and raker 2012): colorimetric method with molybdenum in sulphuric acid. Total potassium was determined after digesting the sample in di-acid mixture concentrated HNO3: concentrated HCl, by flame photometer. After 60th day, the compost was sieved and sugar canewaste vermicompost were collected.

Result and Discussion:

During vermicomposting process physiochemical parameters were measured, and also the population of earthworms were counted the results are given in figure 1 and 2.

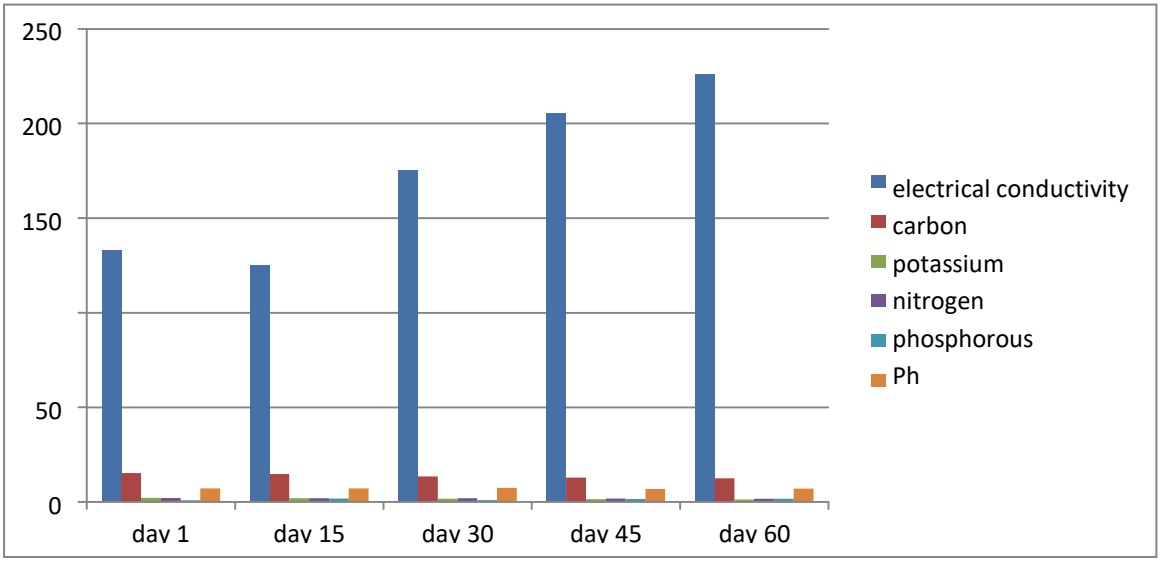

Figure 1: physicochemical parameters analyzed on 1st,15th, 30th , 45th ,60th days

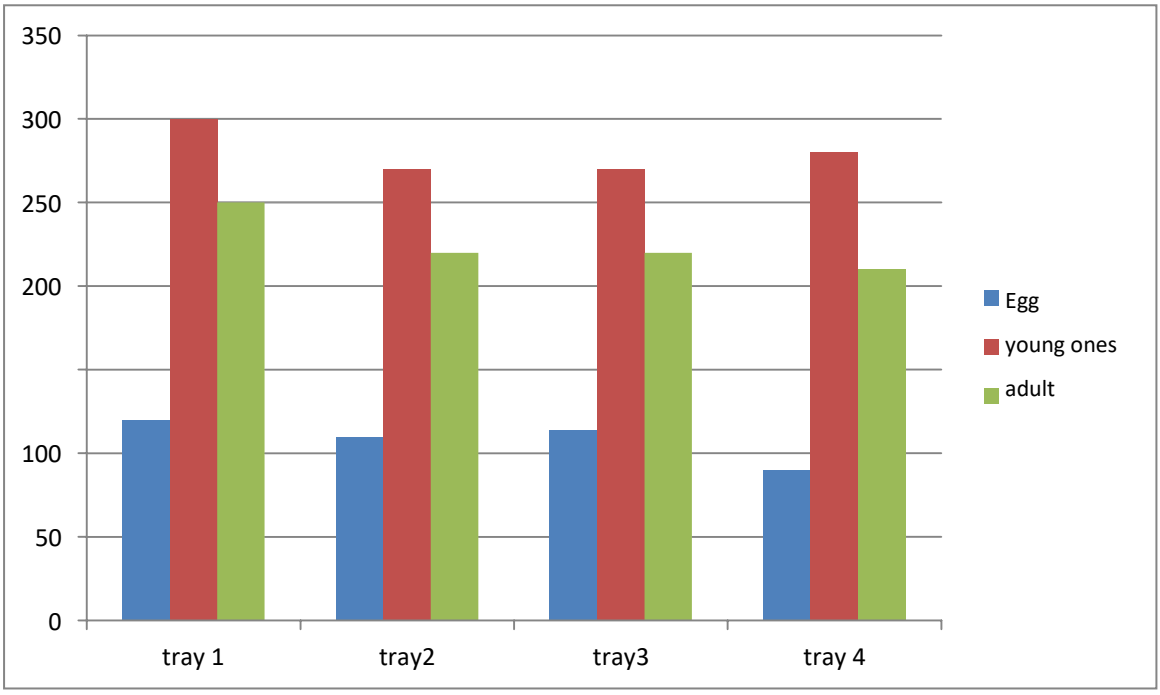

Figure 2: Enumeration of Earthworms population in vermicompost

Tray 1 –Egg (120), Young ones (300), Adult (250); Tray 2 – Egg (110), Young ones (285), Adult (235); Tray 3 - Egg (114), Young ones (270), Adult (220); Tray 4 - Egg (90), Young ones (280), Adult (210)

After vermicomposting process the final compost is given in plate 4.

Plate 3: Predecomposition of sugar cane

Plate 4: Vermicompost

The physic chemical parameters of sugarcane vermicomposting for I day such as the pH (7.2), organic carbon (15.3), nitrogen (2.05), phosphorous (0.85), potassium (2.32), C:N ratio (10.96). Vermicomposting of presented from sugarcane were there physic chemical parameters in 15 day such as pH (7.3), organic carbon (14.8), nitrogen (2.01), potassium (2.10) phosphorus (1.85) C:N ratio (14.37), Vermicompost practices were the physic chemical parameter 30 days such as pH (7.5), electrical conductivity (4.4) and nitrogen (2.02), organic carbon (13.5), potassium (1.85), phosphorus (1.05), C:N ratio (10.92). Vermicomposting of sugarcane physio chemical parameter 45 days such as pH (7.0), organic carbon (13.0), nitrogen (1.50), potassium (1.50), phosphorus (1.50), C:N ratio (12.33). Vermicomposting of sugarcane physico chemical

character 60 days such as the pH (7.1), organic carbon (12.5), nitrogen (1.45) potassium (1.45), phosphorous (1.39), C:N ratio (13.3). The conclusion of the study was that earthworm Eisenia fedita is well adapted to sugarcane waste.

In higher content of physico chemical characteristic such as the pH, EC, Organic carbon, Na, Ca, K was observed when *Eisenia fedita* introduced vermicomposting Ismail (1995) and Kale 1998. The vermicomposting (*Eisenia fedita*) the enumeration of growth rate of worms (egg, young ones, adult) from 60 days worked at vermicompost. The growth rate of earth worms after 60 days in Tray I (egg 120, young ones 300, adult 250) was analyzed. The growth rate of earth worms after 60 days in tray II (egg 110, young once 205, adult 235) was analyzed. The growth rate of earth worm after 60 days in tray III (egg 114, young ones 270, adult 220) was analyzed. The growth rate of earth worms after 60 days in tray IV (egg 90, young ones 280, adult 210) was analyzed. In the high rate young ones has been analyzed in the tray I, II, III and IV. In the high rate of eggs has been analyzed in the tray I. In the high rate of adult has been analyzed in the tray IV (Talashilkar and Power, 1998; Stoffella and Kahn, 2000).

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BLACK SOLDIER FLY, *HERMETIA ILLUCENS* L.: A NUTRITIVE INSECT AND A SOLUTION TO LIVESTOCK FEED

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Abstract:

The food industries require new supportable sources of protein and fats which can be received by the use of insects like *Hermetia illucens* L at an industrial scale. Black soldier fly larvae have a potential to feed on any organic waste matter and convert it into insect biomass so it can be utilised as a livestock feed. Livestock sector like poultry, aquaculture, piggery, duck culture etc. always struggling to fulfil the demands of nutritious feed. Insects like black soldier fly have been identified as potential alternatives to the predictably used protein sources in livestock feed due to their rich nutrient content and the fact that they can be reared on organic wastes streams. Products made from insect biomass resultant from the organic waste streams are better suitable for industrial large-scale production of insect meal, for this purpose systematic study of nutritive value of these larvae is needed. Present study confirmed that different nutritive parameters like crude protein, fats, amino acids, fatty acids, vitamins, flavonoids, minerals etc. gained by of Black soldier fly larvae fed on different organic wastecan be utilized as an alternative feed for livestock sector.

Keywords: Black soldier fly, *Hermetia illucens* L., livestock feed, nutritive value, organic waste, insect biomass

Introduction:

The food demand will probably increase by 70% by the year 2050 worldwide so as to meet the demands of 9.7 billion people who are expected to inhibit the world by that time (Tilman *et al.,* 2011). While the world population continuously growing along with resource consumption and waste generation, the food resources are becoming limited, causing various responses from different sectors of society (Paulin *et al.*, 2018). The use of livestocksectors like poultry, fish, shrimp, piggery etc. utilized as food is the solutionin the demanding and developing

world population which can solve the poverty and provide food security (Thornton *et al.,* 2010; Otte *et al.,* 2012).

India's poultry industry signifies a major success story. Poultry production has been growing at the rate of around 8 percent per annum, with an annual turnover of US$ 7500 million, while the agricultural production has been increasing at the rate around 2 percent per annum over the past two to three decades. In India since 1970s, Universal production, consumption and trade of poultry meat has grown faster than any other meat. During 1990s demand growth for poultry meat enhanced when demand growth reducedfor other meats and continued to lead the expansion of poultry meat trade (Maurice et al.)

As India received its vast coastal area, it offers immense opportunities for fisheries in both marine and inland waters. India has the second largest share in global aquaculture market, mainly due to its 2.36 million hectares of ponds and tanks, a coastline of 7,500 kms and brackish water area of 1.1 million, which offers a great opportunity for fish farming. In India, the fisheries sector is a means of income for ~ 28 million people in the country. The Food and Agriculture Organisation (FAO) 2020 report discovered that Asia has the largest share in the global fish production at 89%, since the last 20 years. Fish production in the country reached a record high of 14.16 million metric tonnes between 2019 and 2020.

Poultry farming and aquaculture are the two sectors which can fulfil the demand of protein diet in future. As these two sectors increasing day by day feed demand for these sectors willalso increase. Soyabean, corn, fish meal prices are high cannot fulfil the demand of flourishing livestock farming due to food feed competition. Increase of soybean production in tropics resulted in land grasping and deforestation in addition to other negative social and environmental penalties (Foley *et al.*, 2011). For reducing such impacts there is an urgent need to replace these traditional feed ingredients such as soybean and fish meal with innovative, economically valuable and environmentally sustainable ones (Tschirner and Kloas, 2017).

Several insect species feed on various types of organic waste streams can be used as an alternative feed for livestock as these insects gain proteins, fatty acids, micronutrients and gain high amount of energy (Van Huis *et al.,* 2003; Rumpold *et al.,* 2013; Finke and Oonincx, 2014; Nowak *et al.,* 2016). The Black Soldier Fly (BSF) *Hermetia illucens* L. is recognised as promising alternative source of protein for animal feed (Hale, 1973; Hopley, 2016). The larvae of this fly can feed on any organic waste like vegetable and Kitchen residues, municipal organic waste, farm waste, dried distillers' grain etc. and reduce the organic waste 50-60% and turn them into high protein biomass (Craig *et al.,* 1994). Black soldier flies dry weight containsupto 50%

crude protein (CP), up to 35% lipids and have amino acid profile that is similar to that of fishmeal (Elwert *et al.,* 2010). These larvae are discovered as an alternative source of protein for feed of livestock like poultry, piggery, several types of fishes, shrimps etc.

Black soldier fly (BSF)

Black soldier fly (*Hermetiaillucens* L) belongs to order Diptera,family Stratiomyidae, life cycle divided into four phases: eggs, larva, pupa and adult stage. Last larval stage is prepupa where it migrates from wet food substrate to dry place and converts into pupa. Larval stage lasts for 14 to 18 days, depending on environmental temperature and pupal stage lasts for about two weeks. Adult flies emerge form pupal stages prefer to fly towards bright light. Adult flies do not feed on any food but gravid female flies attract towards smelly food for laying eggs. A single female can lay 500 to 800 eggs at a time in a bunch in cracks or crevices above the garbage. BSF larvae reduce the plant or animal waste up to 80%, digest harmful bacteria, adult flies donot transmit any pathogens from waste food or garbage so these flies can be reared and utilised for industrial use.

Why Black soldier fly larvae (BSFL) for livestock feed

During meal more than one -third of the edible parts of food gets lost or wasted, insects play an important role in managing and converting food waste as they can be reared on large variety of such bio-waste substrates and can be converted into insect biomass (Spranghers *et al.,* 2017; Cappellozza *et al.,* 2019). Black soldier fly is one of the most effective bio converters reduce organic waste up to 75% and convert it into biomass havinghigh-quality nutrients like protein and lipids (Gold *et al.,* 2018).

Zhenghui Gao *et al.* (2019) fed BSF larvae on maize straw fermented with *Aspergillus oryzae* for 24hrs in ratio of 4000:1 and found that larvae contained 41.76% crude protein, 8.24% crude ash, 30.55% crude fibre, monounsaturated fatty acid 24.86%, polyunsaturated fatty acid 25.37% while saturated fatty acid 45.41% and concluded that the black soldier fly have the ability to bio convert maize straw and after BSFL bioconversion the maize straw residue can be used as organic fertilizer.

Andrea Scala *et al.* (2020) reported that the rearing substrates impacts growth and macronutrient composition of BSF larvae reared at an industrial scale. Author used three organic waste streams e.g. banana, appleand spent grainfrom brewery. BSF larvae fed on only fruits diet as well as on combination of these food diets. BSF larval crude protein content was high i.e. 47.83%, 48.01% and 45.61% when fed on spent grain, Apple +spent grain and banana + spent grain mixed diet respectively as compared to fed only on fruits. This diet also affected crude

lipid content of BSF larvae. Crude lipid content of larvae fed on fruits i.e., apple, banana and apple +banana was 36.1%, 27.9% and 33.4% respectively.

Marwas Shumo *et al.* (2019) took the advantage of easily available organic waste streams in Kenya to produce nutrient rich black soldier fly larvae. Nutrient value was checked when larvae were fed on three organic wastes i.e., chicken manure, brewers spent grain and kitchen waste. Crude protein in larvae fed on these wastes were 41.1%, 33% and 41.3% when fed on chicken manure, kitchen waste and spent grain respectively. Ether extract of larvae fed on same waste diet was 30.1%, 34.3% and 31% respectively. Author also evaluated minerals, fatty acids, amino acids, flavonoids and vitamins and concluded that BSFL can be effectively used as a high-quality feed as a substitute for other animal or plant derived protein sources in commercial livestock feed.

The growing demand for fish and soyabean meal in livestock and aquaculture feeding gives alternative protein carriers to animal feed industry and use of insect-based feeds is gradually is a matter of interest among fish-feed producers, scientists and policy makers (Andreas *et al.,* 2014). Different levels of fishmeal replacement by using black soldier fly larvae meal had been tested on rainbow trout showed neither signs of higher mortalities nor of nutrient deficiencies observed and concluded that BSF meal can replace fish meal up to alevel of 50% in trout feeds.

To search alternative sources of poultry feed is still a big concern by nutritional researchers, by finding balance between high performance and low cost (Khoramabadi *et al.,* 2014). BSF larvae can be a high value feed as they are rich in protein (37% to 63%) and lipids (15%-49%) and have better amino acid profile than soybean meal (Barragan-Fonseca *et al.,* 2017). Schiavone *et al. (*2017) demonstrated that defatted BSF meals can be excellent source of apparent metabolizable energy and digestible amino acids for broilers, results into a better efficient nutrient digestion.

BSFL can replace all the time more expensive protein sources used in poultry, aquaculture and livestock composite diet formulation, such as fish meal and soybean meal, which holds the potential to improve future food and feed insecurity. Xiu Liu *et al.* (2017) studied metabolic changes in nutrition composition of BSF from egg to adult. Author found a rapid increase of crude fat content since the development of 4-14 days of larvae with its maximum level reached 28.4% in dry mass while crude protein reached highest level of 46.2% at early pupa stage.

Conclusion:

It is possible that black soldier fly larvae generated protein and fat could be used as a replacement for other protein and fat resources in livestock feed industries. Protein and fat content in larvae very much depends on the type and quality of organic waste fed to the larvae. By providing different types of organic waste to the BSF larvae we can get essential nutrients like crude protein, crude lipids, amino acids, fatty acids, vitamin, minerals etc as an insect biomass and such nutritious insects can be utilised as an alternative feed to the livestock or can be used its by-products.

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AN ASSESSMENT OF INVERTEBRATE SPECIES IN WETLAND ECOSYSTEMS

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Abstract:

The inland water resource on the surface of earth such as Rivers, Lakes, Reservoirs and Ponds became the focus of special attention in the early stage of development of science of ecology. The sources and the nature of fresh water, its motion and changing condition as it flows to the sea and the life it support to all aquatic living things, are now the subject of Limnology, the science of fresh water and Hydrobiology the science of aquatic life. Anthropogenic activities of human beings such as bathing, cleaning, household activities, washing of the livestock, will increase the nutrient load. This may be stopped and some other alternative may be made for this to reduce the nutrient loads. So there is need of hour for monitoring the quality and status of wetland ecosystems in order to check the quality of water. The impact of human activities on these aquatic ecosystems and to improve freshwater ecosystems conservations should be given emphasis on these aspects.

Introduction:

The inland water resource on the surface of earth such as Rivers, Lakes, Reservoirs and Ponds became the focus of special attention on the conservation of wetland ecosystems. The sources and the nature of fresh water, its motion and changing condition as it flows to the sea and the life it support along the way, are now the subject of Limnology, the science of fresh water and Hydrobiology the science of aquatic life.

The hydrobiological studies of Indian waters in relation to the seasonal fluctuation of water characteristics and biological conditions of lakes, reservoirs, tanks and ponds are scanty in comparison to the vast area of inland freshwater available. The study of seasonal condition of pond life in Punjab by Prasad (1916) was perhaps the first attempt towards the study of aquatic ecosystem in India. Later on, Pruthi (1933) studied seasonal fluctuations physical and chemical conditions of a tank located in Indian Museum Compound, Kolkatta while Gonzalves and Joshi

(1946) tried to correlate abiotic and biotic factors of a tank at Bandra (Mumbai). Fresh water ecology is an intriguing field because of the great diversity of aquatic habitats.

The wetland ecosystems carrying great loads of biodiversity as it is very much rich in its nutritional value and productivity (Gibbs, 1993; Paracuellos, 2006) and are one of the ecologically important method of conservation concern sites owing to its tropic dynamics. Wetlands are generally less than 3 m deep over most of their area which are usually rich in nutrients and have abundant growth of aquatic macrophytes. They consists of high densities and diverse fauna mainly planktons, macrophytes, macro invertebrates which have high value for biodiversity conservation. Excessive growth of macrophytes in wetlands affects the water quality adversely and interferes with the utilization of the water body. However, marginal aquatic vegetation is desirable as it controls erosion, beneficial for wildlife habitat and also supports to improve water quality of aquatic ecosystems. Aquatic ecosystems are variously studied for the physico-chemical and biological aspects for their conservation and management.

In an aquatic ecosystem, the physico-chemical environment has profound influence on its biotic components, as it controls its diversity, biomass and spatial distribution in time and space. The physico-chemical parameters exert their influence both individually and collectively and their interaction produces an abiotic environment which ultimately conditions the origin, development and finally the succession of the biotic communities. Fluctuations in physico-chemical parameters produce adverse effects on the biotic communities which limit their production and reduce their ability to compete with other population within the environment. All aspects of biological heterogenicity i.e. structural, functional or taxonomic were included in the scope of biodiversity (Sarkar, 2008).

According to the study on the biological diversity, that means the variability among the living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexities of which they are the important part; which includes diversity within species, between species and of ecosystems. The physical and chemical characteristics of water affect the abundance, species composition, their diversity, stability and productivity of the indigenous population of aquatic organisms. The biological methods used for assessing quality of water include collection, counting and identification of aquatic organisms means quantitative and qualitative analysis, biomass measurement etc. and are very essential for its effective management and conservation of the wetland ecosystems.

Planktons:

Planktonic organisms are found in all aquatic ecosystems except in torrential rivers. They are small plants and animals whose powers of locomotion are so limited that they cannot

overcome currents in their ecosystem. Most plankters (phytoplankton and zooplanktons) can move a bit, however, either, to control their vertical distribution or to seize prey.

Phytoplankton:

Among biotic communities phytoplankton constitute the first stage in tropic level by virtue of their capacity to convert radiant energy into the biological energy through photosynthetic activity. Also referred to as primary productivity, the magnitude of photosynthetic energy fixation depends primarily on diversity and biomass of phytoplankton. The planktonic photosynthesis play a key role in conditioning the microclimate as it helps in regulating the atmospheric level of oxygen and carbon dioxide. Wanganeo (2007) stated that large amount of algae can adversely affect the quality of water by inhibiting the photosynthesis process for other plants and by reducing dissolved oxygen levels. Thus, excessive amounts of algae usually lead to poor quality. A major contribution to unwanted algal species growth is the influx of nutrients such as nitrates, phosphates, sulphates etc from non-point sources.

Apart from primary production, phytoplankton also plays an important role as food for herbivorous animals. They are also considered as biological indicators of water quality in pollution studies. To summarize, because of their definite role in cycling of energy and matter in an ecosystem, evaluation of phytoplankton population in term of their diversity, density, biomass, spatial and temporal distribution, periodicity and productivity and population turnover, is of vital significance in the management of an ecosystem.

Phytoplankton constitutes the very basis of nutritional cycle of an aquatic ecosystem. The maintenance of a healthy aquatic ecosystem depends on the abiotic or physical properties of water and the biological diversity of the ecosystem (Harikrishanan *et al.,* 1999). The planktonic study is a very useful tool in understanding the basic nature and general economy of the lake (Pawar *et al.,* 2006). The phytoplankton belonged to Myxophyceae, Bacillariophyceae, Chlorophyceae and Euglenophyceae.

Zooplanktons:

Zooplanktons are important in fresh water ecosystem as they indirectly convert the food energy due to their role as preys of economically important fishes. The zooplankton because of their short life period respond quickly, but also because of their small size and often their great numbers are also useful in determining the origin or recent history of given water mass (APHA, 1980). The physical and chemical factors play important role, which for the most part are responsible for the distribution of the animal life in fresh water habitat. The seasonal cycle of

zooplankton are affected by the size, distribution of algae, bacterial prey and pressure of predatory fishes (Kalff, 2002; Chavan *et al.*, 2006).

Zooplankton diversity is one of the most important ecological parameters in water quality assessment. The zooplankton study has been a fascinating subject for a long time. Water bodies rich in phytoplankton are also rich in zooplankton diversity and biomass. Vijaykumar (1999) stated that in an aquatic ecosystem, zooplanktons play an important role not only in converting plant food into animal food but also provide an important food source for other higher organisms including fish.

Zooplankton belonged to Rotifers, Copepodes, Cladocerans and Ostracodes. Biological assessment of the water quality provides a number of advantages over chemical assessment. Among fresh water communities, zooplankton and phytoplankton are most common living organisms and many of them are the bioindicator representatives of pollution. However, the benthos and macrophytes also helps to show the trophic status of the ecosystem.

Benthic Macro-invertebrate:

The community of organisms living on the bottom of a water body is called the benthos. It includes benthic macro invertebrate communities of Nematodes, Oligocheates, Insects and Gastropodes. The benthic macro invertebrates can be used as the barometer of overall biodiversity in the aquatic ecosystem. The invertebrate communities respond to change in water quality, integrates impact over a period of times and presence or absence of species can be an indication of specific environmental condition (Anitha *et al.*, 2004).

The benthic macroinvertebrate biological communities are most frequently used to evaluate water quality in aquatic environments, and occupy variety of trophic levels, acting on the nutrients, bottom detritus and water column dynamics (Rodrigues and Capitulo, 2002). The benthic macroinvertebrates belonged to Nematoda, Annelida, Insecta and Gastropoda. Benthos are important in the secondary productivity of fresh water ponds and lakes, and playing significant role in exchange of autochthonous and allochthonous material in an ecosystem. It has a great ecological importance because they form the food of fishes and their productivity plays a significant role in sustaining food chain and web. Further the qualitative studies of the benthic macroinvertebrates have also been stressed by many workers (Naidu, 2005; Sharma *et al.*, 2007; Sisodia, 2007).

Macrophytes:

The macrophytes stimulate the growth of phytoplankton and help in the recycling of organic matter. The submerged species of macrophytes at the margin also act as green manure favouring the occurrence and abundance of zooplankton and benthic fauna. The macrophytes

also provide suitable breeding and sheltering place for macroinvertebrates and fishes and other aquatic organisms (Meshram, 2003).

Narayana *et al.* (2007) considered that Vallisneria spp. and Ceratophyllum spp., Chara vulgaris are the species that preferably grow at unpolluted pond. Among the macrophytes, free floating, submerged, marginal and Emergent are the different types of varieties of aquatic weeds. Some macrophytes grow rapidly in summer season. When growth slows down the algae occupy progressively the surface of macrophytes. Water chemistry especially nutrient concentration, influence the colonization and species composition of periphyton communities (Charachlis *et al.*, 1990; Guasch *et al.*, 1995). The biodiversity of Macrophytes has been intensively studied by various workers.

Periphyton:

The assemblage of organism growing on the submerged object such as plants pebbles and animal shells etc are called 'aufwuchs' or periphyton. The term periphyton (synonym with haptobenthos) was first used by Behning in 1924 for the plant growth on Buoys, Ships and moorings of river Volga. Haptobenthos have much assumed considerable importance as these organisms occur in appreciable and sufficient abundance in some situations and have found to form the food of some fishes and other aquatic animals. Periphyton belonged to Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophyceae and Protozoa, Rotifera, Cladocera, Ostracoda, Gastrotricha, Oligochaeta, Nematoda and Mollusca with reference to the macrophytes studied such as Eichhornia, Pistia, Lemna minor, Hydrilla, Azolla, Potamogeton and Nymphoides species. The study of haptobenthos or periphyton has received very little attention in India.

Summary:

Wetland ecosystems such as ponds and lakes are considered as natural ecological resources. The management of such wetland ecosystems has become the need of the hours as community activity and other sources of pollution as enumerated which can alter limnological characteristics. Desilting of the pond may be emphasized on some alternatives or solutions could be worked out in collaboration with local municipality for diversion of untreated domestic sewage and slaughterhouse wastes and means to restore recreational activities in the wetland ecosystems.

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