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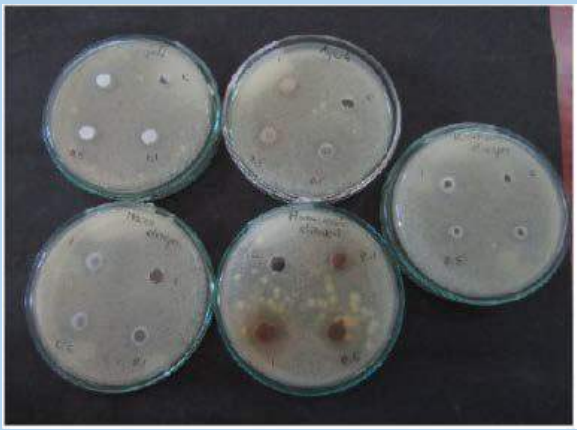
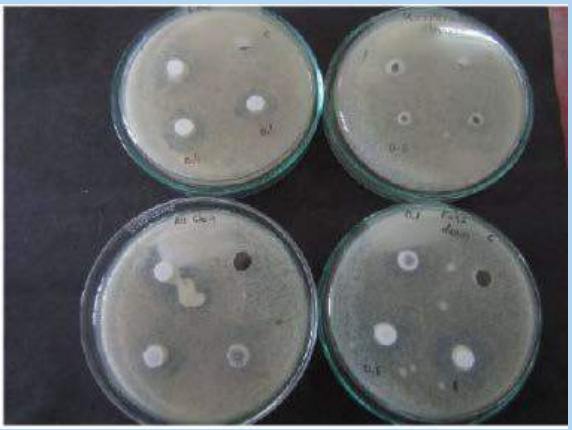

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EDITORIAL

Dear Readers,

We are pleased to launch this issue of *Journal of Marine Biosciences* from the Department of Marine Biotechnology, AMET Deemed to be University, Chennai.

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1. Comparative Study of Zooplankton in Different Limnetic Habitats of Tamilnadu, India.

Janakiraman A.,* Gokula V., Rajanayagam A. and P. Marimuthu

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Abstract

Zooplankton are heterogeneous group of microscopic free-floating drifting aquatic of invertebrates found in different water bodies served as secondary producer and primary consumer in aquatic food chain. Though their range were few to several micrometers, their contribution in aquatic ecosystem are indispensable. In the present study, there are 17 species belonging to 5 groups of zooplankton were recorded from four stations. Maximum diversity was recorded in Pudunagar pond (11 species) and minimum diversity was recorded in Koraiyar River (6 species). Pudunagar Lake (8 species) and Gundur lake (9 species) were recorded. In general, the rotifers *Brachionus calyciflorus,* cladoceran *Moina micrura,* ostracod *Hemicypris sp.*calanoid *Heliodiaptomus cinctus* and cyclopoid copepods *Thermocyclops decipiens* were predominant species of zooplankton in the present study. It was interestingly noted that absence of calanoid copepods species and presence of rotifer *Brachionus* sp. and *Moina* sp.in Koraiyar River is evidence of polluted state. Overall density of zooplankton is ranges from 18.67 to 61.67 ind./L in all the four stations. Minimum density was recorded in riverine environment (18.67±3.26 ind./L) and maximum density was recorded in Gundur lake (61.67±10.08 ind./L). In general, maximum percentage composition was occupied by cyclopoids (33%) followed by calanoids (32%), cladocerans (19%), rotifers (8%) and other zooplankton were below five percent. Investigation concluded that Pudunagar pond and Lake has good water quality than Koraiyar River and Gundur Lake.

Keywords: Zooplankton, Diversity, Ecology, Bio-indicator

Introduction

Freshwater ecosystems provide vital resources for humans and are the sole habitat for an extraordinarily rich, endemic, and sensitive biota, which constitute larger part of our biosphere (Fathibi et al. 2017). Freshwater habitats exist into lentic and lotic ecosystems based on the differences in the water residence time and flow velocity (Wetzel, 2001). These ecosystem water qualities are affected by a wide range of natural and human influences. Water pollution can be analyzed by the changes in physical, chemical and biological properties like colour, organic and inorganic contents and microbial

load. Water quality assessment generally involves analysis of physico-chemical, biological and microbiological parameters and reflects on abiotic and biotic status of the ecosystem. The plankton community is a heterogeneous group of microscopic plants (phytoplankton) and animals (Zooplanktons) adapted to float in the marine and freshwaters habitats. Zooplanktons are microscopic free-floating drifting aquatic of invertebrates found in different water bodies. They are important biotic components influencing all the functional aspects of an aquatic ecosystem, such as food chains, food webs, energy flow and cycling of matter. Zooplankton plays an integral role and serves as bio-indicator and it is a well-suited tool for understanding water pollution status, bioremediation of heavy metals and other toxic materials, bio-monitoring of water pollution and act as biomarker for water quality assessment for fish production, live feed, Bio-control of mosquitoes, important source of industrial products such as chitin and its derivatives, ideal organisms for toxicological studies and also used to find out the evolutionary relationship studies (Pradhan et al.,2008; Purushothama et al.,2011; Tyor et al.,2014). They are also capable of concentrating large quantities of heavy metals from water bodies and depositing on higher trophic levels through the food chain (Hemlata et al.,2013). Untreated domestic wastewater discharge into the pond has resulted in the eutrophication of the pond as evidenced by substantial algal blooms, dissolved oxygen depletion in the subsurface waters, fish mortality. Changes in zooplankton abundance, species diversity and community composition can indicate the change or disturbance of the environment and they can serve as an indicator of changes in trophic dynamics and the ecological state of lakes related to changes in nutrient loading and climate (Kehayias et al.,2014). Therefore, following objectives were made to assess the density, diversity and distribution of zooplankton in different limnetic habitats of Tamilnadu along with their current ecological status.

Materials and Methods

Samples of water and zooplankton were collected during the early hours of the day in the month of February 2021. Water samples were analyzed for physico-chemical parameters and nutrients. Zooplankton was subjected to diversity, density and other ecological studies. Atmospheric and surface water temperature were recorded with the help of a hand-held mercury thermometer, the values were noted after two minutes of stabilization. Estimation of dissolved oxygen (DO) was carried out following Winkler's method (Strickland and Parsons, 1968). pH of water was recorded using pH meter. Other chemical parameters such as nitrate, ammonia and phosphate were analysed using the test kits (Aquarium Pharmaceutical, Canada, Inc.).

For qualitative analysis, zooplankton were fixed and preserved in 5% formalin solution. Species identification was done following Rajendran (1973), Dussart and Defaye (1995), Dhanapathi (2000) and Altaff (2004). For quantitative analysis, 100 litres of subsurface water were filtered through the plankton net 60 µm mesh size of Bolten silk and the plankton concentration was made to 100 ml with the formalin. From this 1 ml is transferred to Sedgwick rafter counting cell using a wide mouth pipette for the enumeration. Animals in each square of the counting cell was counted and recorded. Likewise, enumeration was carried out thrice and the mean was taken. The number of zooplankton in 1 ml represents organisms per litre of the water. As far as the large zooplankton are concerned, such as fish larvae and prawn mysis, they were counted in the entire sample separately. The number of plankton per litre was calculated using the formula of Santhanam et al. (1989).

Results

Physico-chemical parameters of the different water bodies including Pudunagar pond, Pudunagar Lake, Kundur Lake and Koraiyar River water showed variation in their water quality during the study period (**Table 1**). Atmospheric temperature ranges from 26°C to 30°C. Minimum atmospheric temperature recorded in Pudunagar Lake (26°C) and maximum atmospheric temperature recorded in Koraiyar River (30°C). Surface water temperature ranges from 27°C to 29°C. Minimum surface water temperature was recorded in Pudunar lake and Gundur lake (27°C) and maximum temperature recorded in Koraiyar river (29°C). pH of the water ranges from 7.3 to 7.9. Minimum pH was recorded in Pudunagar pond (7.3) and maximum pH was recorded in Gundur Lake (7.9). Dissolved oxygen were ranges from 3.21 (mg/L) to 7.5 (mg/L). Minimum DO recorded in Koraiyar River (3.21 mg/L) and maximum DO recorded in Gundur Lake (7.5 mg/L). Nitrate was ranges from 0.4 mg/L to 3 mg/L. Minimum nitrate were recorded in Gundur lake (0.4 mg/L) and maximum nitrate recorded in Koraiyar river (3 mg/L). Ammonia was ranges from 0.15 mg/L to 4 mg/L. Minimum ammonia was recorded in Pudunagar Lake (0.15 mg/L) and maximum ammonia was recorded in Koraiyar River (4 mg/L). Phosphate of the water ranges from 0.5 mg/L to 5 mg/L. Minimum phosphate was recorded in Pudunagar Lake (0.5 mg/L) and maximum phosphate was recorded in Pudunagar pond (5 mg/L).

In the present study, there are 17 species of zooplankton belonging to 5 groups were recorded from four stations **(Table 2)**. Maximum diversity was recorded in Pudunagar pond (11 species) and minimum diversity was recorded in Koraiyar River (6 species). Pudunagar Lake (8 species) and Gundur lake (9 species) were recorded. In general, the rotifers *Brachionus calyciflorus,* cladoceran*Moina micrura,* ostracod *Hemicypris sp.*calanoid*Heliodiaptomus cinctus* and cyclopoid copepods *Thermocyclops decipiens* were predominant species of zooplankton in the present study.

However, the rotifer species *Brachionus falcatus* found only in Pudunagar pond and *Laccane bulla* found only in Gundur Lake. The cladoceran *Daiapnosoma sarsi* and *Pleuroxus aduncus* found only in Pudunagar pond. *Ilyocryptus spinifer*found only in Pudunagar Lake. *Alona rectangula rectangula* found only in Gundur Lake. The calanoid *Sinodiaptomus indicus* recorded only in Pudunagar pond.

Overall density of zooplankton is ranges from 18.67 to 61.67 ind./L in all the four station **(Table 3**). In general, minimum density was recorded in riverine environment (18.67±3.26 ind./L) and maximum density was recorded in Gundur lake (61.67±10.08 ind./L) and Pudunagar pond (41.33±8.75 ind./L).Station wise density showed much variation according to their habitat. In Pudunagar pond, minimum density belonging to ostracods (1.00±0.00 ind./L) and maximum density belongs to the cladocerans (17.67±3.79 ind./L) and calanoids (14.67±2.08 ind./L) other groups were below five individual per liters of the sample. In Pudunagar lake, minimum density was belongs to the ostracods (1.00±0.00 ind./L) and maximum density belonging to the calanoids (11.33±2.52 ind./L) and others were below five individual per liters of the sample. In Gundur lake, minimum density belonging to the rotifers and ostracods (1.33±0.58 ind./L) and maximum density belonging to the cyclopoids (32.00±5.29 ind./L) and calanoids (23.67±3.06 ind./L) other planktons were below five individual per litres of the sample. In Koraiyar river, minimum density belonging to the ostracods (1.00±0.00 ind./L) and maximum density belonging to cyclopoids (9.67±1.53 ind./L).

Overall percentage composition of zooplankton was presented in **Table 2**. In general, maximum percentage composition was occupied by cyclopoids (33%) followed by calanoids (32%), cladocerans (19%), rotifers (8%) and other zooplankton were below five percent. Individual station percentage composition showed much variation in different habitats. In Pudunagar pond, maximum percentage composition was occupied by cladocerans (43%) followed by calanoids (36%), cyclopoids (10%), rotifers (6%) and other zooplankton groups were below five percent. In Pudunagar lake, maximum percentage composition was occupied by calanoids (56%) followed by cyclopoids (16%), cladocerans (11%), rotifers (7%) and other zooplanktons were below five percentage. In Gundur lake, maximum percentage composition were occupied by cyclopoids (52%) followed by calanoids (38%) and other zooplanktons were below five percentage. In Koraiyar river, maximum percentage composition was occupied by cyclopoids (52%) followed by cladocerans (20%), rotifers (18%) and other zooplanktons were below five percentages.

Discussion

Physico-chemicalparameters are the most appropriate source to measure the water quality of any aquatic body. A slight change in the physico-chemical properties affects the biodiversity of the ecosystem.Various physico-chemical parameters of different waterbodies showed variations in their

habitats. Atmospheric temperature was less in Village habitat like Pudunagar Lake (26°C) and Pond (27°C) than City habitat like Trichy Gundur Lake (28°C) and Koraiyar River (30°C). This could be agricultural habitats surrounding Pudunagar Pond and Lake might reduce temperature whereas Trichy Gundur Lake and Koraiyar River free from agriculture and located Trichy city might be high temperature.

Sharma et al. (2013) referred this to as the surface heating and lesser mixing of the water unlike in the river part where turbulent and fast water current exists and allows an even distribution of the heat throughout the water column. pH of water is a measure of its acidity or alkalinity and most of the natural waters. In the present study the pH was alkalinein all the four stations 7.3 to 7.8. pH was lesser during monsoon monthscould be rain water runoff might reduce the pH while greater during post-monsoon due to no rainfall and dilution. Similar report was observed by Rao et al.,(2012). Srilatha et al.,(2012) reported that dissolved oxygen is one of the most important parameters, which reflects the physical and biological processes prevailing in the water. The dissolved oxygen content depends upon the photosynthetic activities, monsoonal floods and the turbulence caused by the winds. In the present study interestingly noted that, dissolved oxygen of the Koraiyar river was comparatively less than other stations indicated huge untreated sewage directly introduced into the river might increases the nutrience and microbial growth consequences higher metabolic rate as reported by Rao et al.,(2012) and Altaff et al.,(2019).

Nutrients constitute important inorganic components which fundamentally determine the productivity of water body. Nitrates, nitrites, phosphates, ammonia and silicates are the important nutrients. Any change in the concentrations of these nutrients usually promotes growth of some specific taxa of a class and disappearance of some taxa of belonging to another group. In the present study was observed that, accumulation of perennial domestic sewage inputs from Trichy city, agricultural input and Vanaspathi oil refinery company wastes might be reasons for nitrate (3mg/L), ammonia (4 mg/L) and phosphate (3 mg/L) availability during post-monsoon especially January 2021. While in Pudunagar Pond also higher nutrience including nitrate (1mg/L), ammonia (3 mg/L) and phosphate (5mg/L) might be duet input of Cow-dung manure in Fishculture practices. Significant increase in nitrate, phosphate, and ammonia and conversely, a decrease in DO concentration were noticed during the present study especially in Koraiyar River and Pudunagar Pond. These nitrogen compounds were derived from direct discharges of crude sewage and industrial effluents as earlier reported (Jones, 2006; Janakiraman et al.,2017).

Rajagopal et al.,(2010) reported that the presence of certain zooplankton species like *Moina, Keratella, Daphnia* and *Brachionus* are considered to be biological indicator for eutrophication.

Increasing temperature in aquatic systems puts stenothermic animals at risk of population extinction (Portner and Knust, 2007). In eurythermic animals such as the puddle cladoceran *M. macrocopa* increasing temperature to shift of energy allocation in favour of reproduction and away from body maintenance (Engert et al.,2012). Pond has rich diversity than reservoir and riverine ecosystem as reported by Starling (2000). In the present study, maximum diversity was recorded in Pudunagar pond (11 species) and minimum diversity was recorded in Koraiyar River (6 species). Pudunagar Lake (8 species) and Gundur lake (9 species) were recorded. In general, the rotifers *Brachionus calyciflorus,* cladoceran *Moina micrura,* ostracod *Hemicypris sp.*calanoid*Heliodiaptomus cinctus* and cyclopoid copepods *Thermocyclops decipiens* were predominant species of zooplankton in the present study indicating well adaptation. However, the rotifer species *Brachionus falcatus* found only in Pudunagar pond and *Laccane bulla* found only in Gundur Lake. The cladoceran *Daiapnosoma sarsi* and *Pleuroxus aduncus* found only in Pudunagar pond. *Ilyocryptus spinifer* found only in Pudunagar Lake. *Alona rectangula rectangula* found only in Gundur Lake. The calanoid *Sinodiaptomus indicus* recorded only in Pudunagar pond. It was interestingly noted that absence of calanoid copepods species and presence of rotifer *Brachionus* sp. and *Moina* sp.in Koraiyar River is evidence of polluted state.

Minimum density was recorded in Koraiyar riverine environment (18.67±3.26 ind./L) might be lotic water with much disturbance, hydrological variations, nutrience dilution by runoff and lower metabolic rate whereas maximum density in Gundur lake (61.67±10.08 ind./L) and Pudunagar pond (41.33±8.75 ind./L) might be lentic habitat with agricultural and domestic sewage inputs, least disturbance, less dilution, nutrience overload and higher metabolic rate. Similar findings were reported by Ismail and Zaidin2015; Pathani and Upadhyay (2006). , individual stations percentage composition showed variations in their compositions. In Pudunagar pond, the most dominant zooplankton were Cladocerans (43%) followed by Calanoid copepods (36%) indicating oligotrophic nature of the water. In Pudunagar Lake, the most dominant zooplankton were Calanoid copepods (56%) followed by Cyclopoid copepods (16%) indicating oligotrophic nature of the water. In Gundur Lake, the most dominant zooplankton were Cyclopoid copepods (52%) followed by Calanoid copepods (38%) indicating oligotrophic nature of the water whereas in Koraiyar River, the most dominant zooplankton were Cyclopoid copepods (52%) followed by Cladocerans (20%) and Rotifers (18%) indicating eutrophic nature of the water. Investigation concluded that Pudunagar pond and Lake has good water quality and free from domestic sewage and industrial activities whereas Koraiyar River and Gundur Lake received untreated domestic sewage from Trichy city and industrial effluents from Vanaspathi Oil industry pollute the water quality and favoring pollution dominant zooplanktonic species.

Therefore, regular restoration must be taken before discharging these inputs into the river to increase the zooplankton diversity as well as other aquatic biota.

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Table 1. Physico-chemical parameters of different waterbodies of Tamilnadu

	Pudunagar Pond	Pudunagar lake	Gundur Lake	Koraiyar River
Atm. Temperature (C°)	27	26	28	30
Surfacewater temperature (C°)	28	27	27	29
pH	7.3	7.5	7.9	7.8
Dissolved oxygen (mg/L)	6.2	7.3	7.5	3.21
Nitrate (mg/L)	1	2	0.4	3
Ammonia (mg/L)	3	0.15	0.50	4
Phosphate (mg/L)	5	0.5	2.5	3

Rotifers	**Pudunagar Pond**	**Pudunagar lake**	**Gundur Lake**	**Koraiyar River**
Brachionus calyciflorus	+	+	-	+
Brachionus falcatus	-	+	-	-
Brachionus caudatus	+	-	+	+
Laccane bulla	-	-	+	-
Cladocerans				
Moina micrura	+	+	-	+
Moina macrocopa	+	-	-	+
Macrothrix spinosa	+	-	+	-
Daiapnosoma sarsi	+	-	-	-
Pleuroxus aduncus	+	-	-	-
Ceriodaphnia carinata	-	+	+	-
Alona rectangula rectangula	-	-	+	-
Ilyocryptus spinifer	-	+	-	-
Ostracods				
Hemicypris sp.	+	+	+	+
Calanoids				
Sinodiaptomus indicus	+	-	-	-
Heliodiaptomus cintus	-	+	+	-
Cyclopoids				
Thermocyclops decipiens	+	+	+	+
Mesocyclops aspericornis	+	-	+	-
Total (17)	11	8	9	6

Table 2. List of Zooplankton recorded from different stations of Tamilnadu

Table 3. Density and percentage composition of Zooplankton (Mean ±SD) recorded in different water bodies

	Pudunagar Pond		**Pudunagar Lake**		**Gundur Lake**		**Koraiyar River**	
	Mean±SD	Percentage composition	Mean±SD	Percentage composition	Mean ±SD	Percentage composition	Mean ±SD	Percentage composition
Rotifers	2.67±0.58	6.45	1.33±0.58	6.56	1.33±0.58	2.16	3.33±0.58	17.85
Cladocerans	17.67±3.79	42.75	2.33±0.58	11.48	2.33±0.58	3.78	3.67±1.15	19.64
Ostracods	1.00±0.00	2.42	1.00±0.00	4.92	1.33±0.58	2.16	1.00±0.00	5.36
Calanoids	14.67±2.08	35.49	11.33±2.52	55.75	23.67±3.06	38.38	-	-
Cyclopoids	4.00±1.73	9.68	3.33±0.58	16.40	32.00±5.29	51.89	9.67±1.53	51.78
Others	1.33±0.58	3.23	1.00±0.00	4.92	1.00±0.00	1.62	1.00±0.00	5.36
Total	**41.33±8.75**	**100**	**20.33±4.25**	**100**	**61.67±10.08**	**100**	**18.67±3.26**	**100**

2. Biotechnology And its Applications in Aquaculture and Fisheries

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Abstract

Biotechnology provides powerful tools for the sustainable development of aquaculture, fisheries, as well as the food industry. Increased public demand for seafood and decreasing natural marine habitats have encouraged scientists to study ways that biotechnology can increase the production of marine food products, and making aquaculture as a growing field of animal research. Biotechnology in fish breeding Gonadotropins releasing hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. Transgenesis or transgenics may be defined as the introduction of exogenous gene / DNA into host genome resulting in its stable maintenance, transmission and expression. In teleosts, techniques for inducing sterility include exogenous hormone treatment and triploidy induction. The use of hormone treatments, however could be limited by governmental regulation and a lack of consumer acceptance of hormone treated fish products Disease problem area major constraint for development of aquaculture. Biotechnological tools such as molecular diagnostic methods, use of vaccines and immunostimulants are gaining popularity for improving the disease resistance in fish and shellfish species world over for viral diseases, avoidance of the pathogen in very important in this context there is a need to rapid method for detection of the pathogen. The technology of cryopreservation of fish spermatozoa (milt) has been adopted for animal husbandry. The first success in preserving fish sperm at low temperature who fertilizes Herring eggs with frozen thawed semen .The spermatozoa of almost all cultivable fish species has now been cryopreserved.

Keywords: breeding, transgenics, molecular diagnostic, cryopreservation, gene banking

Introduction

Biotechnology provides powerful tools for thesustainable development of aquaculture, fisheries, as well as the food industry. Increased public demand for seafood and decreasing natural marine habitats have encouraged scientists to study ways thatbiotechnology can increase the production of marine food products, and making aquaculture as a growing field of animal research. Biotechnology allows scientists to identify and combine traits in fish and shellfish to increase productivity and improve

quality. Scientists are investigating genes that will increase production of natural fish growth factors as well as the natural defense compounds marine organisms use to fight microbial infections. Modern biotechnology is already making important contributions and poses significant challenges to aquaculture and fisheries development. It perceives that modern biotechnologies should be used as adjuncts to and not as substitutes for conventional technologies in solving problems, and that their application should be need-driven rather than technology-driven. There is a growing demand for aquaculture; biotechnology can help to meet this demand. As with all biotech-enhanced foods, aquaculture will be strictly regulated before approved for market. Biotech aquaculture also offers environmental benefits. When appropriately integrated with other technologies for the production of food, agricultural products and services, biotechnology can be of significant assistance in meeting the needs of an expanding and increasingly urbanized population in the next millennium. Successful development and application of biotechnology are possible only when a broad research and knowledge base in the biology, variation, breeding, agronomy, physiology, pathology, biochemistry and genetics of the manipulated organism exists. .The potential area of biotechnology in aquaculture include the use of synthetic hormones in induced breeding, transgenic fish ,gene banking , uniparental and polyploidy population and health management.

Biotechnology in Fish Breeding

Gonadotropins releasing hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. GnRH is the key regulator and central initiator of reproductive cascade in all vertebrates. It is a decapeptide and was first isolated from pig and ship hypothalami with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH). Since then only one form of GnRH has been identified in most placental mammals including human beings as the sole neuropeptide causing the release of LH and FSH. However, in non-mammalian species (except guinea pig) twelve GnRH variants have now been structurally elucidated, among them seven or eight different forms have been isolated from fish species. The most recent GnRH purified and characterized was by Carolsfeld and Robinson. Depending on the structural variant and their biological activities, number of chemical analogues have seen prepared and one of them is salmon GnRH analogue profusely used now in fish breeding and marked commercially under the name of Ovaprim throughout the world. The induced breeding of fish is now successfully achieved by development of GnRH technology.

Transgenesis

Transgenesis or transgenics may be defined as the introduction of exogenous gene / DNA into host genome resulting in its stable maintenance, transmission and expression. The technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important fishers, mollusks and crustaceans for aquaculture.

The idea of producing transgenic animals became popular when Palmitter et al. (1982) first produced transgenic mouse by introducing metallothionines human growth hormone fusion gene (mT- hGH) into mouse egg, resulting in dramatic increase in growth.

The technique has now seen successfully applied to a number of fish species. An increased resistance of fish to cold temperatures has been another subjectof research in fish transgenics for the past severalyears. Coldwater temperatures pose a considerable stressor to many fish and few are able to survive water temperatures much below 0-1°C. This is oftena major problem in aquaculture in cold climates. Interestingly, some marine teleosts have high levels (10 to 25 mg/ml) of serum antifreeze proteins (AFP) or glycoproteins (AFGP) which effectively reduce thefreezing temperature by preventing ice-crystal growth. Similarly, injection or oral administration of AFP to juvenile milkfish or tilapia led to an increase in resistance to a 26 to 13° C. drop in temperature**.** The most promising tool for the future of transgenic fish production is development of the embryonicstem cell (ESC) technology.

Although significant progress has been made in several laboratories around the world, there are numerous problems to be resolved before the successful commercialization of the transgenic brood stock for aquaculture. To realize the full potential of the transgenic fish technology in aquaculture, there include,

I. More efficient technologies for mass gene transfer
II. Targeted gene transfer technologies such as embryonic stem cell gene transfer
III. Suitable promoters to direct the expression of transgenes at optimal levels during the desired developmental stages
IV. Identified genes of desirable traits for aquaculture and other applications
V. Information's on the physiological, nutritional, immunological and environmental factors that maximize the performance of the transgenics of the transgenics and
VI. Safety and environmental impacts of transgenic fish.

Biotechnology and Fish Healthmanagement

Disease problem area major constraint for development of aquaculture. biotechnological tools such as molecular diagnostic methods, use of vaccines and immunostimulants are gaining popularity for improving the disease resistance in fish and shellfish species world over for viral diseases, avoidance of the pathogen in very important in this context there is a need to rapid method for detection of the pathogen. Biotechnological tools such as gene probes and polymerase chain reaction (PCR) are showing great potential in this area. Gene probes and PCR based diagnostic methods have developed for a number of pathogens affecting fish. In case of finfish aquaculture, numbers of vaccine against bacteria and viruses have been developed. Some of these have been conventional vaccines consisting of killed microorganisms but new generation of vaccine consisting of protein subunit vaccine genetically engineered organism and DNA vaccine are currently under development. In the vertebrate system, immunization against disease is a common strategy. Recent studies have shown that the non-specific defense system can be stimulated using, microbial product such as lipopolysacharides, peptidoglycans or glucans. Among the immunostimulants known to be effective in fish glucans and levamisole enhance phagocytic activities and specific antibody responses

Cryopreservation of gametes or genebanking

Cryopreservation is a technique, which involve long- term preservation and storage of biological material at a very low temperature usually at -196°C, the temperature of liquid nitrogen. The technology of cryopreservation of fish spermatozoa (milt) has been adopted for animal husbandry. The first success in preserving fish sperm at low temperature was reported by Blaxter (1953) who fertilizes Herring(*Clupea herengus*) eggs with frozen thawed semen. The spermatozoa of almost all cultivable fish species have now been cryopreserved. Cryopreservation overcomes problems of male maturing before female, allow selective breeding and stock improvement and enables the conservation (Harvey,1996). One of the emerging requirements for thatcan be used by breeders for evolving new strains. Most of the plant varieties that have been produced are based on the gene bank collections. Aquaticgene bank however suffers from the fact that at present it is possible to cryopreserve only the malegametes of finishes and there in no viable technique for finfish eggs and embryos. Therefore, it is essential that gene banking of cultivated and cultivable aquatic species be undertaken expeditiously.

Conclusion

Biotechnological research and development are growing at a very fast rate. The biotechnology has assumed greatest importance in recent years in the development of fisheries, agriculture and human health. The science of biotechnology has endowed us with new tools and tremendous power to create

novel genes and genotypes of plants, animals and fish. The application of biotechnology in the fisheries sector is a relatively recent practice. Nevertheless, it is a promising area to enhance fish production. The increased application of biotechnological tools can certainly revolutionize our fish farming besides its role in biodiversity conservation. The paper briefly reports the current progress and thrust areas in the transgenesis, use of synthetic hormones in fish breeding, biotechnology in health management and gene banking.

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3. Green Synthesis of Zinc Oxide Nanoparticles Using Medicinal Plant *Acalypha indica* L. And Its Characterization

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Abstract

Nanotechnology distribute with the invention and usage of material with nanoscale dimension. Nanoscale dimension provides nanoparticles a large surface area to volume ratio and thus very specific properties. Zinc oxide nanoparticles (ZnO NPs) had been in recent studies due to its large bandwidth and high exciton binding energy and it has potential applications like antibacterial, antifungal, anti-diabetic, antiinflammatory, wound healing, antioxidant and optic properties. Due to the large rate of toxic chemicals and extreme environment employed in the physical and chemical production of these NPs, green methods employing the use of plants, fungus, bacteria, and algae have been adopted. It is known that the biological synthesis of nanoparticles is gaining importance due to its simplicity, ecofriendliness and extensive antimicrobial activity. Also, in this study we report the synthesis of ZnO nanoparticles using *Acalypha indica* L. leaf extract. The prepared ZnO nanoparticles have been characterized by UV-VIS absorption spectroscopy, Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM). The UV-VIS spectrum of ZnO nanoparticles revealed a characteristic surface plasmon resonance (SPR) peak at 430 nm. The UV–VIS absorption spectra of the pure ZnO NPs exhibited absorption peaks in the UV region which were attributed to the band gap of the ZnO NPs. FTIR spectra revealed the involvement of sulfate and hydroxyl moieties of polysaccharide in the formation of ZnO NPs. SEM analysis shows that the pure ZnO NPs of AI synthesized have hexagonal structures and the average size ranged from 32 to 55 nm.

Keywords: Nanoparticles, Zinc oxide, phytochemical analysis, SEM, UV-VIS.

1. Introduction

The synthesis of nanoparticles has been considered as the preference field in the nanotechnology sector due to the properties of materials based on size (Prakasham et al.,2014). Nowadays, the green synthesis of metal nanoparticles is an interesting issue of nanoscience and also, there is a growing attention to the biosynthesis of metal nanoparticles using organisms. Among these organisms, plants seem to be the best candidates and they are suitable for the large-scale biosynthesis of nanoparticles.

Nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms (Ramesh et al.,2014). Metal oxides with nanostructure have attracted considerable interest in many areas of technology (Sangeetha et al.,2011). Among metal oxide nanoparticles, zinc oxide (ZnO) has received much attention in the recent past. ZnO nanostructures are the forefront of research due to their unique properties and wide applications (Rouhi et al.,2013).

There are different methods used for the synthesis of zinc oxide nanoparticles: direct precipitation, homogeneous precipitation, solvothermal method, sonochemical method, reverse micelles, sol gel method, hydrothermal, thermal decomposition, and microwave irradiation (Kolekar et al.,2013). The biological method of the synthesis of ZnO nanoparticles is gaining importance due to its simplicity, ecofriendliness and extensive antimicrobial activity (Gunalan et al.,2012). According to Maranty *et al.*,2013, the use of eco-friendly biosynthesized nanoparticles as an alternative to the chemically synthesized ones would help control chemical toxicity in the environment.

According to Theodore, 2006, ZnO usage may overtake nano-titanium dioxide (nTiO2) in the near future as it can absorb both UV-A and UV-B radiation while nTiO2 can only block UV-B, and thereby offering better protection and improved opaqueness. Several physical and chemical procedures have been used for the synthesis of large quantities of metal nanoparticles in relatively short period of time. Chemical methods lead to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in medical application (Jain et al.,2009). Currently, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity (Khandelwal *et al.*,2010; Saxena et al.,2010). Biosynthesis of zinc oxide nanoparticles by plants such as Aloe vera (Sangeetha et al.,2011) and gold nanoparticles by *alfalfa* (Gardea *et al.*,2003), *Cinnamomum camphora* (Huang *et al.*,2007), neem (Shankar *f.*,2004), *Emblica officianalis* (Ankamwar *et al.*,2005), lemongrass (Shankar *et al.*,2004) and tamarind (Ankamwar *et al.*,2005) have been reported. Based on the literature, the synthesis of ZnO nanoparticles using plants has been carried out using milky latex of *Calotropis procera*, *Aloe vera* extract (Salam *et al.*,2014), *Ocimumbasilicum L. var. Purpurascens*, *Parthenium hysterophorus L.* (Rajiv *et al.*,2013), *Citrus aurantifolia* extract (Samat *et al.*,2013), *Plectranthus amboinicus* (Vijayakumar *et al.*,2015). Moreover, the synthesis of ZnO nanoparticles using orange juice was reported by Jha *et al.*,2011. Yuvakkumar *et al.*,2015 used *Nephelium lappaceum L. (rambutan)* peel extract in the biosynthesis of zinc oxide nanocrystals. The advantage of using ZnO nanoparticles is that they strongly inhibit the action of pathogenic microbes when used in small concentration (Applerot *et al.*,2006).

Many of the plants used today were known to the people of ancient cultures throughout the world and were highly considered their preservative and medicinal powers. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century. India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant derived drugs have been a part of the evolution of human healthcare for thousands of years .Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced in India such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant based drugs are commonly used in India and China. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. These substances are usually found in several parts of plants like root, leaf, shoot and bark.

Acalypha indica (Euphorbiaceae) is an herb distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in a variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The paste of plant leaves is used for the treatment of skin diseases by rural people. Acalypha indica which belongs to Euphorbiaceae family commonly called Indian Copperleaf grows along the sides of the road which is often mistaken as a weed plant inspite of immense medicinal properties. Indian Copperleaf is a small erect herb, growing up to 60 cm or more. The ascending branches are angled and velvet-hairy. Leaves are broadly ovate, nearly triangular, rather coarsely toothed. Leaf stalks are as long as or longer than the 3-5 cm long blades. Flowers are stalkless, borne on erect axillary spikes longer than the leaves. Male flowers are minute, crowded distally (Indira, 2015).

Female flowers are scattered along the inflorescence axis, each subtended by a conspicuous semicupular leaf-like toothed green bract nearly 7 mm long. Capsule is bristly, 1 mm broad. Some of the medicinal properties used by folk are: Juice of the root and leaves given to children as expectorant and emetic. The leaves, in decoction or powdered form, are used as a laxative. For constipation, an anal suppository of the bruised leaves helps relax the constricted sphincter ani muscle. In Philippines, decoction of leaves used for dysentery. Leaves mixed with common salt applied to scabies. In Indian pharmacopoeia, it is used as an expectorant. Also used for the prevention and reversal of atherosclerotic disease. Used for pneumonia, asthma and rheumatism. In Tamilnadu, India, the Paliyar

tribes of Shenbagathope use the entire plant for bronchitis, a decoction of the herb for toothand ear aches and paste of the leaves is applied to burns.

Poultice of bruised leaves used for syphilitic ulcers, to maggot-eaten sores and as an emollient to snake bites. Decoction of leaves used as instillation for earaches and for periauricular poultice or compress. Leaves mixed with garlic used as anthelminthic Root, bruised in water, and use as a cathartic. Powdered dried leaves used for bed sores. Juice of, fresh leaves, mixed with oil or lime, and are used for rheumatic complaints. Bruised leaves used as "suppository" in constipation, assumed to work through decrease of the sphincter anti contraction (Indira, 2015).

In our present study, we have reported green synthesis of zinc oxide nanoparticles using *A. indica* leaf extract without using any toxic chemicals. To the best of our knowledge, biogenic green approach using *A. indica* leaf extract has been used for the first time as a reducing material as well as surface stabilizing agent for the synthesis of ZnO nanoparticles. The structure, phase and morphology of synthesized product were investigated by the standard characterization technique. In this research paper we have reported biosynthesis of zinc oxide nanoparticles using aqueous extract of *A. indica* leaf. The present study is the continuation to assess the preliminary phytochemical analysis and characterization of *A. indica* mediated zinc oxide nanoparticles.

1.1.Objectives

- To synthesis the zinc oxide nanoparticles of *Acalypha indica* L. aqueous leaf extract
- To characterize the synthesized zinc oxide nanoparticles of *Acalypha indica* L. aqueous leaf extract
- To analyze the preliminary phytochemical analysis of the zinc oxide nanoparticles of *Acalypha indica* L. aqueous leaf extract

2. Materials And Methods

2.1. Collection of plant

Leaves of the plant *Acalypha indica* L. were collected from Thanjavur district, Tamil Nadu, India and taxonomically identified by Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The leaves were picked and washed with water to remove all unwanted debris (Fig 1).

Fig 1: Plant *Acalypha indica* L.

Botanical classification		
Kingdom	**:**	Plantae
Subkingdom	**:**	Tracheobionta
Superdivision	**:**	Spermatophyta
Division	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Subclass	**:**	Rosidae
Order	**:**	Euphorbiales
Family	**:**	Euphorbiaceae
Genus	**:**	*Acalypha* L.
Species	**:**	*Acalypha indica* L.
Vernacular name	:	Kuppaimeni

2.2. Preparation of extract

For aqueous extraction, add 50 g of dried leaf powder immersed in 250 ml of distilled water for 24 hrs at room temperature and then boiled for 15 min on slow heat. Then the residue was removed by filtering through four layer of muslin cloth to obtain filtrate. Again, the filtrate was centrifuged at 5,000 rpm for 15 min and supernatant was collected. Finally, the extract was preserved at 4ºC for further use (Gopalakrishnan *et al.*,2013).

2.3. Synthesis of Zinc oxide nanoparticles

ZnO nanoparticles were prepared by refluxing precursor zinc acetate dihydrate (0.1 M) in diethylene glycol and triethylene glycol at 180 ℃ and 220 ℃ respectively. Reaction time varied for 2 and 3 h with and without sodium acetate (0.01 M). Before refluxing, the solution was kept on a magnetic stirrer at 80 ℃ for 1.5 h. After completion of reflux action, the samples were centrifuged at 8000 rpm for 15 min and washed with distilled water and ethanol for three times. Further, it was dried at 80 ℃ for overnight.

2.4. Preliminary phytochemical screening for plant extract

The presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins in the plant *Acalypha indica* (L.) were analysed by the standard methods of Harborne, 1973.

1. Alkaloids:

Hager's test

To a few ml of filtrate 1 or 2 ml of Hager's reagent (Saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the presence of Alkaloids.

Wagner's test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish – brown precipitate confirmed the test as positive.

Wagner's reagent

Iodine (1.27 g) and potassium iodide (2 g) was dissolved in 5 ml of water and made up to 100 ml with distilled water.

2. Test for Steroids:

Salkowski's test

About 100mg of Pedalium murex dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour ring at the interface was an indicative of the presence of steroid.

3. Test for Cardiac glycosides:

Keller killiani's test

About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de oxy sugar characteristic of cardenolides.

4. Test for Saponins:

Foam Test:

The extracts were diluted with 20ml of distilled water and agitated in graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponins.

5. Test for Resins:

To 20 ml of plant extract, 5 to 10ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5ml of H_2SO_4 was added. Bright purple colour indicates the presence of resins.

6. Test for Phenols:

Lead Acetate Test:

50 mg of extract was dissolved in distilled water and 3ml of 10% Lead acetate solution was added. A bulky white precipitate indicated the presence of phenol compound.

7. Test for flavonoids:

Alkaline Reagent Test

To the 2 ml of aqueous solution of plant extract add few drops of Ammonium hydroxide. Formation of a yellow fluorescence indicated the presence of flavonoids.

8. Test for Tannins:

Lead acetate test

In a test tube containing about 5ml of an aqueous extract, a few drops of 1% solution of lead acetate was added. Formation of a yellow or white precipitate indicated the presence of tannins.

9. Test for Terpenoid:

2 ml of chloroform and 1ml of conc. H_2SO_4 was added to 1mg of extract and observed for reddish brown colour that indicated the presence of terpenoids.

10. Test for Carbohydrate:

Fehling's test

Equal volume of Fehling solution A (copper sulphate in distilled water) and Fehling solution B (potassium tartarate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.

Fehling's Solution:

Solution **A**: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml distilled water.

Solution **B**: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made up to 500 ml.

11. Test for Gum and Mucilage:

The extract (100mg) was dissolved in 10ml of distilled water, then add 25ml of absolute alcohol with constant stirring. White or cloudy precipitate indicated the presence of gum and mucilage.

12. Test for Quinone:

A few drops of conc. H_2SO_4 or NaOH solution was added to the test solution. The change of colour was taken as an evidence for the presence of quinone.

13. Test for phlobatannins:

The test solution was mixed with 1 ml of 1% magnesium acetate solution. A purple colouration was formed to show positive result for the presence of anthraquinone.

14. Test for Coumarins:

One percent of alkaline KOH solution was added to the test solution. The formation of golden yellow colour indicates the presence of coumarins.

2.5. Characterization

UV-VIS Spectral analysis

The synthesized ZnO NPs were characterized using various spectroscopic and microscopic techniques. UV–visible spectrum was evaluated using UV–Visible spectrophotometer (Shimadzu UV-2450) and the spectrum was recorded between 300 and 800 nm (Huzaifa *et al.*,2019).

FTIR Analysis

Fourier transform infrared (FTIR) analysis of the ZnO NPs of *A. indica* was carried out with Fourier transform spectrometer (Shimadzu FT-IR Prestige-21 Model) at a frequency range of 4,000–500 cm^{-1} (Huzaifa *et al.*,2019).

SEM Analysis

Morphological analysis of the synthesized ZnO NPs of *A. indica* coated with platinum was carried out using scanning electron microscope (SEM) (JOEL JSM 6335-F) equipped with 150 kV acceleration voltage, and energy-dispersive (Huzaifa *et al.*,2019).

3. RESULTS AND DISCUSSION

3.1. Preliminary phytochemical analysis of ZnO NPs of *Acalypha indica* L.

Table 1: Preliminary phytochemical analysis for ZnO Nanoparticles of *Acalypa indica* L.

Compound	PM
Alkaloids	+ +
Flavonoids	+
Steroid	+
Saponins	+
Quinones	+
Resins	+
Phenols	+
Cardiac glycosides	-
Tannin	+
Terpenes	+
Phlobatannins	-
Carbohydrates	+
Gum and Mucilage	+
Coumarin	+

(+) Present; (-) Absent

Qualitative preliminary screenings of extracts were performed initially with different chemical reagents to detect the phytochemical constituents present in ZnO NPs of aqueous extract *A.indica*. The extract shows the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids and phenolic compounds (Table 1 & Fig 2).

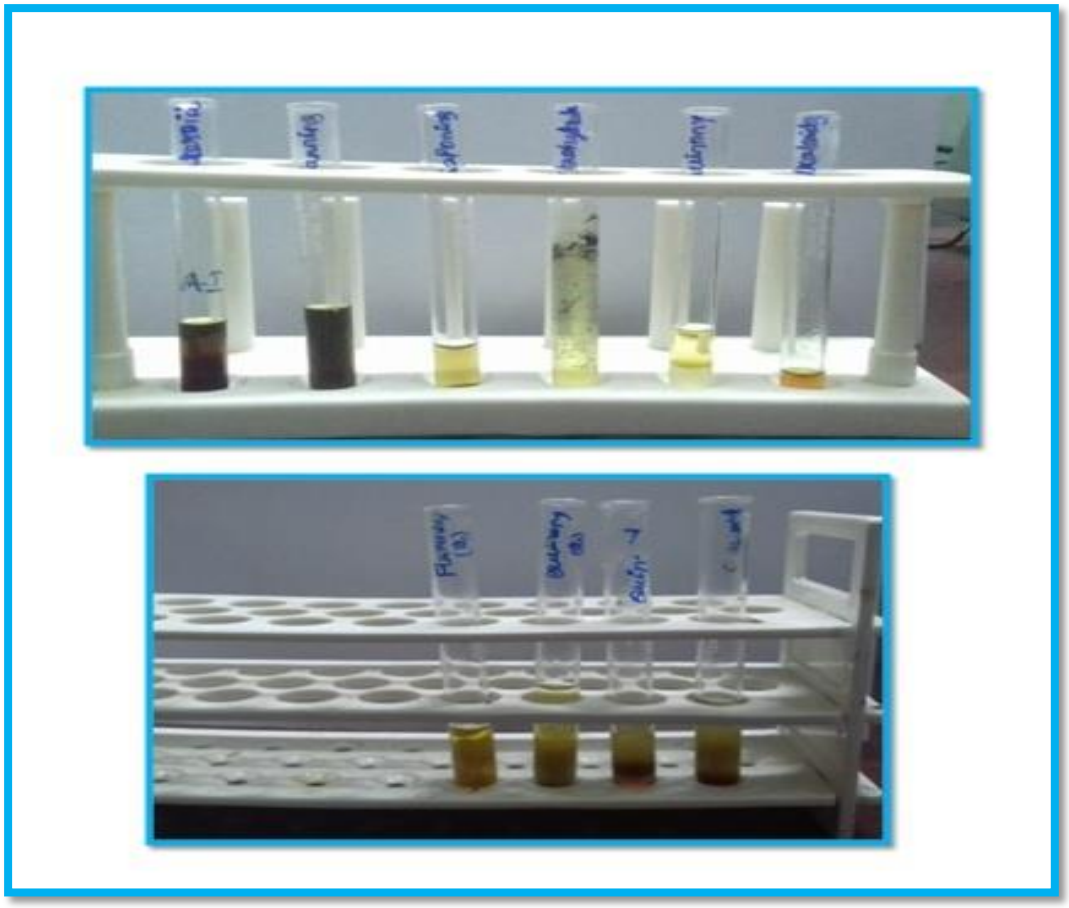


Fig 2: Preliminary phytochemical analysis ZnO NPs of *Acalypha indica* L.

The powdered whole plant of *Acalypha indica* was individually extracted with aqueous solvent. The colours of the extracts were noted. The preliminary phytochemical screening of the extracts *Acalypha indica*, showed the presence of alkaloids, glycosides, phenols, tannins, saponins and Steroids. Preliminary organic analysis of drugs helps to undertake further studies on the isolation and identification of specific chemical constituents. Due to the presence of different phytochemical compounds of *Acalypha indica* leaf extracts has efficient anti-microbial activity against different micro-organisms. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. The water soluble compounds present in the aqueous extract were found to be responsible for efficient stabilization of nanoparticles and reduction of metal ions.

3.2. Characterization of ZnO NPs of *Acalypha indica* L.

3.2.1. UV-VIS Absorbance of ZnO NPs of *Acalypha indica* L.

The absorbance spectrum of ZnO NPs was studied and investigated (Fig: 3). The absorbance spectrum of ZnO NPs synthesized *Acalypha indica* L. shows the absorbance range of 430 nm. The transparency in visible range of the ZnO NPs decreases with increase in leaf extract. The changes in optical transmittance are because of *Acalypha indica* extract concentrations and the UV–VIS spectra the sharpness of the absorption peak is dependent on the concentration of leaf extract, thus being sharper with a higher concentration of leaf extract (Saxena *et al.*,2012).

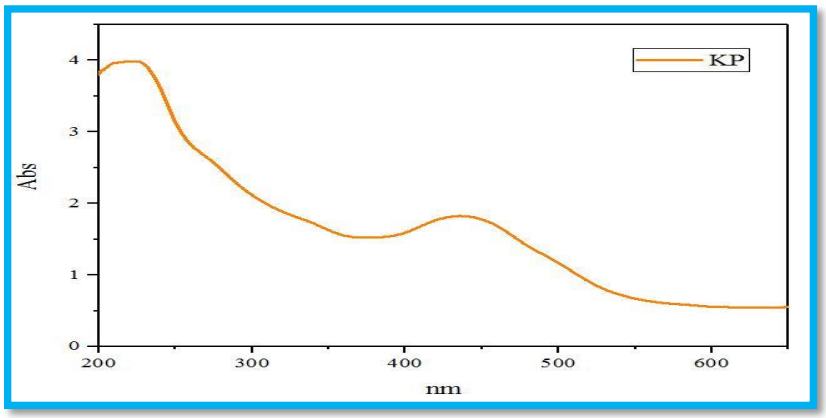


Fig 3: UV-VIS spectra of ZnO NPs synthesized *Acalypha indica* L.

4.2. FTIR analysis ZnO NPs synthesized *Acalypha indica* L.

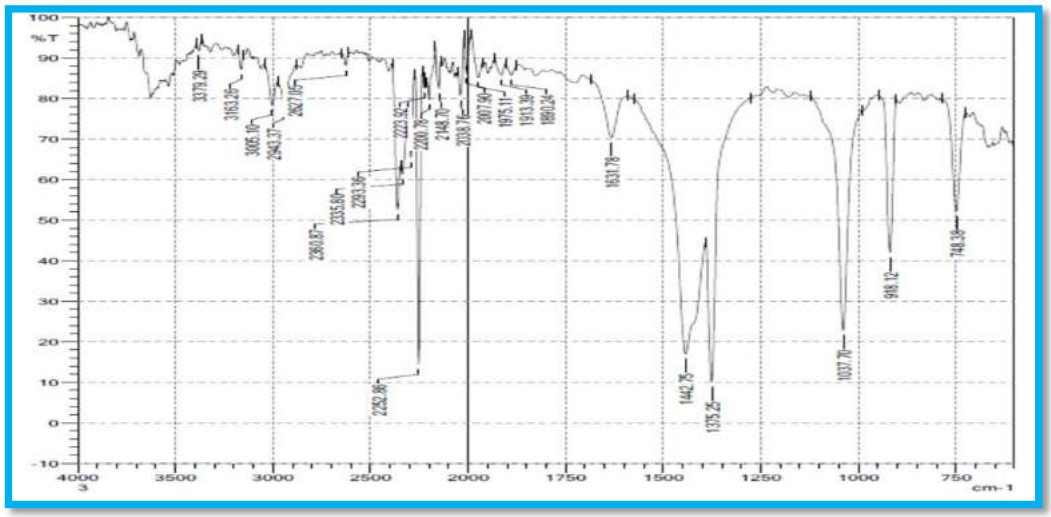


Fig 4: FTIR analysis of ZnO NPs synthesized *Acalypha indica* L.

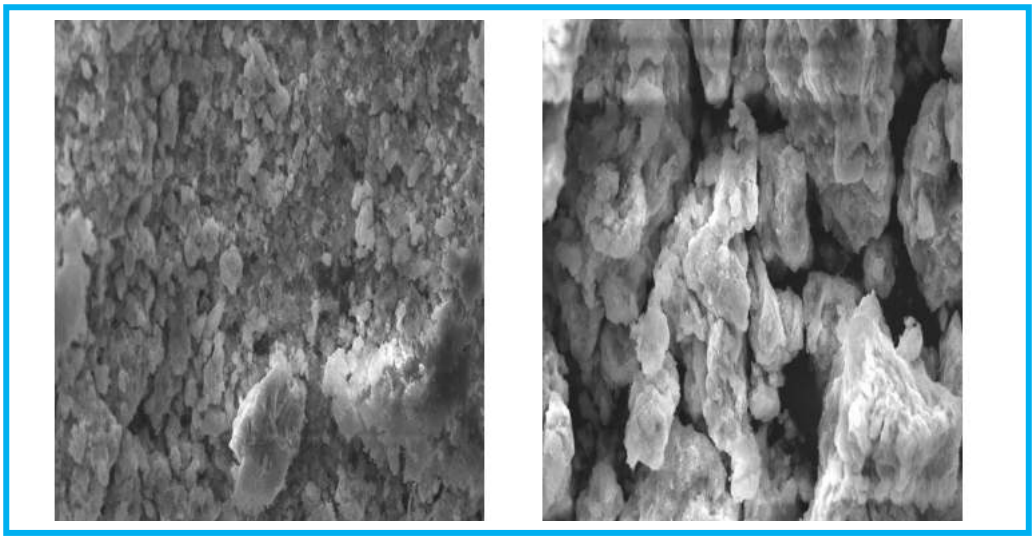
Figure -4 indicates that strong absorbance were observed at peak 3379.29 cm^{-1}, 3163.26 cm^{-1}, 3005.10 cm^{-1}, 2627.05 cm^{-1}, 2252.86 cm^{-1}, 2148.70 cm^{-1}, 1442.75 cm^{-1}, 1375.25 cm^{-1}, 1037.70 cm^{-1}, 918.12 cm^{-1}, 748.38 cm^{-1} which are characteristic of N–H stretch, –C ≡ C–H: C–H stretch, =C–H stretch, H–C=O: C–H stretch, C≡ N stretch, –C≡C– stretch, C–H bend, C–H rock, C–N stretch, O–H bend, C–Cl stretch and Presence of functional groups such as 1°& 2° amines, amides, alkynes (terminal), Alkenes, Aldehydes, Nitriles, Alkanes, aliphatic amines, carboxylic acids and alkyl halides (Fig 4 & Table 2). Our FTIR analysis confirms that phenolic compounds in flavonoids are better able to bind to metal, indicating that a phenolic group may form metal nanoparticles to prevent agglomeration and to thereby stabilize a medium. This suggests that biological molecules may perform dual functions in forming and stabilizing zinc oxide nanoparticles in an aqueous medium. Gnanasangeetha and colleagues found the formation and stabilization of zinc oxide nanoparticles due to the presence of terpenoids present in plant extracts azadirachta and emblica (Gnanasangeetha and Thambavani, 2014). In this work, aglycone steroids may have played a major role in stabilizing and capping zinc oxide nanoparticles.

FREQUENCY, CM^{-1}	**BOND**	**FUNCTIONAL GROUP**
3379.29	N–H stretch	1°, 2° amines, amides
3163.26	–C ≡ C–H: C–H stretch	alkynes (terminal)
3005.10	=C–H stretch	Alkenes
2627.05	H–C=O: C–H stretch	Aldehydes
2252.86	C≡ N stretch	Nitriles
2148.70	–C≡C– stretch	Alkynes
1442.75	C–H bend	Alkanes
1375.25	C–H rock	Alkanes
1037.70	C–N stretch	aliphatic amines
918.12	O–H bend	carboxylic acids
748.38	C–Cl stretch	alkyl halides

Table 2: FTIR analysis of ZnO NPs of *A. indica* L.

3.3. SEM Analysis

The morphology of the ZnO NPs using *Acalypha indica* extract was analyzed by SEM analysis. In SEM images demonstrated that the particles were nanoclusters structure with some surface agglomeration (Fig 5).



4. SUMMARY AND CONCLUSION

- Many of the plants used today were known to the people of ancient cultures throughout the world and were highly considered their preservative and medicinal powers.
- Nanotechnology has energetically developed as an indispensable field of modern research with probable effects in electronics, medicine and biotechnology. Nanotechnology can be defined as a research for the design, synthesis and manipulation of structure of particles with dimension smaller than 100nm
- Nanotechnology combines with biological principles with chemical and physical procedure to spawn nano sized particles with precise functions. An imperative branch of biosynthesis of nanoparticles is the purpose of plant extract to synthesis nanoparticles. The plant phytochemicals like terpenoids, flavonoids, alkaloids present in the aqueous leaf extract with antioxidant property were accountable for the preparation of metal oxide nanoparticles.
- Synthesis of Zinc oxide nanoparticles has attracted considerable attraction owing to their diverse properties like catalysis, magnetic, optical polarazibality, electrical conductivity and antimicrobial activity.

- The rapid green synthesis of zinc nanoparticles using leaf extract of *Acalypha indica* L. provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. The biological production of metal nanoparticles is becoming a very important field in chemistry, biology and materials science. Metal nanoparticles have been produced chemically and physically for a long time; however, their biological production has only been investigated very recently.
- The biological reduction of metals by plant extracts has been known since the early 1900s; however, the reduction products were not studied. The harmful and toxic reducing and stabilizing agents can be avoided by using the plant extracts.
- In the presence of strong oxidizing substances only Zinc nanoparticles can exist as ions. ZnO is non toxic it can be used as photocatalytic degradation materials of environmental pollutants. ZnO nanoparticles prepared from *Acalypha indica* L. leaf extract are expected to have more extensive application in biotechnology, sensors, medical, DNA labeling, drug delivery and water remediation.

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4. Growth and Haematological Status of *Clarias Gariepinus* Fingerlings Fed *Jatropha Tanjorensis* Leaf Powder Meal

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Abstract

Many rural communities in Nigeria use leaves of *Jatropha tanjorensis* as a cheap, safe and biodegradable alternative to antibiotics. This informed the use of different inclusion levels of *J. tanjorensis* leaf powder meal (JLM) in the diets of *Clarias gariepinus* fingerlings to assess their growth and haematological status. Seventy-five (75) healthy and acclimatized fingerlings weighing between 3.5 and 4.5 g were stocked in 5 indoor plastic tanks (100X50 cm) at a density of 5 fingerlings per tank in triplicates. They were fed *ad libitum* with different graded levels of experimental meal (0%, 10%, 20%, 30%, 40%) for 98 days. Basic water quality variables were maintained at their optimal levels throughout the experiment. Results showed that highest mean weight gain (363 g) was obtained from fish fed 30% JLM, while least (200.1g) was obtained from those fed with 0% JLM giving. Similarly, Specific growth rate (SGR) was highest (5.50 % per day) in 30% group and lowest (4.78 % per day) in 0% group. Feed conversion rates (FCR) ranged from 0.83 to 1.50 while mortality rates ranged from 6.67% in 30% group to 26.67% in 0% group. Haematological analysis revealed that Pack Cell Volume (PCV) was highest (32.4%) in 30% group and lowest (24.6%) in 0% group. Red Blood Cell was highest (1.87×10^9/l) in 30% group and lowest (1.16×10^9/l) in 10% group. White Blood Cell was highest (44.4×10^9/l) in 30% group and lowest (27.2×10^9/l) in 0% group. Haemoglobin was highest (11.6g/dl) in 30% group and lowest (9.1g/dl) in 0% group. When modelled for dose and time as factorials, the result revealed that growth was dose- and time- dependent while haematological status was dose- rather than time- dependent. Based on these results, this study considered 30% inclusion level of JLM as best for the culture of *C. gariepinus*.

Keywords: Fish feed, alternative ingredients, dose/time dependence.

Introduction

Jatropha tanjorensis belongs to the family Euphorbiaceae and it shows intermediary phenotypic characters between *J. gossypifolia* and *J. curcas* (Prabakaran and Sujatha, 1999). It is commonly called hospital too far, Catholic vegetable, Iyana-ipaja, lapalapa (Iwalewa *et al.*,2005). In Nigeria, the

leaf of *J. tanjorensis* has been used locally, consumed as vegetable and is popular as a natural remedy against diabetes in this region (Ehimwenma and Osagie, 2007).

J. tanjorensis has received a lot of attention due to its possible health benefit, availability and affordability. Phytochemical screening of *J. tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycoside, anthraquinones and saponions (Omoregie and Osagie, 2007). Pharmacological studies revealed that the plant showed some wide range of biological activities, which include antihypertensive, antioxidant, antimicrobial, antimalarial, hypoglycaemic, hypolipidemic and haematological activities. Although there is a growing popularity efficacy of herbal medicines, scientists still advocate proper physiological and toxicological tests in order to ensure safety in the use of traditional medicines (Oyewole, 2007).

In aquaculture, hormones, antibiotics, vitamins and several synthetic products have been used as growth promoters, anti-bacterial and other purposes, though they have been reported to have positive effects on fish and shrimps, it has been observed that they cannot be recommended in commercial culture operations due to their residual effects in the muscles of fish, shrimps and subsequently man (Obaroh *et al.*,2018). To replace their activity on growth and haematology, there is need to find natural, cheap and biodegradable alternatives.

Haematology is an indicator of immunological status and can provide definitive diagnosis of fish during toxicant exposure (Akinrotimi *et al.*,2007). Haematological indices are of different sensitivity to various environmental factors and chemicals (Akinrotimi *et al.*,2013). Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in values of one or more of the haematological parameters of aquatic organisms (Akinrotimi *et al.*,2007). Sampath *et al.* (1993) noted that there is a possibility that studies on fish blood might reveal conditions within the body of the fish long before there is any outward manifestation of disease. The haematocrit or pack cell volume (PCV), red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb) levels have been reported to vary with diet of the fish (Dienye and Olumuji, 2014). Analysis of the blood samples is important because it helps in confirming disease diagnosis and response to treatment of such diseases. Haematological parameters have found widespread use for the evaluation of physiological environment and husbandry stressors in fishes (Rainza-Paiva *et al.*,2000). Recently, balanced diets have been recommended as effective ways of reducing undue stress in fish culture (Gabriel *et al.*,2007). The change in the blood status of fish caused by stress due to exposure to poor quality feed, feed toxicity, diseases or other environmental stressors have been reported by Ezeri (2001) and Gabriel *et al*. (2001).

Clarias gariepinus (African sharp tooth catfish) is a species of a family Claridae (Teugels, 1996). It is a large eel like fish, usually of dark grey or black coloration on the back fading towards a white belly. An average adult length of 1–1.5 m it reaches a maximum total length (TL) of 1.7 m and up to 60 kg of weight. It cannibalizes on other animals and also feeds on dead animal matter. They are able to crawl on dry ground to escape drying ponds. It is able to survive on shallow mud for a long period of time between rainy season. It can be found throughout Africa, Middle East and inhabit estuaries, lakes, rivers, swamps as well as man-made enclosures.

Fish are sensitive to toxic components present in plants. The dosing of the plant extracts in increasing amounts helps to evaluate the toxicity limits (Parra *et al.*,2001). Sub-acute toxicity studies using animal models provide important preliminary data that helps to select natural remedies with potential health benefits for future work (Rosenthal and Brown, 2007). Toxicity effects of natural remedies in animals and humans are analysed using some physiological parameters like behaviour, body weight, food intake, biochemical, and haematological variables (Ahmad *et al.*,2013). It is against this background that the present study seeks to assess the effect of *J. tanjorensis* leaf powder meal on growth and haematology of *C. gariepinus*.

Aim and Objectives

i. To determine the effect of *Jatropha tanjorensis* leaf powder meal (JLM) on the haematology of *Clarias gariepinus* fingerlings.
ii. To determine the growth rate of *Clarias gariepinus* fed with JLM.
iii. To estimate safe dosage of JLM in feed of *Clarias gariepinus.*

Methodology

Plant sample collection, preparation and extraction

Fresh leaves of *Jatropha tanjorensis* (Figure 1) were obtained from Botanical Garden University of Calabar, Nigeria. Plant leaves were processed in the laboratory to powder as recommended by Musa *et al*. (2000).

Proximate Analysis of *Jatropha tanjorensis* leaf

Moisture and ash contents were determined as described by Naczk *et al*. (2002). The formulae are as follows:

$$\%\, moisture\ content\ = \frac{Weight\ of\ Can\ +\ Sample\ after\ drying}{Weight\ of\ Can + sample\ before\ drying} \times 100$$

$$\% \, Ash \; = \frac{Weight \; of \; ash}{weight \; of \; sample} \times 100$$

The crude fat content was determined using the Soxhlet extract method. The formula is as given below:

$$\% \, Crude \; fat \; = \frac{Weight \; of \; ether \; extract}{Weight \; of \; sample} x \, 100$$

Crude protein content of each of the sample was determined using the Kjeldahl procedure. The formula is as given below:

$$\% \, N \; = \frac{Tr \; x \; n \; x \; 14g}{Weight \; of \; sample}$$

% Crude protein = % N x 6.25

where n = number of samples,

Tr = sample titre,

14g = the molecular weight of Nitrogen

6.25 = protein conversion factor.

Nitrogen Free Extract (NFE)

The nitrogen free extract content was determined by subtracting the percentage of moisture, protein, fat and ash from 100. All determinations were in triplicates and the same was repeated for all the species and the values recorded.

NFE = 100% - (% Moisture + % Protein % Fat + % Ash).

Experimental feed

The powdered meal of *J. tanjorensis* produced were mixed directly with the basal diet. It was added to the diets at concentration of 0%, 10%, 20%, 30%, and 40% of feed labelled D0 – D4 respectively (Table 1). Compounded feeds were pelletized (2mm) using the pelletizing machine, sundried, allowed to cool in an open air, packed and stored in an opaque nylon bag.

TABLE 1: Feed ingredients used at different inclusion levels of *Jatropha tanjorensis* powder (JTP) meal at 45% crude protein for *Clarias gariepinus* fingerlings

	Experimental diets (%)				
Ingredients	**D0**	**D1**	**D2**	**D3**	**D4**
JTP meal	0	10	20	30	40
Soybean meal	40	30	20	10	0
Fish meal	22	22	22	22	22
Groundnut cake	13	13	13	13	13
Yellow maize	15	15	15	15	15

Vitamin premix	3	3	3	3	3
Vegetable oil	2	2	2	2	2
Methionine	1	1	1	1	1
Lysine	1	1	1	1	1
Bone meal	2	2	2	2	2
Salt	1	1	1	1	1
Total	100	100	100	100	100

DO = 0%, D1 =10%, D2 =20%, D3 =30%, D4 = 40% JLM

Fish collection and stocking

A total of seventy-five (75) healthy *C. gariepinus* fingerlings of mean weight ranging between 3.5 - 4.5g were obtained from the University of Calabar Fish Farm. The setup adopted a completely randomized design. Fingerlings were acclimatized for 14 days prior to feeding trials. At the end of the acclimatization period, fish were divided into 5 groups, each containing 3 sub-groups representing the treatments namely; D0, D1, D2, D3, and D4. Fingerlings were stocked in 5 indoor plastic tanks (100X50 cm) at a density of 5 fingerlings per tank in triplicates. They were fed *ad libitum* with different graded levels of experimental meal (0%, 10%, 20%, 30%, 40%) for 98 days. Basic water quality variables were maintained at their optimal levels throughout the experiment.

FIG. 1: Picture of *Jatropha tanjorensis* (Hospital too far)

Monitoring Fish Growth

Fish specimens were weighed in each tank every two weeks using a sensitive electronic weighing scale to monitor the fish growth. Mortality was also monitored daily and recorded.

Growth Variables

The following growth parameters were calculated at the end of the study as demonstrated by Agbebi *et al*. (2012):

1. Mean weight gain (MWG) = Final weight (g) of fish – Initial weight (g) of fish
2. Feed conversion ratio (FCR) = Dry weight of feed fed (g) / fish weight gained

3. Specific growth rate (SGR) = lnW2-lnw1/ Time (days of experiment) x 100
   Where, W1 = Initial weight gained
   W2 = Final weight gained
   Ln = Natural logarithm
4. Survival rate = no. of fish at the end of experiment / no. of fish at the beginning of the experiment x 100

Blood samples were collected with syringe and preserved in disodium salt of ethylene-diamine tetra-acetic acid bottles for haematological analysis. Haemoglobin (Hb) was determined with the cyano-haemoglobin method. Packed cell volume (PCV) was determined with the microhaematocrit method. Red blood cell (RBC) was determined with the improved Neubauer haemocytometer. White Blood Cells (WBC) was determined with the improved Neubauer counter.

Statistical Analysis

Data obtained from the experiment were subjected to analysis of variance to ascertain significant differences in haematological variables of fish fed different inclusion levels (0%, 10%, 20%, 30%, 40%) of *Jatropha* leaf powder meal (JLM). Regression analysis was carried out to determine the relationship between average SGR/FCR and levels of JLM. Acceptable value of $p<0.05$ was considered to be statistically significant.

Results

Chemical composition of raw *Jatropha* leaf and its powder

The chemical composition of raw *Jatropha* leaf and its powder is presented in Table 2. The results showed that the powder contained less moisture content (1.5%) compared with the raw leaf extract (5.3%). Higher crude protein and fat (37.8%, 30.1%) were also observed in powder extract as compared to the raw leaf (31.1%, 28.1%) respectively; higher ash and gross energy (5.5%, 597 kcal g^{-1}) were respectively observed for *Jatropha* powder compared to 5.2% and 570 kcal g^{-1} observed for raw *Jatropha* leaf. Crude fibre was also higher (4.3%) in raw extract compared with 3.9% observed in leaf powder. Generally, both extracts possess relatively good nutritional composition but the powder is preferred in aquaculture because of its increasing shelf life.

TABLE 2: Chemical composition of raw *Jatropha* leaf extract and its powder

Chemical Composition	Raw *Jatropha* leaf extract	*Jatropha* leaf powder
Moisture contents	5.3	1.5
Crude protein	31.1	37.8
Crude fat	28.1	30.1
Crude ash	5.2	5.5
Crude fibre	4.3	3.9
Nitrogen Free Extract (NFE)	31.3	24.7
Gross energy (kcal g^{-1})	570	597

Nitrogen Free Extract (NFE) = 100- (sum of crude protein, fat, ash and fibre)

Growth response of *C. gariepinus* to experimental diets

Weekly average weight gain ranged from 1.7 g to 201.0 in the control group, 1.5 g to 279.2 g in group fed with 10% JLM, 1.7 g to 320.7 g in group fed with 20% JLM, 1.5 g to 363 g in group fed with 30% JLM, 1.7 g to 251.4 g in group fed with 40% JLM. As shown in Figure 2, average weight gain showed both dose and time dependency.

At the end of the 96-day experimental period, results showed that highest average weight gain (363.0 g) was obtained from fish fed 30% JLM, while least (200.1 g) was obtained from those fed 0% JLM. Similarly, Specific growth rate (SGR) was highest (5.50 % per day) in 30% group and lowest (4.78 % per day) in 0% group. Feed conversion rates (FCR) ranged from 0.83 to 1.50 while mortality rates ranged from 6.67% in 30% group to 26.67% in 0% group (Table 3).

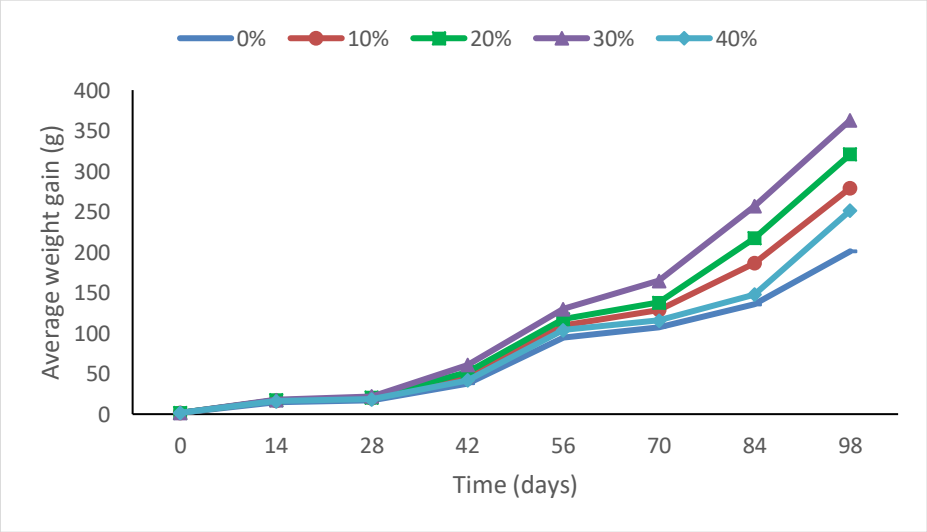

FIG. 2: Average weight gain of *C. gariepinus* fingerlings fed different inclusion levels of JLM
Plotted points represent means (n=5)

TABLE 3: Growth variables and mortality of *Clarias gariepinus* fingerlings fed *Jatropha* leaf powder meal

Dose/ Growth variables	0%	10%	20%	30%	40%
Initial length (cm)	5.5	5.6	5.6	5.5	5.4
Final length (cm)	31.8	36.1	36.7	38.1	33.7
Initial weight (g)	1.7	1.5	1.7	1.5	1.7
Final weight (g)	205.6	279.2	320.7	368.5	251.4
Mean weight gain (g)	200.1	273.6	315.1	363.0	246.0
Specific Growth Rate (%/day)	4.78	5.22	5.23	5.50	4.99
Feed Conversion Ratio	1.50	1.10	0.95	0.83	1.22
Mortality rate (%)	26.67	13.33	13.33	6.67	6.67

Regression model I

There was significant relationship ($r^2 = 0.9145$) between mean specific growth rate and levels of JLM (Fig. 3). Similarly, mean feed conversion ratio showed significant association ($r^2 = 0.8126$) with concentration of JLM (Fig. 4). However, the models could not account for the respective 8.55% and 18.74% variation flagged by the association between SGR/FCR and inclusion levels of JLM in diets of *C. gariepinus*.

Haematological status of *C. gariepinus* exposed to experimental diets

Statistical analysis of variance revealed significant differences ($P<0.05$) in the levels of haematological variables at different inclusion levels of JLM in diets of *C. gariepinus* (Table 4). According to the haematological analysis, Pack Cell Volume (PCV) was highest (32.4%) in 30% group and lowest (24.6%) in 0% group. Red Blood Cell (RBC) was highest (1.87×10^9/l) in 30% group and lowest (1.16×10^9/l) in 10% group. White Blood Cell (WBC) was highest (44.4×10^9/l) in 30% group and lowest (27.2×10^9/l) in 0% group. Haemoglobin (Hb) was highest (11.6g/dl) in 30% group and lowest (9.1g/dl) in 0% group.

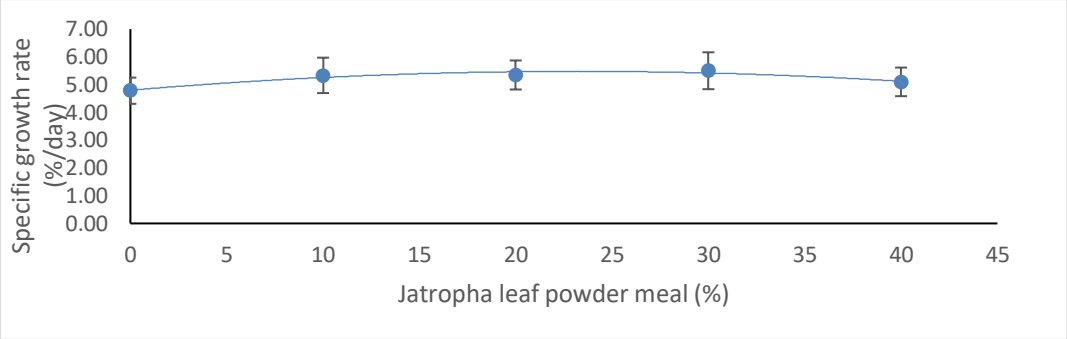

FIG. 3: Relationship between mean specific growth rate and levels of JLM in diets of *C. gariepinus*.
Plotted points represent means ± SEM (n=5)
Regression equation: $y = 4.7981 + 0.0586x - 0.0013x^2$; $r^2 = 0.9145$

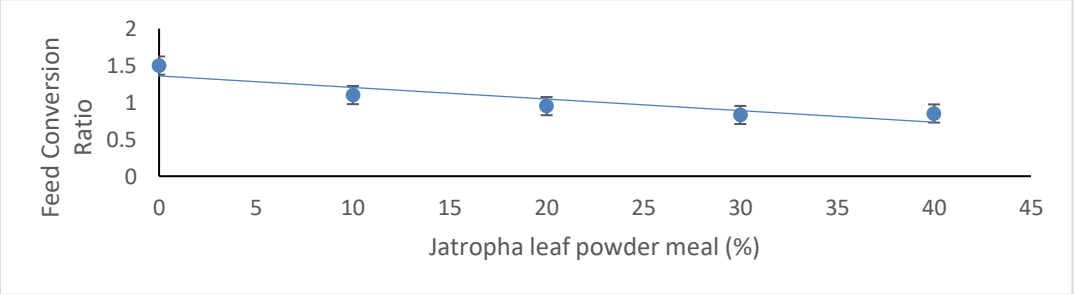

Fig. 4: Relationship between mean feed conversion ratio and levels of JLM in diets of *C. gariepinus*.
Plotted points represent means ± SEM (n=5)
Regression equation: $y = 1.36 - 0.0157x$; $r^2 = 0.8126$

TABLE 4: Some haematological parameters of *Clarias gariepinus* fingerlings fed different inclusion levels of *Jatropha* leaf powder meal

	0	10	20	30	40
PCV (%)	24.6±0.12^{a}	30±0.21^{b}	26.2±0.10^{c}	32.4±0.22^{d}	26.2±0.12^{c}
RBC (× 10^9/l)	1.49±0.07^{a}	1.16±0.10^{b}	1.6±0.08^{ac}	1.87±0.08^{d}	1.76±0.07^{cd}
WBC (×10^9/l)	17.2±0.24^{a}	24.2±0.13^{b}	34.4±0.19^{c}	44.4±0.08^{d}	35.6±0.11^{e}
Hb (g/dl)	9.1±0.12^{a}	10.1±0.10^{b}	10.1±0.14^{b}	11.6±0.10^{c}	10.4±0.05^{d}

^{abcd}Means (±SEM) with different letters in the same row are significantly different at P<0.05

PCV = Pack Cell Volume, RBC = Red Blood Cell, WBC = White Blood Cell, Hb = Haemoglobin

Regression model II

Multivariate regression analysis revealed significant relationship ($r^2 = 0.606$) between haematological variables and dose levels of JLM. However, the haematological variables did not show any significant association ($r^2 = 0.018$) with culture period (Table 5). The results therefore revealed that haematological status was dose- rather than time- dependent.

TABLE 5: Relationships between haematological variables and dose level of JLM/culture time

Model	Independent variable	R	R^2	R^2 (Adjusted)	Standard error	F	Sig.
1	Dose	.778[a]	.606	.592	0.90715	44.161	0.000
2	Time	.133[a]	.018	-.016	2.31980	0.517	0.723

[a]Dependent variables: Hb, WBC, RBC, PCV

PCV = Pack Cell Volume, RBC = Red Blood Cell, WBC = White Blood Cell, Hb = Haemoglobin

Discussion

The present study on growth and haematological status of *Clarias gariepinus* fingerlings fed different inclusion levels of *Jatropha tanjorensis* leaf powder meal (JLM) revealed significant differences ($P<0.05$) among treatments. Fish fed 30% JLM was considered best in the culture of *C. gariepinus*. This finding agrees with a previous study of Al-Thobaiti *et al*. (2018) which showed significant higher growth response to plant meal when compared with commercial fish meal. However, the report of Dienye and Olumuji (2014) showed that haematological parameters of fish fed with Moringa leaf meal decreased with increasing inclusion levels. This is contrary to the present finding which showed otherwise increase in haematological status as the inclusion levels of JLM increased. Reason for the observed difference could be due to the phytochemical contents present in the different plant species used in the present and past studies.

All the haematological variables recorded in this study increased as the inclusion levels of JLM increased and were found to be within the recommended physiological ranges reported for *C. gariepinus*. The range of PCV (24.6 to 32.4%) observed in this study is within the range (21.00 to 32.00%) reported by Dienye and Olumuji (2014). White blood cells (WBC) and red blood cells (RBC) counts were observed to be higher than those reported by Sotolu and Faturoti (2009). White blood cells are the defence cells of the body. Douglas and Jane (2010) demonstrated that their counts have implication in immune responses and the potency of the animal to withstand toxic or unconducive environment or fight infection. The haemoglobin range (9.1 – 11.6 g/dl) observed in the present study

was within the range reported by Sowunmi (2003) but higher than 4.46 g/100 ml reported for *Heterotis niloticus* (Fagbenro *et al.*,2000). The high range of haemoglobin concentration observed in the present study may be due to the capability *C. gariepinus* to undergo anaerobic metabolism. The increase in the level of haemoglobin as JLM increased in the diets of *C. gariepinus* could simply mean that diets with higher inclusion levels of JLM had positive effect on the blood.

The present study reveals the possibility of utilizing *J. tanjorensis* leaf powder in the diet of *C. gariepinus* fingerlings. As shown in the study, the proximate composition of the raw *J. tanjorensis* leaf and its powder agrees with the findings of Kumar *et al.* (2008) who reported 35.3% and 35.6% crude protein for prepared and raw *Cyprinus carpio*. Alatise *et al*. (2014) also reported similar findings of 31.23% and 37.75% crude protein for raw and boiled *Jatropha* kernel.

Previous studies have also reported similar growth rate in the diet of *C. gariepinus* replaced with portentous feeds of plant origin. A comparison between *Jatropha* leaf powder meal and soybean revealed similar pattern for all essential amino acids except lysine and sulphur amino acid in the work of Alatis *et al*. (2014). It is a well-established fact that protein is given priority in fish feed and production. This is because among other nutritional requirements, protein is the most required in large amount. It is also the most expensive of all, so replacement with low cost product without compromising the health of culture organisms or general production output has been a thing of great concern to aquaculturists.

In their study, Fagbenro *et al*. (2010) showed the need to adopt plant based protein diets in the culture of fish and other aquatic organisms. Although toxic and other anti-nutritive substances in plant based feed can affect feed intake with attendant poor growth, drying and conversion to powder is one of the methods adopted in this study to remove anti-nutritional factors in *Jatropha* leaf meal. This resulted in improvement of quality feed, reduction in cost of production and growth of fish.

Conclusion

Based on the findings of this study, it was concluded that *C. gariepinus* fingerlings exhibited the best growth rate and haematological status when fed with 30% inclusion level of JLM.

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5. Anti-dandruff Activity of Commercially Available Shampoos

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Abstract

Anti-dandruff shampoos like synthetic or herbal are marketed to combat the problem of dandruff. To test the effect of synthetic and herbal shampoos on dandruff causing fungus to isolate the species Malassezia. The fungal species was isolated using Potato Dextrose Agar medium. Total six synthetic and herbal shampoos were selected to check the effectively against dandruff by agar cup method. Different level (0.1 ml, 0.5 ml, 1ml) of shampoos were taken to check the Zone of Inhibition. The selected shampoos were effective as they showed inhibition against dandruff. Synthetic shampoos were more effective when compared to herbal shampoos. The highest zone of inhibition was obtained by Head &Shoulders > Pure derm > All clear and Dove. Herbal anti-dandruff shampoos were found to be an effective but their anti-dandruff activity was less compared to synthetic one. Currently, herbal hair is high demand for strengthen for negligible side effects.

Keywords: Malassezia, Synthetic, Dandruff, Shampoo, Scalp.

Introduction

Dandruff is a common scalp condition that can affect anyone and it is a huge problem found in people who exposed to dust and other pollutants in day-to-day life, 5% of the population are affected from dandruff after puberty in the range of 20- 30 years, it is a common scalp disorder and also for a cosmetic problem as it leads to hair fall, and dandruff affects in males than the females. Excessive shedding of skin cells and flaking from the scalp is the reason for causing the dandruff [1-3] for Seborrheic dermatitis, Psoriasis, fungal infection or excoriation are associated with infestation of head lice. The pathogenesis of dandruff involves hyperproliferation, resulting in deregulation of keratinization. Inflammation on scalp skin along with the appearance of oily scales of dirty yellow color that can form lesions. These lesions can be associated with pruritis of different intensity. The most affected area of dandruff is scalp, but it also occurs between eyebrows, along the side of nose, behind the ears, over the breastbone and sometimes in the armpits [4]. Many factors are responsible for the dandruff such as fungal infection, hormonal deficiency, weather conditions, poor hygienic, strain, anxiety, excessive

use of hair spray, gels, alkaline soaps, share combs, hair brushes, inadequate rinsing of hair and irregular shampooing [5]. Adequate exposure of sunlight is also the reason for dandruff, it is known as desquamation of the scalp [6]. Dandruff usually occurs as small, round, white to grey patches on the top of the head. It is not a serious problem but make you feel socially uncomfortable and mortified. It is not a spreadable disease and can be controlled. Dandruff is normal for skin cells to die and flake off is common. Depending on the severity the symptoms can vary from mild to severe. It is determined by the flakes on the scalp [7]. Malassezia furfur is a type of fungus belongs to the Malassezia species. It is the causative organism for dandruff [8-10]. Malassezia is a lipophilic, dimorphic fungus occurs in the human skin cause superficial and deep mycoses such as Pityriasis versicolar, Seborrheic dermatitis [11,12] Being an opportunistic pathogen it causes a disease such as dandruff. Malassezia species formerly known as Pityrosporum [13]. Dandruff medically known as Pityriasis capitis is caused by Malassezia furfur, Malassezia globasa and Malassezia restricta [14,15].

Today, many treatment options are available for the control of dandruff includes therapeutic use of antidandruff shampoos containing Miconazole, Ketoconazole, Keratolytics, antimicrobials like Zinc pyrithione, selenium sulphide, salicyclic acid, lmidazole derivatives,

- Fungicidal substance - Eg. (Pyrithione andimidazoles)
- Cytostatic substance - Eg. (Tar, selenium sulphide and octopirox)
- Keratolytic substances- Eg. Salicyclic acid and sulphur compounds) (Adamsk) [24].
- Synthetic anti-dandruff shampoo

Herbal anti-dandruff shampoo sulphur and tar derivatives [16]. They have certain limitations because poor clinical efficacy or due to acquiescence issues. In market the number of anti-dandruff shampoos is readily available in various forms such as powder, clear liquid, lotions, solid gel, medicated and liquid herbal shampoo [17-19]. Based on the type of ingredients used, it may be simple shampoo, anti-dandruff shampoo, anti-septic shampoo, shampoo containing vitamins, amino acid and protein hydrolysate called as a nutritional shampoo. It may require for the washing of hair and scalp packed in a form which is fitting for use they may be anionic, cationic, and non- ionic surfactants [20,21]. The gentle shampoos to reduce oiliness and mild dandruff but regular shampoos are not valid in dandruff, so they are used antidandruff shampoos, but all the antidandruff shampoos are not alike. Shampoo is hair care product the primary role of shampoo is rinsing or detergent action, lubrication, conditioning, prevention of static charge gradually builds up in hair, but the removal of dandruff also one of the important characteristics of a good shampoo, the complete formulation of shampoos is must be

medically safe for long term usage is important for the hairy region. In Worldwide, so many traditional systems are available, practiced and prove that throughout the areas including Ayurvedic medicine, Unani, and Traditional Chinese Medicine can be used as medicine and maintaining good health [22]. They also represent an excellent source of secondary metabolites for growing food additives and pharmaceuticals industries. The demand for natural products are highly increased in Worldwide. The use of medicinal plants and cosmetic purposes is inextricably linked to ancient and modern cultures of Asian countries. Skin is outer covering and protecting of the human body that provides the environment from unfavourable external factors [23].

Depending on the formulation shampoos, oils, creams, lotions, and other cosmetic products are available in the market. These formulations include therapeutic use of antidandruff agents that are classified into three groups according to their mechanism of actions.

Two types of antidandruff shampoos are available commercially, the word herbal or Ayurveda is a symbol of safety when compare to synthetic one which has adverse effects on human health. In Ayurveda, the several plants are useful effectively against various causes of dandruff [25]. Today the plants have a major part that involve in skincare and cosmetic, WHO estimates that up to 80% of the people used natural products.

Commonly used plants in herbal shampoos	
S. No	Name of the plants
1	Piper betle
2	Hibiscus rosa-sinensis
3	Datura metal
4	Citrus aurantifolia
5	Camellia sinensis
6	Ficus bengalensis
7	Lawsonia inermis
8	Phyllanthus emblica
9	Ocimum sanctum
10	Zingiber officinalis

Materials and Methods

Culture: The collected samples were cultured on PDA medium which was incorporated with chloramphenicol to get rid of the bacterial contaminants. Small amount of the samples collected were

introduced into petri dishes containing media using sterile forceps. The petri plates were labelled appropriately and incubated at 30℃ for 2days.

Biochemical test

Catalase test: Catalase test was carried out to confirm the presence of fungal species to test whether it is positive or negative. 3 ml of 3 % hydrogen peroxide (H_2O_2) solution was poured into a test tube. T - the isolated fungal colonies were immersed into the test tube using a sterile glass rod.

Agar Cup Method: ACM was performed to check the antifungal activities of shampoo. Two days' prior small amount of culture was inoculated into Potato Dextrose Agar was used to prepare plates was maintained for this assay. Each plate contained a well of 0.6 cm in diameter the different shampoos were added to the well using pipette. Experiments were done using suitable controls.

Anti-fungal Activity

Table 1: List of Anti-dandruff shampoos used during the experiment work

S.No	Name of Anti-dandruff shampoo	Active Ingredients	Manufacturer
1	Head & Shoulders	Zinc Pyrithione	Procter & Gamble
2	All Clear	Zinc Pyrithione	Hindustan Unilever
3	Himalaya	Tree Tea Oil	Himalaya Herbs Health care
4	Dove	Zinc Pyrithione	Hindustan Unilever
5	Pure derm	Oxy fused micro bubble	Hindustan Unilever
6	Ayush	Rose marry oil	Lever Ayush
7	Panjanli	Hibiscus, Biringarag	OEM Manufacture
8	Karthika shikakai	Hibiscus, shikakai	Cavinkare
9	Meera shikakai	Green gram, Thulsi	Cavinkare
10	Homemade shikakai	Hibiscus, shikakai	Home
11	Karthika shampoo	Hibiscus, Fenugreek	Cavinkare
12	Meera shampoo	Sodium lauryl sulpate	Cavinkare

Zone of Inhibition (ZOI)

ZOI was done on PDA plates by agar cup method before two days' active culture was inoculated into the PDA medium. All the shampoos were dissolved in 9 ml sterile distilled water. The same procedure

is done to check the zone of inhibition by incubating at 30°C for 24hours. After incubation, the plates were observed. The zone of inhibition was measured using a zone measuring scale and the results were observed.

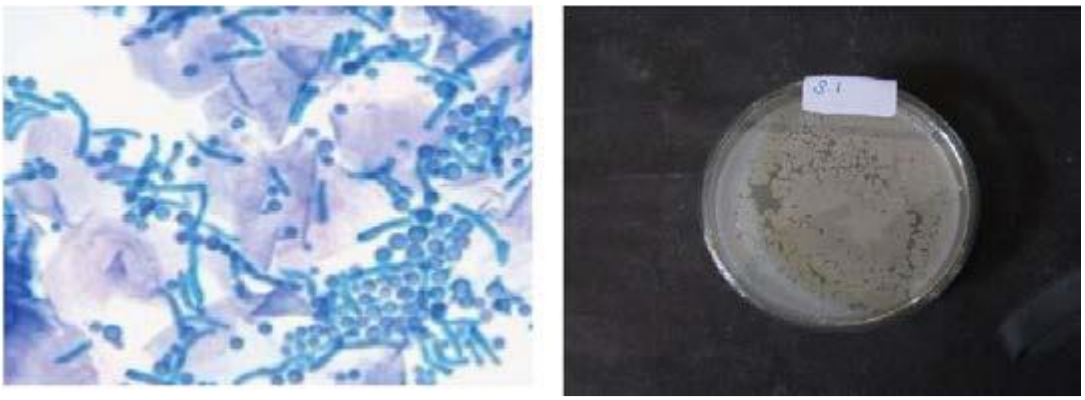
Statistical analysis

All the analyses were calculated using the Statistical Package of Social Sciences (SPSS) software package version 16.0. Results with $P < 0.05$ were considered to be statistically significant.

Results

Isolation of fungal species: The collected samples were streaked over the surface of Potato Dextrose Agar and 3 different colonies were obtained. Further, these colonies were individually streaked in separate potato dextrose agar plates. According to the morphological features. Malassezia was identified and then inoculated in PDA medium. The sample was analyzed on the basis of direct microscopy of the collected sample of scalp containing dandruff ovoid shaped cells.

Figure 1: Isolation of fungal species from the Potato Dextrose Agar plates



Biochemical test: Catalase test was performed which showed active bubbling, the isolated colonies of fungi were inoculated in a test tube containing 3 ml of 3 % H_2O_2, to indicating positive result.

Figure 2: Catalase test (anti-fungal activity)

Anti-fungal Activity

Agar Cup Method: Agar cup method was performed throughout the experiment for different samples (Shampoos) to check the inhibition levels of dandruff

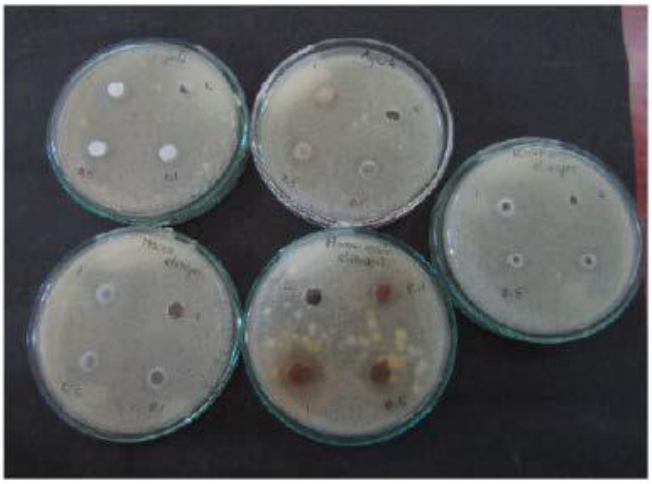
Table 2: Shampoos and its Zone of Inhibition

Name of shampoo	Zone of Inhibition in diameter (in cm)
Head & Shoulders	2
All Clear	2.3
Dove	1.8
Pure derm	2.3
Homemade shikakai	1.3
Karthika shikakai	0.4
Meera shikakai	0.4

Figure 3: Preliminary test of anti-fungal activity

Zone of Inhibition (ZOI): Zone of Inhibition was performed with different levels of anti-dandruff shampoos and to check efficiency of shampoos in terms of inhibition.

Figure 4: Synthetic and herbal based shampoos were tested



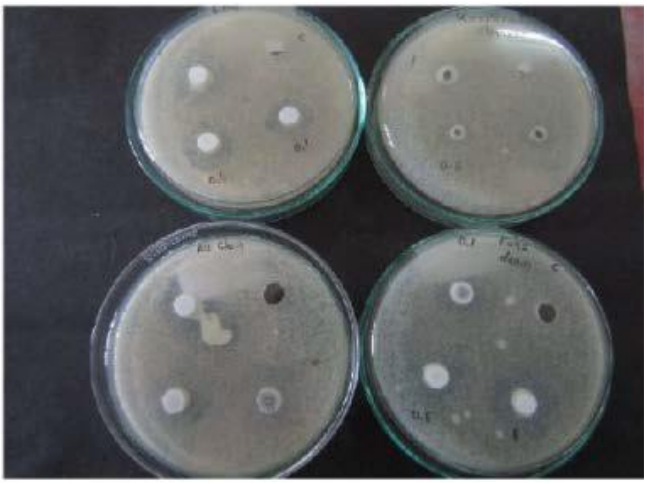


Figure 5: Confirmatory test of anti-dandruff activity using different shampoos

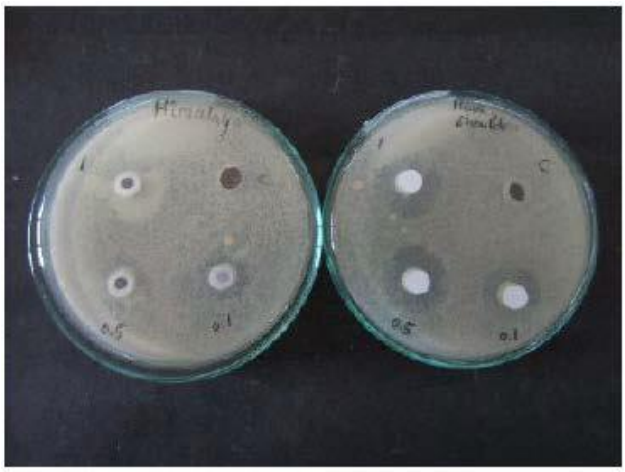


Table: 3 Measurement of their zone of different shampoos

S.No	Name of shampoo	0.1 ml	0.5ml	1ml
1	Head & Shoulders	1.5 cm	1.9cm	2.3 cm
2	All Clear	1.8 cm	2cm	2.4 cm
3	Himalaya	0.1 cm	0.5cm	1.8 cm
4	Dove	1.3 cm	1.8cm	2.2 cm
5	Pure derm	1.6 cm	2cm	2.7 cm
6	Ayush	_	0.3cm	0.5 cm
7	Pantanjali	_	0.1cm	0.3 cm
8	Karthika shikakai	_	0.1cm	0.2 cm
9	Meera shikakai	_	0.2cm	0.3 cm
10	Homemade shikakai	_	0.6cm	1 cm
11	Karthika shampoo	_	_	0.3 cm
12	Meera shampoo	_	_	0.2 cm

Discussion

Plant extract shows moderate zone of inhibition and local herbal shampoo shows potent antifungal activity, due to the presence of Shikakai, *Aloe vera*, Methi, Soap nuts and *Amla* Prakruthivanam shampoo [29]. But, our study reveals that the synthetic shampoos show more antifungal activity when

compare to natural hair wash. Herbal extracts area valid alternative for the chemical preparations. Herbal shampoos and poly herbal hair oils have excellent results due to their synergistic, antifungal, anti-inflammatory & immuno-stimulatory action. In the present study, we reveal that the zone of inhibition was find more in pure derm, Head & shoulders etc., whereas in natural products the zone of inhibition was less. All the ingredients used to formulate shampoo are safer than silicones and polyquaterniums to synthetic conditioning agents. Instead of using cationic conditioners, shikakai, hibiscus reetha, and various plant extracts have conditioning effect. The formulation of anti-dandruff hair shampoo provides a method for treating a scalp dandruff or seborrheic dermatitis. Polyherbal anti-dandruff hair shampoo containing different concentrations of herbal extract of pomegranate, reetha, shikakai, orange peel, licorice, curry leaves, neem leaves, and hibiscus flower used as an effective agent. Pomegranate is the key ingredient for *M. furfir* species as antifungal. It shows good antifungal effect in combination with increased concentration [30]. In our study we also did the biochemical test such as catalase to know the active bubbling, but we got the good result in synthetic shampoos due to the presence of ketacozinole. The *C. crenata* shell and oil-soluble Glycyrrhiza extracts shows high anti-Malassezia activity and used in the treatment of *Seborrheic dermatitis*. The extracts also showed fungi static activity against other common facultative pathogenic yeasts, *Cryptococcus* and *Candida* [31]. The current study reveals that we did a comparative study for both natural and synthetic shampoos available in market. We got a moderate result in natural products when compare to synthetic ones. Plant extracts showed better activity against dandruff causing organism *Malassezia furfur*. From these results, we came to the conclusion, that plant extracts have antifungal activity and could be safely used for treating dandruff. Synthetic drugs are unable to prevent recurrence [32]. In our study, we didn't use any plant extract, we used the home-made shikakai and also commercially available powder. But we got a promising result in synthetic shampoos. The study was significant and efficient known plant products with anti-dandruff activity could be compared with commercially available shampoos but also their good efficacies at minimum concentrations could be identified. This can help make a polyherbal mixture that could be incorporated in hair oil or shampoos for better anti-dandruff activity [33]. Our study also reveals that we got better result in shikakai in minimum concentration but good result in shampoos [34]. The shampoos are mainly responsible for improving and enhancing hair growth, quality, minimizing eye irritation and other factors.

Medicinal plants are strong evidenced usage of various secondary metabolites. Therefore, the plants can be explored and studied further for their therapeutic utility.Anti-Dandruff shampoos are widely used for removal of dandruff. All the antidandruff shampoos had better antifungal activity but there are considerable variations in the potency of their antifungal activity depending on the active

compound. The formulation contains therapeutic use of anti-dandruff agents such as Fungicidal substances, Cytostatic substances and Keratolytic substances. Herbal shampoos could be used to combat dandruffs and their efficacy was not questionable. Antifungal activity against dandruff with Zone of inhibition ranging from 1 ml, 0.5 ml and 0.1ml. The highest zone of inhibition was observed by All clear, pure derm, Head & shoulder and Dove. Comparatively the synthetic shampoos showed a high zone of inhibition than the herbal shampoos. Herbal anti-dandruff shampoos were found to be an effective but their anti- dandruff activity was less compared to synthetic one. The usage of various medicinal plants in herbal formulations has to promote the hair growth. The medicinal plants are used and treatment with many herb based medications is progressive in the field. Based on the above results indicated that all the tested shampoos they chemically strong. Due to slight modifications or differences between brands of various manufacturing processes, laboratory conditions and other reasons. Herbal based shampoos are more effective, safety and easily manufacturing and its economic value.

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6. Finish And Shellfish Farming

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Abstract

Aquaculture has been depicted as both the workmanship and study of developing sea-going creatures and plants. Aquaculture can possibly grow further and to satisfy an expanding human need for protein. Shellfish culture is a significant area of hydroponics creation around the world, and zoonoses and medication build ups related with shellfish ranch practice are of worry to general wellbeing. Modern sea fish cultivating otherwise called untamed sea, seaward, or marine finfish aquaculture is the concentrated development of hostage finfish in the sea, in net pens, units, confines, or different gadgets. We rely upon a solid marine biological system to supply quality, plentiful wild fish stocks. Farmers gain shellfish and finfish seed at different phases of its turn of events relying upon the necessities of the residency and cultivating activities. The seed is placed into a nursery climate where it is sustained into adolescent creatures. To meet the prerequisite of fish fingerlings for confine cultivating in ocean, saline water and beach front aquaculture, it is important to build up marine balance fish nurseries for the larval raising and fingerling creations. In this study, we are going to enlighten the best farming techniques for finfish and shellfish.

Keywords: finfish, shellfish, untamed sea, ocean, farming.

1. Introduction

Aquaculture has been described as both the art and science of growing aquatic animals and plants. It has been practiced for thousands of years by different societies using a wide variety of approaches to growing aquatic organisms in water. The origins of aquaculture will probably never be known because there are no ancient aquaculture-specific artifacts or even the remains of ponds or dams that can be distinguished from other uses such as water storage or for growing crops. Worldwide aquaculture production of finfish and shellfish species is an ever-increasing sector of food production and now represents nearly half (48%) of all aquatic species intended

for human consumption. It is thought that the increased aquaculture production is driven primarily by the vacuum created as a consequence of the static (or declining) status of wild capture fisheries allied to an overall greater demand for fish food products1. This generalization is broadly accepted as the primary driver for increased aquaculture production; however, more specific drivers may be, increased profit as a consequence of targeted marketing allied with development of new species in developing countries and as a means of providing more self-sufficient mechanisms to grow fish food and provide a regular income in developing countries. Typically, aquaculture production has been dominated by the culture of finfish species; however, shellfish production has shown a steady increase in production over the last number of decades1 and represents 27% by weight and 15% by value of worldwide aquaculture production. The culture of shellfish in aquaculture is comprised primarily of species of crustaceans (e.g., shrimp and crab) and molluscs (e.g., oysters, clams, mussels). A major distinction between the crustaceans and molluscs is that crustaceans, as omnivores, typically require the input into their culture system of feed, usually derived from external sources (e.g., fish protein or oils). Consequently, issues surrounding the sustainability of crustacean culture as activities are more akin to those encountered with finfish aquaculture. Molluscs, particularly those identified above, are filter feeders. Filter feeding organisms, for the most part, feed at the lowest trophic level, usually relying primarily on ingestion of phytoplankton. The process in extractive in that it does not rely on the input of feedstuffs in order to produce growth

2. Methodologies

There are many different types of farming methods used for finfish aquaculture, including land-based recirculation, flow-through tanks, nearshore cages, coastal ponds, and offshore cages. The focus of this module is on cages and pens since this method is often used for finfish aquaculture in reef areas.

Production Cycle

In order to have a comprehensive understanding of finfish aquaculture, it is important to understand the production cycle of finfish from hatchery to grow-out cages. Broodstock fish are usually collected from the wild and used to produce fingerlings that are used to stock cages. Once the adult fish produce a viable spawn, the eggs are collected and incubated until the larvae hatch. These larvae are fed a combination of live feeds (rotifers, artemia, copepods) until they

are big enough to be moved into the nursery. Once in a nursery, the fish are fed artificial feed until they reach the appropriate size for transport.

Hatchery fry production tends to be a bottleneck for new species, as the demand is greater than the supply. For example, in Palau in 2019, the amount of rabbitfish fry that the farmers demanded was greater than what the National Aquaculture Center could supply to them, forcing farmers to stock less fish than they wanted

Downstream supply chain of aquaculture which consists of post-harvest processing, distribution, value-added processing, marketing, and wholesaling. Source: opens in a new windowTowards A Blue Revolution: Catalyzing Private Investment In Sustainable Aquaculture Production Systemsopens pdf file. Image © Alison Bradley

Cage Culture Methods

Finfish cage culture can be used for a variety of species and generally has a smaller environmental cost than coastal ponds. Ponds, being on land rather than in a marine environment, directly compete for space and have historically been a driver of negative environmental impacts on sensitive nearshore coastal habitats, such as mangroves and estuaries.

Cage and pen cultures are types of enclosures where the animals being farmed are enclosed in an area by a structure, usually a net or cage. Cages have netting on all sides, in some cases even the top to prevent predators from entering the cage. Pens can use the seafloor as the bottom of the pen and they only have nets on the sides.

Cages and pens are built from different types of materials. In Asia, small to mid-scale farms have used materials such as bamboo and wood for decades; switching to new materials such as nylon, plastic, polyethylene, and steel mesh, which although have a higher cost, present a much longer life span and allow better water exchange.

In coastal areas the design that is most often utilized is a cage that is constructed with locally sourced materials (wood or bamboo) that floats due to buoyant materials (drums filled with air or styrofoam blocks). A synthetic fiber net is then hung from the wood platform to become the enclosure that holds the fish.

Farming Fish in Open Ponds

Catfish, trout, tilapia, striped bass and its hybrids for several decades, the commercial production of such fish in farm ponds in the United States has continued to expand. Like agriculture, success in raising fish depends first of all on knowledge and commitment.

Cage Culture

Raising Fish in Ponds. Farmers interested in growing fish commercially can choose whether to raise fish in open ponds, in recirculating tanks, or in cages and net pens.

Sea cage culture

Sea cage culture involves growing fish in the sea in the enclosed net cage which allows free flow of water. It is a production system comprising of a floating frame of varying dimensions and shape, net materials and mooring system, to hold and culture a large number of fish. Cage culture can be undertaken in open seas, sheltered bays or lagoons having suitable water quality and with prior permission from concerned government authorities.

Producing Striped Bass in Hatcheries

The striped bass that farmers rear in ponds begin life in hatcheries, where eggs from ripe females are removed for fertilization by male stripers or, if hybrids are to be made, by such species as white bass. This technique carries out the capture and handling of brood stock from spawning grounds, then follows biologists who determine when female fish are ready to ovulate, and finally how to remove mature eggs and fertilize the eggs with sperm from male fish.

Pond preparation and water treatment

Step 1: Dry the Pond with lime

Step 2: De-weeding, cleaning and desilting (after drying generally 10-12 cm mud to be removed)

Step 3: Apply lime (quick lime @ 250 kg/ha)

Step 4: Fill the pond with water up to a depth of 30-50 cm and apply fertilizer (SSP /Urea @30-50 kg / ha)

Step 5: After the plankton bloom increase (approximately after 7 days), fill the pond at a depth of 1.5-2 m

Step 5: Stock the fingerlings after acclimatization

Feeding schedule

Feeding rate, frequency and time of feeding are important factors to be considered in cage farming. Feeding rate and frequency are related to age and size of the fish. Fish larvae and fry need to be fed with a high protein diet frequently. When fish grows bigger, feeding rates and frequencies can be reduced.Feed consumption is influenced by time of feeding, season, water temperature, dissolved oxygen levels and other water quality parameters. Also feeding depends on biomass, protein content, feeding frequency etc.

Feed contains the following five major constituents viz.

(i) Protein

(ii) Carbohydrate

(iii) Fat

(iv) Mineral and

(v) Vitamin. Protein is the most essential element for growth of the fish.

Marine Fin Fish Culture in Brackish Water Pond

Brackish water aquaculture in India is an age-old practice confined mainly to the bheries of West Bengal, similar to gheris in Odisha, pokkali/rice fields in Kerala, kharlands in Karnataka and Maharashtra, and khazans in Goa coasts. For boosting up Brackish water aquaculture, quality of fish seed is a limiting factor for intensification of fish production, which is now being taken care of by establishing Marine Fin Fish Hatcheries. PMMSY aims to boost up Marine fish species production and also to cover more area under Brackish water sector. Fish species such as Seabass, Cobia, Silver Pompano, Indian Pompano, Orange spotted grouper have shown a lot of promises for commercial aquaculture in Brackish Water area. In India, about 13% of 1.24 million ha potential brackish water resource is under utilization at present, mainly for Shrimp culture. The country has large potential for the development of Marine Fin Fish culture in brackish water.Nursery rearing of seed is essential for all species and it can be done as a separate activity, in land based nursery ponds or hapas held in ponds or in floating nursery cages, Healthy, uniform-sized fingerlings should be procured for stocking in Brackish water ponds for grow out culture.

1. Good quality fish fingerlings should be stocked to obtain maximum survival.

2. Pond should be fertilized to maintain water quality and water color. Optimum increase in phytoplankton will allow development of zooplankton, which in turn will help to reduce feed cost and enrich the fish with high EPA and DHA (n 3 fatty acids).
3. Creation of feeding zone with the help of feed tray will acclimatize the fish to feed in particular area, which will reduce the feed cost.
4. Water exchange should be done during the culture period to maintain water quality.
5. 2-paddle wheel aerators in a single pond can be used depending upon the Dissolved Oxygen (DO) level.

3. Results and Discussion

Marine fin fish culture has been increasingly resorted as means of enhancing the fishery resources, replenishing natural stocks whose populations have declined through over-exploitation or environmental degradation. It also maximizes the productivity of water body in an open bay/ coastal lagoon / brackish water pond.

Marine fin fish has gained much popularity due to its high nutritional profile and great demand in seafood basket both in domestic and international fish market. The goal is also to ensure doubling the income of the coastal fishers and fish farmers.

Preventive measures to increase the survival rate during nursery rearing:

- ✓ Fish fingerlings are reared at nursery rearing unit up to 5-15 cm (depending upon the species)
- ✓ Transport the fingerlings through oxygenated polythene bag
- ✓ Avoid stocking during winter season
- ✓ Water salinity is to be maintained above 20 ppt

Advantage of Marine fin fish culture

- ➢ Socio-economic upliftment of coastal fishers by generating employment
- ➢ Enhanced production of seafood for human consumption
- ➢ Enhanced production of high value marine fin fish
- ➢ Increasing national seafood export
- ➢ Substitution of seafood imports
- ➢ Opportunity for commercially viable business opportunities for the entrepreneurs
- ➢ Alternate livelihood option for coastal fishers as catch from sea is dwindling

Good Management Practices (GMPs):

- Avoid over-stocking of fish fingerlings
- Monitor growth rate at appropriate time intervals
- Feed fish with pellets of good quality and right quantity
- Regular cleaning and exchange of net cages for effective water exchange
- Avoid use of antifouling paints/ chemicals
- Timely removal and proper disposal of dead fish if any
- Periodic monitoring of water temperature, Dissolved Oxygen, pH, etc.
- Close observation of fish behavior while feeding, to assess health status
- Integrated Multi-Trophic Aquaculture (IMTA)/ Polyculture of compatible species in cage

4. Conclusion

Marine Fin Fish Rearing

The key factor for successful marine fish culture is good quality seed. To meet the requirement of fish fingerlings for cage farming in sea, brackish water and coastal aquaculture, it is necessary to establish marine fin fish nurseries for the larval rearing and fingerling production of Cobia, Silver/Indian pompano, Sea bass, Grouper, Snapper etc. Fingerling size is the ideal stocking stage for marine fin fish in sea cages/ponds to avoid crop loss. Hence, marine fin fish seed rearing up to desired size for achieving better marine fish seed growth is the need of the hour.

Despite such complications, strong evidence has accumulated over the past 10–15 years for significant biological impacts, mostly adverse. Thus, there is generally good agreement between results from a range of approaches and information sources:

•Experimental studies in the laboratory, usually single species, involving well-controlled manipulations of pH and carbonate chemistry

•Mesocosm studies in the field, using medium to large enclosures (up to $75m^3$) to investigate the responses of natural planktonic or benthic communities

•Natural CO_2 vent systems, where water chemistry conditions already closely match those anticipated to occur much more widely in future

•Observations of natural populations, particularly those living near the deep-water threshold for carbonate saturation state (affecting the physical integrity of unprotected shells and exoskeletons)

•The palaeo record of past extinctions and evolutionary changes associated with natural ocean acidification events

•The management experience of the mariculture industry, particularly for shellfish farming on the US Pacific coast.

When you choose sustainable seafood, you support the fishermen and farmers who are leading the way. The environmental impact of different fishing and farming methods vary choosing fewer damaging methods supports a healthy ocean.

5. Acknowledgement

We, herewith acknowledge that this is fully our work and we have referred to some pages in online for reference.

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