

**EFFECTS OF THE PISCICIDES, MAHUA OIL
CAKE AND CROTON SEED ON THE
PRAWN CULTURE SYSTEM**

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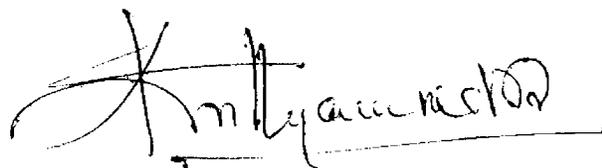
AUGUST 1997.

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Shri. K. ASOKAKUMARAN UNNITHAN under our Scientific supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology, and no part thereof has been presented before for the award of any other degree, diploma or associateship in any University.



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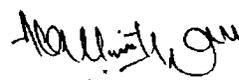


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DECLARATION

I, K. ASOKAKUMARAN UNNITHAN, do hereby declare that this thesis entitled "EFFECTS OF THE PISCICIDES, MAHUA OIL CAKE AND CROTON SEED ON THE PRAWN CULTURE SYSTEM" is a genuine record of research work carried out by me under the supervision and guidance of Dr. N.R. Menon, Director, School of the Marine Sciences and Dr. V.J. Kuttyamma, Reader, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin - 16, and that it has not previously formed the basis of the award of any degree, diploma or associateship in any University.



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INTRODUCTION

Fish including many species of shellfish represent an excellent source of animal protein and are bioenergetically cheaper to produce when compared to agricultural livestock. Qualitatively human being can utilise at least 83% of the raw weight of a fish (Bell and Can Terbery, 1976). much more than agricultural livestock. Fishery holds considerable promise for enhancing world protein production.

The physical geography of our country with its long coastline along the east and west and the network of rivers, backwaters, lakes and lagoons have been responsible for fishing becoming an important occupation providing employment and income for the rural poor, principal source of food for the people and valuable foreign exchange for the country. The estimated average per capita availability of total proteins in the Indian diet is only about 48.5 g of which 5.3 comes from animal source with a mere 0.8 g from fish (Bardach and Santerre, 1981). This strongly indicates that food quality as well as total calorie intake is a major problem in India. Per capita nutritional food fish requirement is about 11 kg/ year with an availability of only about 4 kg/capita/ year (Sinha, 1979). Obviously, supply does not meet the demand. While this is the national scenario, the demand for quality fish and fish products in international markets is also ever increasing. Fishing industry also plays a vital role in providing livelihood for thousands of families by way of extending employment opportunities at different stages such as production, processing, transportation, marketing etc. Given this national and international importance to fish and fish products, the relevance of any step taken towards increasing the fish production cannot be overlooked.

Generally, attempts towards achieving increased fish production are aimed at (1) exploiting new resources, (ii) increasing the exploitation of under-exploited resources

shrimp industry as an organised industry of considerable importance. These changes have raised India to the status of one of the foremost prawn exporting countries of the world and till recently India had the proud privilege of being the world leader in prawn production and export by virtue of rich prawns grounds in the sea. However, the situation has changed, pushing down India's position because of the sudden spurt in the supply of prawns through aquaculture from other countries (Anon 1990 & '95).

Healthy growth and survival of the prawn industry depend on the uninterrupted production and supply of quality prawns. It is highly essential to safeguard the production trend against any fluctuation or decline, and at the same time effective steps have to be taken to boost the production to meet the fast increasing demand. In view of this, priority attention has been given to the development of coastal aquaculture and utilisation of brackishwater areas for productive purposes in our Fisheries plan Schemes.

The yield from the traditional brackishwater aquaculture, by 'trapping and holding', practised over decades in the impoundments of the extensive backwater systems of the Sunderban mangrove swamps of West Bengal and in the low-lying fields adjoining the Vembanad Lake in Kerala, is only nominal. This practice is not dependable as far as the present and future demands are concerned. In this context innovative approaches of raising commercially important species of prawns by selective farming of the desirable ones alone is a solution. The programme envisages efficient utilisation of the culture systems by way of producing maximum quantity of the most desirable species of prawns such as *Penaeus monodon* and *P. indicus*.

Encouraged by the expansion of prawn market year after year and the resultant hike in the price structure in spite of the increased supply from many other nations, introduction of selective farming in the traditional culture systems and adopting the

package of practices for extensive, semi-intensive or intensive pattern, is gaining momentum in India. In addition to this, efforts are also under way to convert the vast stretches of coastal land strips, saline swamps and shallow backwater areas into productive prawn farms along the east and west coasts. Equally rewarding is the entrepreneurship developing presently in setting up hatcheries in different parts of the country for the mass production of seeds of commercially important species for selective farming.

To obtain maximum survival and growth of the prawn seeds stocked in the culture pond, it is absolutely essential that pests including predators, competitors and other weed organisms be eliminated from the culture system before stocking with the desirable species. Prawn farmers have long been aware of the fact that production from the pond is adversely affected by pests. Simple innovative practices for the removal of pests have been tried with varying degrees of success. The traditional method of netting the pond with a fine meshed drag net does not ensure satisfactory results as good many of the pest organisms escape from being caught. The possibility of draining the fields completely for eliminating the undesirable organisms is also dependent on topographical aspects of the locality. Therefore it becomes necessary to apply fish toxicants.

Generally, piscicides are either inorganic chemicals or toxins of plant origin. Inorganic chemicals like organochlorine insecticides, even in lower doses affect majority of the pond organisms and their toxicity lasts for a longer duration. There is also a great risk in the use of such materials because of the residual toxicity, if given in overdose or applied repeatedly. Increasingly widespread use of chlorinated hydrocarbon insecticides is becoming a source of real danger to fish and other aquatic and wild life (Chaudhuri, 1975). In view of this grave situation it is advisable to look for piscicides of

plant origin which at certain concentrations destroy pest organisms and are naturally degradable and environment friendly.

A large number of plant species growing in the wild in India are reported to be poisonous to fishes. Though many of these plants or plant products have been subjected to research on their piscicidal properties under freshwater conditions, the information available are fragmentary. Similar studies concerned with brackishwater environment are also sparse.

The present research programme envisages a comparative study of the effects of two piscicides of plant origin, viz., mahua oil cake, a derivative from the plant Bassia latifolia and croton seed, a product from the plant Croton tiglium. Although some reports on the effects of mahua oil cake and croton seed on fresh water pond culture systems are available, information on their effect on brackishwater culture systems are rather scanty. This was the guiding principle for launching the present study. It is hoped that the findings will enable aquaculturists to make use of the piscicides in a more rational and efficient way, and will go a long way towards realising the maximum return from culture systems without hampering the environment.

The thesis is presented in seven chapters such as Introduction, Review of literature, Materials and Methods, Results, Discussion, Summary and Bibliography.

The Introductory chapter details out the importance, traditional practices, present status and prospects of prawn farming in India and also the relevance of the present study. Reports on the effects of various piscicides under laboratory and field conditions and the information available on the environmental aspects of prawn culture systems including prawn culture operation are briefly reviewed in the second chapter. The third chapter on Materials and Methods provides detailed descriptions on the different test

organisms, toxicants, test media employed and the methodology followed during the laboratory and field experimentation. The results of the experiments conducted under the laboratory and field conditions are presented separately under the chapter on Results. Under laboratory investigations, the results of experiments such as, refinement of the method of preparation of the toxicant for application, study of the toxic effects of the two piscicides on selected finfish, penaeid prawn and clam species; study of the effect of the piscicides on the physio-chemical parameters of the test media; assessment of the persistence of toxicity of the piscicides in the ambient media under different concentrations; haematological effects of the piscicides on the selected test fish species etc., are covered.

Results of the experiments on the toxic response of different fin fish and shell fish species, zooplankton and macrobenthos under different concentrations of the piscicides; delineation of the effective dose of the two piscicides for absolute mortality of weed fishes under field conditions; effects of the piscicides on the hydrographic parameters of the culture systems under different concentrations; persistence of toxicity and progressive degradation of the piscicides under different concentrations; field culture of the penaeid prawn, Penaeus indicus, in ponds treated with the optimum dose of the two piscicides with regular monitoring of the hydrographic parameters including depth, temperature, salinity, dissolved oxygen, pH, nutrients, primary production etc. and soil characteristics such as texture, pH, nutrients, dynamics of benthic fauna and also the growth, survival and production of prawn etc. are described under the section on field studies. Under the chapter on Discussion, the results of the present study carried out under laboratory and field conditions are critically discussed and explained in the light of the information available. The salient findings made during the present study, including the laboratory and

field investigations are summarised under the chapter on Summary, followed by the Bibliography containing the list of literature cited in the text.

2 REVIEW OF LITERATURE

Historically, the development of Indian aquaculture can be divided into three phases (Bimachar and Tripathi, 1966) beginning from 1147 A.D. The rearing of Indian major carps in natural or man-made impoundments were the primary activity. The second phase between 1850's and the early 1960's was a period characterised by the introduction of exotic freshwater fishes for culture. During the later stages of this phase a number of important innovative aquacultural procedures were adopted or developed for the first time in India. These include the use of hypophysation for induced spawning, artificial fertilisation of fish ponds, and the use of sewage for enhancing the primary productivity of the fish ponds. It was also at this stage that systematic efforts were initiated to establish and improve brackishwater aquaculture in certain parts of India. Alagarwami (1990) has made an elaborate review of literature on the origin and development of brackishwater aquaculture in India over the past decades. The immense scope for an organised system of salt water fish farming in our country was originally conceived by James Hornel who suggested the development of coastal saline swamps, backwaters, estuaries, deltaic marshes and even salt pan channels for the purpose of fish farming (Tampi, 1958).

Among marine products, prawns occupy the most prominent place both in the domestic and international markets. In India, traditionally a system of prawn farming popularly described as 'trapping and holding' has been prevalent in the low-lying brackishwater impoundments adjoining the Vembanad Lake in Kerala known as 'pokkali fields' and in the Sunderban mangrove swamps of West Bengal known as 'bheries', since decades. In this system juveniles of prawns and fishes ascending from the sea along with the

tidal current are periodically let into brackishwater impoundments during the high tide and they are harvested periodically during low tide. The system followed in Kerala, popularly known as prawn filtration was described as early as 1937 by Panikkar (1937) and later on redescribed by others (Menon, 1954; George *et al.*, 1968 and George, 1974).

The traditional system of trapping and holding followed in West Bengal, locally known as bhasabadha or bheries has been described by Hora and Nair (1944), Pillai (1962) and Saha *et al.*, (1986). Prawn farming practices in traditional lines had also spread to certain other maritime states of the country such as Karnataka, Goa and Orissa also in due course (Alagarwami, 1990).

In view of the fact that the commercially more important and fast growing species of prawns are represented only in small proportions in the yield by the traditional practice, Menon (1954) remarked as early as 1954 that 'unless prawn can be shown to lead to an improvement in production, it has little chance of being adopted by those engaged in the industry'. He has also suggested that improvement could be effected if the proportion of Penaeus indicus could be appreciably raised, or if they could be made to grow larger than at present in the fields.

While evaluating the merits and demerits, ecological and techno-economic aspects of the traditional practices, Muthu (1978) highlighted the scope for improving the culture practices and production trend by way of propagating the method of selective farming of the desired species of prawns. Among the different species of commercially important penaeid prawns, P. indicus and P. monodon are the prize species because of their fast growth, large size and high economic value (Alagarwami, 1981). The principle of the improved method of selective farming can be summarised as the technology that involves the exclusive

stocking of the seeds of commercially more important species of prawns such as P. indicus or P. monodon proportionate to the area and productivity of the fields and growing them for definite periods to achieve good quality and maximum quantity of prawns for more profitability than the conventional practice. Operational guidelines for selective farming have been presented in various publications (Ramamurthy, 1978; Kartha & Nair, 1980; Rajyalakshmi, 1980; Unnithan, 1985 & 1996 and Anon, 1992). Based on the quantum of input requirement, package of practices and the resultant production target, selective farming systems are classified as extensive, semi-intensive and intensive in different parts of the world, although there is no clear cut demarcation among these systems.

Selective farming operations are done in the seasonal and perennial fields which had been used formerly for the conventional trapping and holding system and also in other backwater and estuarine areas including the shallow brackishwater canals in coconut groves, the derelict water bodies in salt pan areas along the coastline etc. The dynamics of such brackishwater ecosystems including hydrographic as well as faunistic aspects have been studied by many workers. Primary productivity and related hydrographic parameters, the epifauna and benthic fauna, chemical constituents of the bottom soil etc of the prawn culture fields adjacent to the Vembanad Lake, the largest in Kerala, have been studied in detail by Gopinathan *et al.*, (1982), and on the basis of the observations on the primary production, the fields have been classified as highly productive (>1500 mg C/m³/day), moderately productive (500-1500 mg C/m³/day) and low productive (<500 mg C/m³/day). Sheeba (1992) studied the ecological characteristics of the prawn culture fields of Cochin area. Anirudhan (1980) investigated into the nutrient chemistry of Vembanad Lake. Nutrient distribution in the Cochin harbour and its vicinity, forming part of the

Vembanad Lake, have been studied by Sankaranarayanan and Panampunnayil (1979) and Murthy and Veerayya (1972). Nair et al., (1988) looked into the environmental conditions of paddy-cum-prawn culture fields of Cochin backwaters.

The organic carbon content of the bottom soil of the three brackishwater culture system in Cochin region, namely the seasonal fields, perennial fields and canal systems in coconut groves has been reported to be 4.44%, 2.37% and 1.67% respectively, indicating the order of fertility standard of the three systems (Easwara Prasad, 1982). Following the method developed by Pillai and Boyd (1985), Joseph Gilbert and Pillai (1987) estimated the lime requirement of different seasonal and perennial prawn culture ponds around the Cochin backwaters for the pre-monsoon and monsoon seasons, based on exchange and potential acidity of the bottom soil. Suseelan (1978) explained the environmental parameters conducive for the culture of marine prawns. Water quality management in aquaculture systems has been dealt with by Pillai and Boyd (1985) and Boyd (1989). Sivakami (1988) demonstrated the beneficial effects of fertilizer and feed application on the growth of *P. indicus* in marine microcosms.

Reports on the production and distribution of plankton in relation to hydrographic parameters of the Vembanad Lake are also available (Haridas *et al*, 1973; Madhupratap and Haridas, 1975; Pillai *et al*, 1975; Madhupratap and Rao, 1979; Madhupratap, 1979 and Jose *et al* 1988). Phytoplankton and zooplankton of paddy-cum-prawn culture fields around Cochin have been studied by Gopalakrishnan *et al* (1988). Gopalakrishna Pillai (1977) studied the distribution and abundance of macrobenthos of the Cochin backwaters.

Requirements of prawn seeds for culture are met either by the natural wild resources or through hatchery production. Considerable work has been done on the distribution and

seasonal abundance of penaeid prawn larvae along the coasts of India (Kuttyamma, 1975; Rao, 1980; Kuttyamma and Kurien, 1980 & 1982; Victor Chandra Bose *et al*, 1980; Thampy *et al*, 1982; George and Suseelan, 1982; Suseelan and Kathirvel, 1982; Ramamurthy, 1982 and Rao, 1983). Mathew *et al* (1982) developed a simple device for the quantitative assessment of prawn seed resources in the estuarine areas. Mohamed *et al* (1968) and Muthu (1978a) outlined the identification characters of postlarvae of penaeid prawns found in brackishwater areas. Simple methods of collection, sorting, counting and transportation have been described by Selvaraj *et al*, (1980) and Unnithan (1985).

The rapid and widespread expansion of prawn farming along the east and west coasts of India necessitated large scale production of seeds of commercially important species under controlled conditions. The success achieved in the hatchery production of prawn seeds in India has been reviewed by Mohamed (1983).

Detailed studies have been made in India on the food and feeding habits of prawns (Gopalakrishnan, 1952; Panikkar, 1952; Panikkar and Menon, 1956; Thomas, 1972 & 1973 and Kuttyamma, 1974). Considerable differences have been noticed in the food preferences of the larval stages, juveniles and adult prawns. Panikkar (1952) stated that the food of young penaeids consisted of organic detritus found in the mud, algal material and other extremely small organisms contained in the mud. Adult prawns are reported to feed on a variety of animal and plant material available in the area where they live. They feed on crustaceans, polychaetes, molluscs, radiolarians, foraminiferans, pisces, diatoms, algae etc along with considerable quantities of organic detritus from the bottom of the sea or backwater (Thomas, 1978). According to Gopalakrishnan (1952), food of *P.indicus* includes vegetable matter, crustaceans, polychaetes echinoderm larvae, hydroids,

trematodes etc. or whatever suitable material they come across. Hall (1962) found that the food of the juveniles of P.indicus from Malayan prawn ponds consisted of crustacea, vegetable matter and polychaeta.

Farming trials carried out by various agencies in India during the past two decades have yielded valuable information on the production profile of commercially important species of prawns under different eco-geographical conditions. Suseelan (1975) reported the production data out of two culture operations of P. indicus undertaken during the period, Jan-Dec., 1973 in the salt pan area near Manakkudy estuary in Kanyakumari District. The first crop yielded 625 kg/ha with a survival rate of 82% while the second crop yielded 509 kg/ha with a survival rate of 71%; total production being 1134 kg/ha/year with a stocking density ranging from 38000-50000 nos/ha. No feeding was done. George (1980) obtained a production of 521 kg of P.indicus /ha/105 days without feeding, from a brackishwater pond at Narakkal, using wild collection of juveniles, stocked @ 40000 nos/ha, recording a survival of 75%. Culture of P. indicus during 1978-79 @ 5 seeds/m² in the coastal ponds at Mandapam, Tamilnadu, fed with clam meat and trash fish showed a growth of 121 mm/11g in 158 days recording a survival of 44.05% and a total yield of 231.53 kg/ha/5 months (Nandakumar, 1982). P.indicus juveniles cultured in polyethylene lined beach ponds at Calicut attained mean size of 124.3 mm/13.3g in 115 days (Lazarus and Nandakumaran, 1986). Culture of P.indicus in newly developed ponds adjacent to the salt pan areas along the Kallar River at Veppalodai, north of Tuticorin in Tamilnadu under a stocking density of 1.2-1.5 lakh nos/ha yielded production upto 1604 kg/ha/224 days with a survival of 95.4% (Marichamy and Motha, 1986). Poultry manure @ 750 kg/ha was applied at the bottom at the preparation stage of the pond and later the optimum

productivity was maintained by applying organic manure @ 20 kg/ha and fertilizers like urea and superphosphate, each @ 5 kg/ha, whenever required. The prawns were fed with pelletised feed twice a day @ 7-10 % of body weight. Lipton (1995) reported a production of 4.5 tonnes of P.monodon /ha/crop under a stocking density of 1.4 - 1.5 lakh seeds/ha with Taiwanese feed and paddle wheel aeration, in a private semi-intensive farm at Kanjiramkudi in Ramanathapuram District of Tamilnadu.

Shrimp farmers have long been aware that production from their ponds is adversely affected by pests. To obtain the maximum survival and growth of the prawn or fish seeds stocked in the culture pond, it is absolutely essential that the existing population of pest organisms be eliminated before stocking the culture system with the desired species. Therefore, eradication of pests including predators, competitors and other weed organisms from the culture system is an essential prerequisite for a scientific management of the culture operation. Pest organisms may be native to the culture systems or may be entering the system through the mesh screen at the sluice gate while in the egg or larval stages. Simple practices for their prevention and eradication have been tried with varying degrees of success. However, in recent years more scientific methods have been applied to the problem. The traditional method of netting the pond with a fine meshed drag net does not ensure satisfactory clearance as good many of the pest organisms escape being caught (Bhuyan, 1967). This necessitates the application of fish toxicants which at certain concentration specifically destroy pest organisms and are naturally degradable.

Das (1969) has suggested that fish toxicants should have the following qualities; (a) effective in killing the fishes at low doses, (b) not injurious to men and cattle, (c) may not render the affected fishes unsuitable for consumption, (d) leaves no cumulative adverse

effect in the pond, (e) quick detoxification of the pond water and (f) easy availability. Generally, piscicides may be either inorganic chemicals or toxins of plant origin.

Among chemical piscicides, RADA (Rosin Amine Dacetate), PCP-Na (agricultural chemical) and Malachite green are commonly used as fish-removing agents in Japan (Shigueno, 1975). Until recently synthetic insecticides like Tafdrin- 20 with 20% Endrin was commonly used as piscicides (Shirgur, 1975). Chaudhuri (1975) has studied the suitability and economics of organochlor insecticides for clearing nursely ponds of miscellaneous predatory and weed fishes and other harmful organisms like predatory insects, tad poles, prawns, crabs, etc.; the presence of which is highly undesirable in nursery ponds. (Alikunhi *et al.*, 1955). His observations indicated that organochlor insecticides such as Aldrin, Dieldrin and Endrin are highly toxic to fish, prawns and insects. Even lower doses of the chemicals affected majority of the pond organisms and the toxicity lasted for a long time even at slightly higher doses. He is of the view that there is a great risk in the use of endrin in nursery ponds because of the residual toxicity of the chemical, if given in over dose or applied repeatedly.

Increasingly widespread use of the chlorinated hydrocarbon insecticides viz., DDT, Benzene hexachloride (BHC), Lindane, Gammexane, Chlordane, Methoxychlor, Toxaphene, Heptachlor etc and the organochlor insecticides like Aldrin, Dieldrin and Endrine is becoming a source of real danger to fish and other aquatic and wild life (Chaudhuri, 1975). Studies on the effects of these insecticides on fish, fish food organisms and wild life are many (Cottam & Higgins, 1946; Cope *et al*, 1947; Surber, 1948; Hoffman & Surber, 1949; Lawrence, 1950; Young & Nicholson, 1951; Dondoroff *et al* 1954 and Harrington & Bidlingmayer, 1958).

Ramachandran (1963) observed that the practice of directly applying anhydrous ammonia into water bodies for weed control is also effective in killing fishes and other aquatic animals. He recommended this technique for eliminating pest fishes in aquaculture management. From his observations he arrived at the conclusion that the toxic effect of ammonia is marked by stoppage of photosynthesis; the chlorophyll seeming to be quickly destroyed even at lowest doses. Ammonia is reported to remain for several days at higher concentration in the water. Another clue he has arrived at is that the toxicity of ammonia seems to be due to the unionised molecular ammonia. He also suggested the technique requires well- experienced judgement to give satisfactory results. The toxicity of ammonia has been ascribed (Hasan & Macintosh, 1986) to the fact that the unionised form of ammonia can readily diffuse across gill membranes due to its lipid solubility and lack of charge, whereas the ionised form occurs as a larger hydrated form with charged entities which cannot readily pass through the hydrophobic micropores in the gill membrane. However, it has been shown that ammonium may also have considerable toxicity under low pH conditions. The toxicity of ammonia varies among species. Increased ammonia concentrations adversely affect enzyme-catalysed reactions, membrane stability and gill function, resulting in fish mortality (Colt and Armstrong, 1979).

Modifying the earlier technique of direct injection of anhydrous ammonia as described by Ramachandran (1963), Subramanian (1983) described a simple method for the eradication of undesirable fishes from fish culture ponds by application of ammonia, in which ammonia is released by the application of solutions of calcium hydroxide and ammonium sulphate in the ratio, 1:1.8. Ammonia released at a concentration of 15 ppm (12.4 ppm N) killed plankton, minnows and predatory fishes including the air breathing

species. He is of the view that the usefulness of this method is limited to unbuffered, soft water environments, where ammonia will raise the pH and remain unionised and toxic to kill the fish.

Utilisation of commercial bleaching powder as a fish toxicant has been described by Tripathy *et al.* (1980). Free chlorine, even at low concentrations (0.028 to 0.079 mg/l) in natural waters has been reported to be toxic to fish by upsetting osmotic imbalance (White, 1955; Tompkins and Tsai, 1976). With a view to accentuate the effects of liberated chlorine in the presence of ammonia, Ram *et al.*, (1988) made an attempt to develop an appropriate combination of bleaching powder and urea as a fish toxicant. Combination of commercial bleaching powder (at 5 mg chlorine/l) and urea (at 5 mg total ammonia = $\text{NH}_4^+ + \text{NH}_3$ /l) proved effective in killing murrel fry (*Channa punctatus*) under laboratory conditions. When a similar combination was tried under field conditions, the best results were obtained in ponds where urea had been broadcast 24 to 47 hr before the application of bleaching powder.

In shrimp farms, the necessity of applying a toxicant which at certain concentrations kills only the fin fishes, retaining the prawns and which is naturally degradable has been thought of by various workers. None of the inorganic pesticides meets the requirement of specificity. Further more, these chemicals, particularly the chlorinated hydrocarbons remain persistent in the environment, resulting in cumulative effects on other organisms (Minsalan & Chin, 1986). Toxicants which naturally occur in plants are degradable and fin fish can be more sensitive to its toxic properties than crustaceans.

Considerable number of plant species growing in the wild in India are reported to be poisonous to fishes by Chopra *et al.* (1956 & 1965), Nayar (1955), Nadkarni (1954) and Kirtikar & Basu (1975).

Conventionally, fishery workers in India had been using imported "Derris" powder, produced from the plant Derris elliptica (Family; Leguminosae) till import restrictions were enforced. Roark (1932) and Shepard (1951) have dealt with the commercial exploitation of D. elliptica species (South East Asian countries, East Indies and Latin American countries) for production of insecticidal preparations. Derris powder contains 5-7% rotenone, which is the toxic principle (Shirgur, 1972). Rotenone, derived from Derris sp. was demonstrated to eradicate Oreochromis mossambicus without affecting the survival of shrimps (Peterson, 1976). Shirgur (1972, '74 & '75a) worked on the feasibility of developing Derris powder from the Indian strain of Derris elliptica (Roxb) Benth. He also made a comparative evaluation of the powder prepared from D. elliptica (Roxb) Benth, D. trifoliata var uliginosa Lour; (Roxb. ex Wild) and the imported Derris powder.

Shirgur (1975) made preparations of piscicidal powder from different parts of a number of indigenous plants like Albizia lebbek (Linn.) Benth, Balanites roxburghii planch and Randia dumetorum Lam. Babu (1965) has reported the results of his laboratory studies on the use of Croton tiglium Linn. as a fish poison. Bhuyan (1968) described the use of C. tiglium seed as fish poison in field trials. According to Horra & Pillai (1962) powdered croton seeds were used to by Chinese fish culturists for eradication of unwanted fishes from nurseries before stocking of prawn and fry.

Bhuyan (1967) carried out laboratory and field investigations using the plant 'Rulei' (Milletia pachycarpa). In its effects on fish, the toxic principle in the roots of M. pachycarpa

appeared more or less the same as rotenone. As in Derris, the poisonous part of 'Rulei' is also the roots. According to Nandy and Chakraborty (1976), the unripe fruits of the plant, R. dumentorum being a cheap source of fish poison, can be collected without destroying the plant. Chakraborty *et al.* (1972) and Bhuyan and Lakshmanan (cited by Nandy and Chakraborty, 1976) studied the usefulness of Barringtonia acutangula and Milletia piscidia, respectively, as fish poisons at the Pond Culture Division of the Central Inland Fisheries Research Institute, W. Bengal.

Sharma and Simlot (1971) studied the piscicidal properties of the fruit of bitter temru (Diospyros cordifolia Roxb. Syn. D. montana Roxb.), a shrub of the family Ebenaceae, which is distributed in many parts of Rajasthan, commonly used to stun fishes. The plant is distributed throughout tropical India extending to Ceylon, Burma and North Australia (Anon, 1944). The experiments were conducted using partially purified active component of the fruit. The material was found to be quite effective in killing many types of fishes including the hardy air-breathing species like Channa striatus and Heteropneustes fossilis at a concentration of 6.6 ppm. It also lowered the dissolved oxygen content of water appreciably at higher concentrations. Skin appeared to be the most affected part at all concentrations tested, showing decolourisation, peeling off and also slime secretion at higher concentrations just prior to death. It has also been suggested that the application of temru extract is quite promising as a piscicide. At low concentrations (0.9 ppm) the fish was not killed, but remained on the surface and therefore could be easily netted out. This has been pointed out as an advantage especially with fishes like C. striatus, which normally remains hidden in the mud and hard to catch. The results obtained with temru is comparable with the findings of Babu (1965) in Croton tiglium. Temru did not seem to have any

deleterious effect upon the health of workers. It has been suggested that since this material can be inactivated in a highly alkaline solution, it is possible to destroy its activity, so that the ponds may be made inhabitable after its use without resorting to dilution or changing of the pond water.

Jena (1986) studied the effect of powdered tamarind (Tamarindus indica,L) seed husk, as a piscicide. The studies indicated that at a dose of 5-10 mg/l, it was effective in obtaining a total kill of a wide variety of fishes like Indian major carps, O. mossambica, Channa marulius etc. within 2 hrs under laboratory conditions. It was also observed that the lethal action of tamarind seed husk was independent of water temperature. There was no significant difference in pH, dissolved oxygen and carbondioxide throughout the experiments. According to Chopra *et al* (1949) the tamarind seed husk contains Saponin like ingredient, possessing strong haemolytic properties markedly toxic to fish. Requirement in relatively small quantities coupled with its effectiveness both at fairly low and high temperatures, quick action and short duration of toxicity have been cited as advantages in favour of this material (Jena, 1986).

Culturists in Taiwan have customarily used the tea seed cake as a toxin to kill undesirable fishes in ponds before stocking with fingerlings. The cake is made from the brewer's grains of a wild tea (Camellia sp.) after extraction of its oil (Tang, 1967 and Terazaki *et al* 1980) and it contains 5.2 - 7.2% Saponin. Recommended levels for use in eradicating undesirable fish in shrimp ponds is 10 - 25 ppm (Cook, 1976). Tarasaki *et al* (1980) studied the toxicity of crude saponin extracted from the cake to the shrimp Penaeus merguensis; fishes, Scatophagus argus, Tilapia mossambica, Mugil tade, Eleutheronema tetradactylum and Mystus sp; crab Uca sp and food organisms, Brachionus plicatilis

(rotifer), Colurella sp. (rotifer), Schizopera subteranea (copepod), and Artemia salina (brine shrimp). Experiments indicated that LT 50 for T. mossambica and Mystus sp. was more than 6 hr. in 1.1 ppm of saponin (salinity: 15‰) and E. tetradactylum died within 1 hr. The LT 50 for T. mossambica shortened as the concentration of saponin increased, and was less than 1hr. at concentrations above 4.7ppm. Larger fish had greater resistance than smaller ones under same concentration. Resistance of Tilapia mossambica to saponin weakened as salinity increased. Shrimp and crab survived more than 30 hr. in concentration exceeding 10ppm. The 24-h Tlm of shrimp was 50.4 ppm. For larval shrimps (PL 11) less than 30ppm was harmless while a concentration less than 7ppm was harmless to rotifers. The lethal dose for Artemia salina was higher than that of shrimp and crabs.

Minsalan and Chin (1986) made a series of studies to refine the methods of applying tea seed cake in shrimp ponds. The experiments were conducted with two species of fin fishes, Oreochromis mossambicus and Glossogobius giurus and two species of crustaceans, Metapenaeus ensis and Penaeus monodon. Results indicated that 15ppm was required for the complete eradication of finfishes within six hours. It is also suggested that a concentration of 10ppm can be used with the same effects if the toxicant is applied about noon time when the temperature is highest. This would result in savings by 33% of the cost of tea seed cake. As the rate of degradation was found to be slow, it was also advantageous to dilute pond water as soon as possible, so that shrimp production will not be affected by the application of tea seed cake. It is recommended that the water level in the pond be reduced to one third before application, that the cake be applied in minimum quantity towards noon when water temperature is higher and the water depth be restored after about six hours of application.

Lakshman (1983) described the usefulness of mahua oil cake as a fish poison and manure in freshwater environments. Bhatia (1970) reported the threshold concentration for the effect of cake to several species of freshwater fishes to be 60 ppm. Nath (1979) described the changes in hydrographic parameters and the time taken for the detoxification of the cake in fresh water under laboratory conditions. Sumit Home Chaudhuri *et al.*, (1986) studied the effect of mahua oil cake on the blood cells and blood values of an air-breathing fish and a carp species.

3. MATERIALS AND METHODS

3.1. MATERIALS

Materials such as piscicides, test animals and test media having relevance with field applications were employed for the laboratory as well as field investigations and were procured locally.

3.1.1. Piscicides

Mahua oil cake and croton seed were selected as piscicides for the present study.

3.1.1.1. Mahua oil cake

Mahua oil cake used for piscicidal purposes is a product from the perennial madhuka (*Bassia Koenig ex Linn.*) tree species, *Bassia latifolia* Roxb (Syn. *Madhuca latifolia*; *M.indica* J. F. Gmel) belonging to the family Sapotaceae (Bhatia, 1970 and Lakshman, 1983).

Mahua tree is known by different names in the different parts of the country: English - mahua; Hindi - mahua; Telugu - ippa, ippachettu madhuukamu etc; Tamil - iluppai, iruppai etc; Malayalam - iluppa, iruppa etc Canarese - ippe mara; Bengali - maua and Sanscrit - madhuka.

Mahua, a deciduous tree reaching 12 to 15m high, distributed in Central India, Gujarat, Bengal, Konkan, North Kanara and other South Indian forests, and is cultivated and self-sown (Kirtikar et al., 1975 and Nadkarni, 1954). The fruit of mahua is a berry containing 1-3 seeds. The thick, soft and sugary pericarp forming 70% of the weight of the berry is edible. The seed is oblong, dull brown in colour and contains almond shaped creamish yellow kernel which form 70% of the weight of the seed. Flowers, seed oil and

cake, leaves, bark etc are also used. Flowers contain sugar, cellulose, albuminous substances, enzymes, ash, water etc. Seeds contain fatty oil, fat tannin, extractive matter, bitter principle saponin, albumen, gum starch, mucilage and ash. Ash contains salicylic, phosphoric and sulphuric acids, lime and iron, potash and traces of soda. Oil is a mixture of stearin and olein. Leaves also contain a glycosidic saponin different from that obtained from the seeds (Nadkarni, 1954).

The various parts of mahua tree have been known in ancient Indian and folk medicines for curing various ailments. The bark is used for the treatment of rheumatism, ulcers, itches, bleeding and spongy gums, tonsillitis and diabetes mellites. The roots are also employed to treat ulcers. The dried flowers are used for fomentation in orchitis for their sedative effect. The flowers fried in ghee are eaten by persons suffering from piles. The sugary syrup or honey obtained by extracting the flowers is reported to be useful for treating eye diseases.

The flowers containing high amount of fermentable sugars are used for distilling country liquor. The distilled spirit from the flowers is an appetising, cooling nutritive tonic used for coughs in the form of decoction (Wealth of India, 1962). The oil from the seeds has emollient properties and has been used in skin diseases, rheumatism and head ache (Chopra et al., 1956). It is also used as laxative in piles and haemorrhoids and as an emetic. Because of tannin content, leaves and bark have astringent properties. The oil is used by natives for cooking. Major part of the oil, however, goes for soap making and cosmetic industry. Suitable modifications of its fat give products which are regarded as potential cocoa butter substitutes that may be useful as extenders in high-priced confectionery fats, vanaspathi and margarine (Bhattachary and Banerjee, 1983; Ghosh *et al*, 1983). The cake is used as a

manure either alone or in mixture with other cakes and fertilisers and also as a piscicide. The piscicidal property of mahua oil cake is attributed to its saponin or mowrin content. Saponins are poisonous towards the lower forms of life and are used for killing fish by the aborigines of South America (The Merck Index IX Edn., 1976 - Review and Bibliography :R.J.McIlroy; The plant glycosides (Edward Arnold & Co, London 1951 Chapter IX).

Saponins are toxic bitter principles present widely in plant kingdom and a few lower classes of animals like echinodermata and in snake venom. They are found in various plant parts like leaves, stem, roots, flowers and fruits. The content may vary from 0.1 - 30% in plants or in different parts of the same plant (Tschesche and wulff, 1973). Chemically they are glycosides with a steroid (C27), triterpenoid (C30) or steroid - alkaloid ring structure called the 'Sapogenin' or aglycone, with a carbohydrate moiety attached to it. Saponins are present in more than 90 plant families, of which triterpenoid saponins constitute the major group (Chandel and Rastogi, 1980).

Mahua oil cake contains 6-8 % saponin (Mulky, 1976) which is soluble in water (Lakshman, 1983). Mahua oil cake procured from the Marine Products Export Development Authority, Cochin was used for the present studies.

3.1.1.2. **Croton seed**

Croton seed is a product from the plant genus Croton, belonging to the family Euphorbiaceae. Four species, viz., Croton reticulatus, C. oblongifolius, C. caudatus and C. tiglium are common in India. Among these, C. tiglium Linn. found throughout India, and plentiful in eastern Bengal, extending to Assam and Burma yields the croton seed having piscicidal properties (Nadkarni, 1954).

Croton tiglium is an evergreen shrub, the young shoots sprinkled with stellate hairs; bark smooth, ash coloured; flowers small; capsules oblong and obtusely three lobed and seeds smooth, about 13 mm long or longer (Kirtikar *et al*, 1975).

C. tiglium is known by different names in the different parts of the country : English - Croton oil seed, purgative croton etc; Sanskrit - Naepala, Jayapala, Kanakaphala, Titteriphala etc; Gujarati - Nepal ; Bengali Nepala vitua etc; Tamil and Malayalam- Neervalam (Nadkarni, 1954)

Seed kernels contain 55-57 % croton oil (Chopra *et al*, 1956). Croton oil is composed of : (I) Crotonoleic acid, (ii) Tiglic acid or Methyl crotonic acid, (iii) Crotonol which is nonpurgative, but an irritant to the skin, (iv) several volatile acids to which the odour is due and several fatty acids (Nadkarni, 1954).

Fats present in croton oil are glycerides of stearic, palmitic, myristic and lauric acids and of several volatile acids of the same series like acetic, butyric, valerianic and tiglic acids (Nadkarni, 1954).

Seeds, leaves, bark and root, all possess drastic purgative properties. Seeds are powerful drastic purgative and vermifuge; in over doses it acts as an acronarcotic poison. Oil is a powerful hydrogogue cathartic and externally vesicant producing irritation, inflammation, popular and pustular eruption. The activity of croton oil as a vesicant externally and as a purgative internally is attributed to the presence of crotonoleic acid which is said to occur in the free state in which it is freely soluble in alcohol and in combination as a glyceride. The glyceride does not possess poisonous properties, but the free acid acts as a powerful irritant to the skin and as a purgative in the intestine. The crotonol glyceride is attacked and split up like other glycerides by the juices of the stomach

and the crotonoleic acid is set free which then exercises its purgative influence (Nadkarni, 1954). The oil from the seed is useful in diseases of the abdomen, mental troubles, convulsions, fever, insanity, inflammations, bronchitis (Ayurveda). The oil is cathartic, tonic; removes pus and bad matter from the body (Yunani). It is also useful in dropsy, obstinate constipation, intestinal obstructions, lead poisoning and as a preliminary laxative in leprosy and as a rivulsive in apoplexy. The oil is applied to the scalp in acute cerebral diseases and to the cord in spinal meningitis. The oil has been tried as a counter irritant and vesicant in rheumatism, synovitis, paralysis and painful conditions of joints and limbs (Nadkarni, 1954). Seeds have bitter bad taste, causing a burning sensation; expectorant, emetic; good in sore eyes, excessive phlegm and leucoderma.

When eaten, the seeds cause nausea and ecuctation, followed by flatulent distentions of the abdomen, colic and diarrhoea. A single seed itself has been proved fatal. The oil in a dose of one drop causes burning sensation in the oesophagus and stomach, nausea and vomiting. In an hour or two some gurgling or slight colic is perceived in the bowels, followed somewhat suddenly by a watery stool and heat about the anus. Within 24 hours eight or ten more stools follow with considerable weakness. Also cause epigastric uneasiness and oppression, palpitation of the heart, headache, feverishness, perspiration and sleep (Nadkarni, 1954).

On account of their drastic purgative properties, the seeds and oil were regarded by the Chinese as entirely poisonous. According to Hora and Pillai (1962), powdered croton seeds are used by Chinese fish culturists for eradication of unwanted fishes from nurseries before stocking of spawn and fry. The fruits are employed by Dayaks in Borneo to poison fish and in Lakhimpur the seeds are ground in water and the infusion is used to kill insect

pests (Kirtikar, *et al.*, 1975). The seeds are reported to be used in Java for killing fish (Nadkarni, 1954). In Assam (local name: Konibin) and N.E.F.A. (local names : Engosinum and Kusere) it is frequently used by tribals for killing fish in streams and ponds (Bhuyan, 1968). In Kerala it is used by rural people to catch fishes from streams and pools (Babu, 1965).

The piscicidal property of C. tiglium seed is attributed to its content of the toxalbumin, Croton, as cited by Babu (1965). However, according to Chopra *et al.*, (1956) seeds of croton contain 2 toxic proteins, Croton globulin and Croton albumin, which are essentially blood poisons (Chopra *et al.*, 1949).

For the present experimental purpose, C. tiglium seeds were purchased locally from hill produce merchants at Alwaye.

3.1.2. Test animals

While selecting the test organisms, weed fishes having widespread distribution in brackishwater prawn culture systems adjoining Cochin backwater and their ready availability for collection were taken into account. Since pilot experiments revealed that larger specimens were more tolerant to the toxicants than smaller ones, only larger individuals were employed for the study.

To delineate the relative tolerance of finfishes to the selected toxicants, toxicity studies were carried out employing common weed fishes such as Tilapia mossambica (17-20cm), Etrophus maculatus (8-9cm), Tachysurus maculatus (16-18cm), Ambassis gymnocephalus (6-7cm), Glossogobius giuris (6-8cm), Magalops cyprinoides (20-24cm), Elops saures (22-26cm), Macropodus cupanus (5-6cm), Aplochylus lineatus (5-7cm), Gambusia affinis (5-6cm), and the borrowing snake eel Ophichthys boro(30-40a) and

O. microcephalus (45-50cm). T. mossambica was then chosen for further detailed studies and represented the most tolerant group of finfishes. Among prawns, individuals of postlarval stages of the species, Penaeus indicus (1.4 - 2.0 cm) were selected for the study; while Villorita cyprinoides var. cochinensis (4 - 4.5 cm) was the animal of choice from among the molluscan species.

Healthy individuals of the test species were collected from the brackishwater areas, causing minimum stress and transported to the laboratory in well aerated water. The animals were acclimated to the laboratory condition in large collapsible plastic pools containing well aerated water of habitat salinity. The lots showing disease symptoms or any abnormal behaviour were totally discarded. During the acclimatisation period of two weeks, the animals were fed regularly and the salinity was gradually adjusted to the experimental salinity (15 ‰).

3.1.3. Test medium

Laboratory experiments were carried out in brackishwater of salinity, 15 ‰ collected from Cochin backwaters. The water was transported to the laboratory in large plastic carbouys and kept in total darkness for ageing. The water was filtered through a glass wool filter, aerated to full saturation before use and the optimum salinity was maintained throughout the experiments. Dilution or concentration of the test medium to the experimental salinity was done by adding tap water or sea water as required.

Field experiments were conducted in Vypeen Island that form a part of Ernakulam District. The island, about 25 km long with an average breadth of 2 km is bordered by the Arabian sea and Cochin backwaters along the western and eastern side. The Azhikkodu

and Cochin bar mouths form the northern and southern boundaries respectively. The extensive prawn culture systems in the island including the perennial fields, seasonal pokkali fields and canal systems in coconut groves are fed by a net work of canals running transversely and longitudinally having confluence with the Cochin backwaters which in turn is confluent with the Arabian sea through the two bar mouths.

Brackishwater impoundments, forming part of a canal system in a coconut grove at Narakkal village ($76^{\circ} 14' E$ and $10^{\circ} 3' N$) situated in Vypeen Island about 10 km north of Cochin barmouth were selected for the present study. The ponds were confluent with one of the main feeder canals running longitudinally along the island.

3.2. METHODS

3.2.1. Laboratory experiments

3.2.1.1. Preparation of the toxicants for application

Mahua oil cake:

With a view to standardise the method of preparation of the maximum potent form of the toxicant for application, different preparations of the material such as (a) freshly powdered cake and (b) aqueous suspension from pre-soaked cake were employed during the study. Lethal time for absolute mortality of the fish was used as an indicator of the efficiency of the preparation. Since pre-soaked material was found to be more toxic than the other, experiments were carried out to evaluate the influence of soaking time on the potency of the toxicant.

Croton seed:

Toxicity studies involving pre-soaked as well as unsoaked croton seed as aqueous suspension were carried out to evaluate the relative potency. Pre-soaking was found beneficial in that it helped easy and effective grinding of the seed. Experiments were also carried out to evaluate the influence of duration of soaking on toxicity as in the case of mahua oil cake.

3.2.1.2. Toxicity studies

Laboratory conditioned animals of uniform size were exposed to test solutions containing graded concentrations of the toxicants following standard method (Sprague, 1973). In order to evaluate the relative tolerance of different fish species, selected finfishes were separately exposed to a lethal concentration of 200 ppm of mahua oil cake and 4 ppm of croton seed. The lethal time (LT100) of each species was considered as the indicator of its tolerance against the toxicant. Among the different species tested, those which required the maximum and minimum time to reach lethality were considered the most and least tolerant, respectively.

Experiments were carried out in fibre glass tanks of 50 l capacity coated with chemical resistant epoxy resin inside and ten animals each of the test species were exposed to the selected toxicant concentrations. The experimental tanks were covered to minimise external disturbances. The experiments were carried out at room temperature ($28^{\circ}\text{C}\pm 1^{\circ}\text{C}$) and the animals were not fed during the course of experimentation. Appropriate duplicates and controls were maintained for all the experiments. The animals were inspected at

regular intervals and all dead individuals which failed to respond to mechanical stimulation were removed.

Behavioural responses such as body movements, swimming pattern, opercular movements and maintenance of equilibrium were observed to identify stress symptoms among individuals of the test species exposed to the toxicants. Failure to respond to physical stimulus and stoppage of gill movements were accounted for ascertaining mortality.

Studies were also conducted to determine the lethal dose (LD 100) of the most tolerant fish species tested, within 6 hours. A period of over 6 hrs involves the risk of any dilution of the medium under field conditions which may occur due to an increase in tide level and seepage, likely in low lying backwater impoundments, influencing the toxicity of the piscicide. On the other hand, a duration of less than 6 hrs was also not preferred since it may necessitate a higher dose of the toxicant which may be disastrous to the entire ecosystem including the desirable species. Further, biodegradability of the piscicide also may be delayed in the case of higher doses.

The toxic responses of postlarvae of P. indicus and the clam V. cyprinoides were also studied following exposure to different concentrations of mahua oil cake and croton.

Test media containing different concentrations of the two toxicants were observed for a period of 96 hrs to assess the impact of the toxicants on the physico-chemical parameters of the test media such as temperature, pH and dissolved oxygen. While mahua oil cake was applied in the form of pre-soaked powder, in the case of croton seed, its aqueous suspension from pre-soaked material was put to experimentation. With a view to

understand the nature of restoration of dissolved oxygen in the test medium, experiments were carried out employing mahua oil cake and croton seed and the medium covered by liquid paraffin to prevent contact with atmosphere.

The persistence of toxicity of mahua oil cake and croton seed suspension in the ambient medium was determined by exposing the finfish A. gymnocephala which represented the least tolerant group of fin fishes, to selected concentrations of the toxicants every 24 hours till the media was no longer lethal to fishes due to progressive degradation of the toxicants.

3.2.1.3. Haematological studies

For haematological studies blood samples were collected from the caudal vein in aseptic condition by severing the caudal peduncle (Hesser, 1960). The collected blood samples were treated with 3:2 mixture of ammonium oxalate and potassium oxalate at the rate of 0.5 - 1.0 ml per ml of blood to prevent coagulation. Aliquots of pooled blood samples from 3 to 5 fishes were used for the different estimations. The different haematological parameters were estimated employing standard techniques (Hesser, 1960; Blaxhall and Daisley, 1973).

The technique employed for the erythrocyte counts of fish blood were similar in most respects to those used in mammalian counts except for a change in the RBC diluting fluid. Hendrick's RBC diluting fluid was used during the present study (Hendrick, 1952). Neubauer type of haemocytometer was used for the purpose of RBC counting. The total erythrocyte count is expressed in millions of RBC per cubic mm of blood.

Cyanomethaemoglobin method described by Ortho Diagnostic Systems (1986) was followed for estimating the haemoglobin content. To 0.02 ml of blood 5 ml of aculte reagent (modified Drabkin reagent) was added and stirred well. The potassium ferricyanide present in the reagent converts the haemoglobin iron from the ferrous to ferric state to form methaemoglobin and this in turn combines with potassium cyanide of the aculte reagent to produce a stable pigment or the cyanomethaemoglobin which represents the sum of oxyhaemoglobin, carboxihaemoglobin and methaemoglobin. The cyanomethaemoglobin formed was measured spectrophotometrically at 540 nm. The calibration curve was prepared using the Human Haemoglobin standard provided with the aculte reagent. The haemoglobin content is expressed as g % (or gm/dl).

Haematocrit values (or packed cell volume - Ht %) was measured by applying the method of Mcleay and Gordan (1977). Blood was drawn into heparinised microhaematocrit tube (0.55 ± 0.05 mm diameter). One end of the tube was sealed and centrifuged in microhaematocrit centrifuge at 11500 rpm for 5 minutes. Haematocrit value was estimated after measuring the red cell column using a haematocrit counter provided along with the microhaematocrit centrifuge, and expressed as the percentage of whole blood.

From the values of Hb content (Hb %), haematocrit (Ht %) and total erythrocyte count (millions/mm³) the following erythrocyte constants were calculated using the respective formula (Lamberg and Rothstein, 1978). a. Mean corpuscular volume (MCV) : MCV represents the average volume of individual erythrocyte in cubic microns (μ^3) and computed by the formula,

$$\text{MCV} = \frac{\text{Ht \%}}{\text{RBC (in millions/mm}^3\text{)}} \times 10$$

b. Mean Corpuscular Haemoglobin (MCH) : MCH represents the average weight of haemoglobin in individual erythrocyte in picograms (pg) and calculated by the formula,

$$\text{MCH} = \frac{\text{Hb \%}}{\text{RBC (in millions/mm}^3\text{)}} \times 10$$

3.2.2. Field experiments

Preparation of the ponds for experiment

Ponds for the present study were prepared by erecting earthen bunds across the canals in the coconut grove and placing wooden sluice gates of 0.5 m width for regulation of tidal flow. Ten ponds prepared on similar lines were used for experimentation. The area of the ponds ranged from 229-447m² having an average depth range of 0.41m - 0.52m with salinity ranging from 15.81 ‰ - 18.07 ‰

On the previous day of the experiment, water in the ponds was let out to the maximum possible extent during the low tide and the sluice gates were sealed with hard clay and the volume of water in each pond was calculated by multiplying the area of pond by average depth, for the purpose of quantifying the piscicides.

Close-meshed nylon net enclosures (hapa) were erected within the ponds for introducing the finfish and prawn species to study their toxic responses during the experiment. Specimens of the clam species, *V.cyprinoides* were maintained buried at the bottom in perforated plastic bins with lid, filled with pond soil.

Preparation and application of the piscicides

The piscicides quantified on the basis of the volume of water in the experimental ponds were taken in separate containers on the previous day of the experiment and kept

mahua oil cake and *croton seed* for 6 hours. soaked in water for 12 hours. The pre-soaked mahua oil cake was used as such for the experiment, whereas the pre-soaked croton seed was ground well in a wet grinder and the aqueous suspension used for application.

Mahua oil cake was broadcast all over the water surface. The aqueous suspension of the croton seed was diluted with pond water and sprinkled uniformly throughout the pond surface. After application of the piscicides, the pond water was mixed thoroughly by dragging a nylon net all along the water area.

3.2.2.1. **Determination of the minimum concentration of the piscicides effective for the absolute mortality of weed fishes**

In order to determine the minimum concentration of the piscicides effective in destroying the weed fishes completely, graded concentrations of the piscicides, namely 150, 200, 250 and 300 ppm in the case of mahua oil cake and 2,3,4 and 5 ppm in the case of croton seed were applied in different ponds and the toxic responses of the test organisms were examined. Among the different concentrations tested, the minimum concentration of the piscicides capable of killing all the fishes tested was selected as the optimum one for application in the prawn culture fields.

3.2.2.2. **Progressive degradation of the piscicides and restoration of the normal conditions in the field culture system.**

In order to evaluate the persistence of toxicity of the piscicides in the ponds, test fishes were introduced every 24 hours, into nylon hapas erected in the experimental ponds treated with different concentrations of the piscicides till the water ceased to be lethal to the fish life. Postlarvae of *P. indicus* were also introduced into the medium every 24 hours

to study their toxic response and survival rate till the water was ascertained to be ready for stocking with prawn seeds for culture.

Hydrographic parameters of the ponds treated with different concentrations of the piscicides were studied daily for a period of ten days from the time of application to assess their fluctuation and final restoration to the normal condition. Temperature of water was measured using a 0 - 50^o C mercury thermometer. pH was determined using a digital pH meter. Mohr - titration method (Strickland & Parsons, 1977) was adopted for determining the salinity of water, using the formula,

$$\text{Salinity of water sample (‰)} = \frac{V_2}{V_1} \times S$$

where, V_1 = Volume of Silver nitrate required for 10 ml of Standard Sea Water

V_2 = Volume of Silver nitrate required for 10 ml of water sample

S = Salinity of Standard Sea Water.

Dissolved oxygen content was estimated by Winkler method (Strickland & parsons, 1977)

3.2.2.3. Effects of the piscicides on the zooplankton and benthos

Samples of zooplankton and macrobenthos were taken from the experimental ponds treated with the two piscicides at intervals 0,24,120 and 240 hours.

Zooplankton samples were collected using a 0.75m long conical plankton net with a 25 cm square mouth. The anterior 10 cm portion of the net was formed of canvas followed by a 50 cm long organdy cloth of mesh size, 0.3 mm. The cod end of the net was stitched to

a polythene bucket with a lid functioning as a filter made of organdy cloth of 0.3 mm mesh size, stitched to the bucket with the help of a canvas portion of 5 cm length (Menon *et al.*, 1977)

The net was hauled manually from the shore of the pond for 5 minutes covering a distance of 20 meters. The samples collected in the bucket portion were transferred into a container by removing the lid, followed by repeated washing. The quantity of water filtered was determined by multiplying the mouth area of the net by the distance covered; i.e., $0.625 \text{ m}^2 \times 20 \text{ cm}$. The samples were preserved in 5% formalin and stored in plankton bottles for later analysis. The organisms were identified upto group level and quantified in terms of numbers per cubic metre.

Collection of macrobenthos was made using a Van Veen Grab of 20 X 20cm size. Fauna retained by a sieve of 0.5 mm mesh size were preserved in 5% formalin containing Rosebengal and were subjected to qualitative and quantitative analysis. Organisms were identified upto group level. Quantification was made in terms of the numbers and wet weight of each group per m^2 of pond area.

3.2.2.4. Experimental culture of prawn in ponds treated with the optimum concentration of the piscicides.

Culture of the penaeid prawn Penaeus indicus in ponds treated with the optimum concentration of 200ppm and 4ppm in the case of mahua oil cake and croton seed respectively was carried out for a period of 3 months in duplicates.

The ponds were stocked with early juveniles of P.indicus collected from the wild,

on the fifth day of the treatment with the piscicides after ascertaining that the water characteristics had returned to the normal condition and that no more weed fishes were present in the treated ponds.

Studies on the water and soil characteristics were made at fortnightly intervals starting from the date of application of the piscicides. Water characteristics such as depth, temperature, salinity, pH, dissolved oxygen, nutrients and primary production and soil characteristics such as organic carbon, phosphate, potassium and pH were also studied at fortnightly intervals. Correspondingly, zooplankton and macrobenthos samples were also taken for study. Samples of prawn were collected at the same intervals for analysing their growth in length and weight. Specimens were released back into the pond after taking the measurements. Determinations of temperature, salinity, pH and dissolved oxygen were done following standard methods as described earlier.

Among water nutrients, Nitrite-Nitrogen ($\text{NO}_2\text{-N}$) was determined by the Azo-dye method (Bendschneider and Robinson, 1952), Nitrate- Nitrogen ($\text{NO}_3\text{-N}$) by the method of Morris and Riley as described by Strickland and Parsons (1977), inorganic phosphorus by the method given by Murphy and Riley (1962) and Ammonia - Nitrogen ($\text{NH}_3\text{-N}$) by the phenol - hypochlorite method (Solarzano, 1969). The light and dark bottle technique given by Gaarder and Gran (1927) was used for the estimation of primary production. Water samples collected in light and dark bottles were incubated for 3 hours from 11 am to 2 pm. Assuming that photosynthesis takes place for 10 hours during a day, primary production was calculated using the formula:

$$\text{Primary production (mg C/ m}^3\text{/ day)} = \frac{\text{O}_2 \text{ (ml)} \times 0.536 \times 1000}{\text{PQ} \times \text{T}}$$

where PQ (Photosynthetic Quotient) is taken as 1.25, T, the number of hours of incubation.

4 RESULTS

4.1. LABORATORY EXPERIMENTS

Standardisation of the method of preparation of mahua oil cake and croton seed for application in water, evaluation of their effect on the hydrography and selected brackishwater organisms including haematological effects, and the pattern of their progressive degradation and persistence of toxicity in the test media were among the laboratory experiments carried out during the present study. The results obtained are presented in Tables, 1 - 9 and Figures, 1 - 6.

4.1.1. Standardisation of the method of preparation of the piscicides for application.

While standardising the method of preparation of mahua oil cake for application, it was observed that pre-soaking of the material in water prior to application was beneficial (Table 1a & Fig. 1a-e). The effect of the duration (hours) of pre-soaking on the toxic potency of the cake was tested by exposing Tilapia mossambica to the media containing 200 ppm of the piscicide pre-soaked for different durations.

As is evident from the Table 1a & Figs. 1a-e, no mortality occurred within 30 minutes among different preparations of the cake pre-soaked for varying duration of time ranging from 6 to 48 hours. After 60 minutes, varying percentages of mortality were observed among individuals of the

Table 1a. Tilapia mossambica: Time course of percentage mortality against a constant concentration (200 ppm) of mahua oil cake pre-soaked for different durations

Duration of pre-soaking (hours)	Periods of observation (minutes)			
	30	60	90	120
0	-	30	75	100
6	-	50	100	
12	-	60	100	
24	-	45	100	
48	-	50	75	100
Control	-	-	-	-

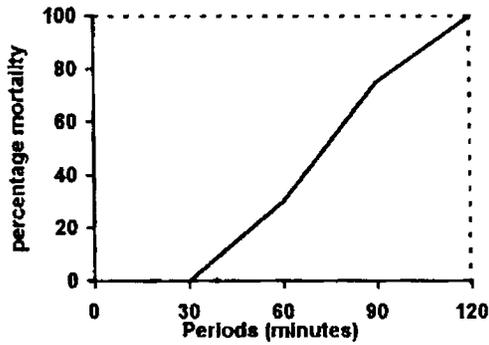
- No mortality

Table 1b. Tilapia mossambica: Time course of percentage mortality against a constant concentration (4 ppm) of croton seed pre-soaked for different durations

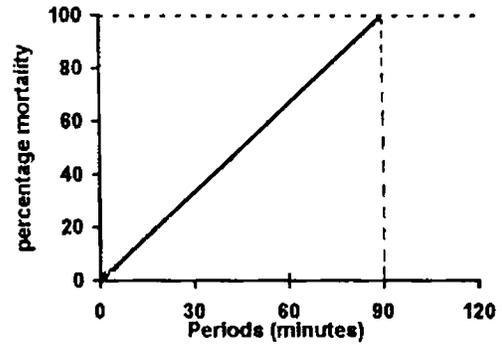
Duration of pre-soaking (hours)	Periods of observation (minutes)												
	30	60	90	120	150	180	210	240	270	300	330	360	
0	-	-	-	-	-	35	60	100					
6	-	-	-	-	-	45	80	100					
12	-	-	-	-	-	25	65	100					
24	-	-	-	-	-	20	45	80	100				
48	-	-	-	-	-	-	-	50	55	75	75	100	
Control	-	-	-	-	-	-	-	-	-	-	-	-	

- No mortality

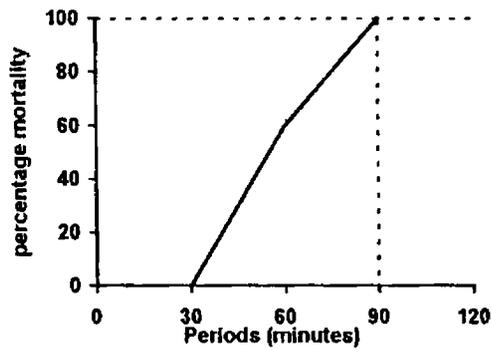
Figures 1 a - e Tilapia mossambica: Time course of percentage mortality against a constant concentration (200 ppm) of mahua oil cake pre-soaked for different durations



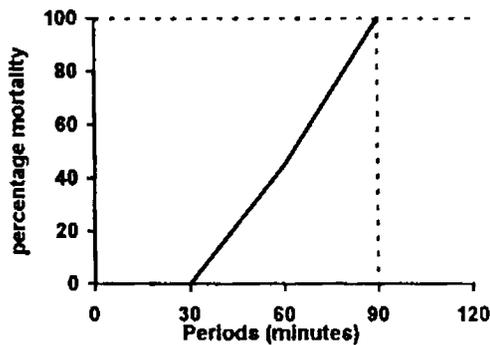
1a. Unsoaked mahua oil cake



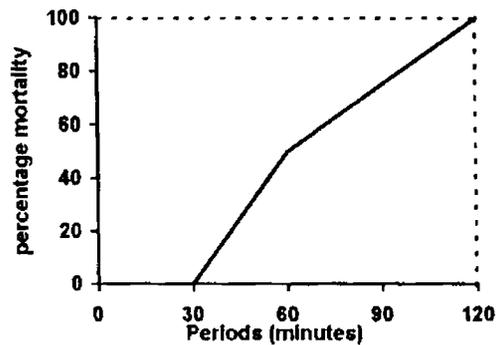
1b. 6 hrs pre-soaking



1c. 12 hrs pre-soaking

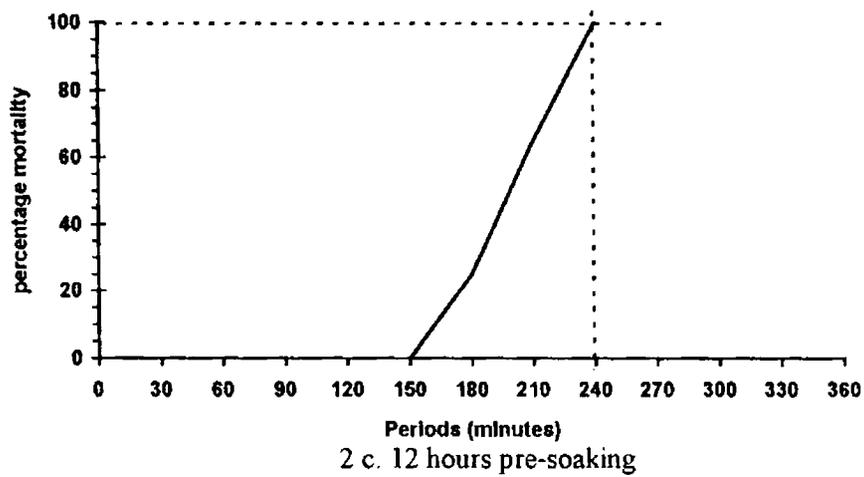
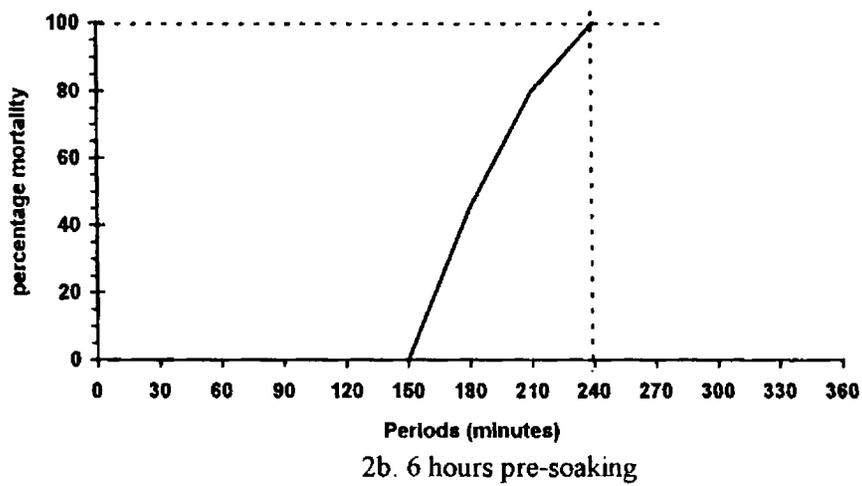
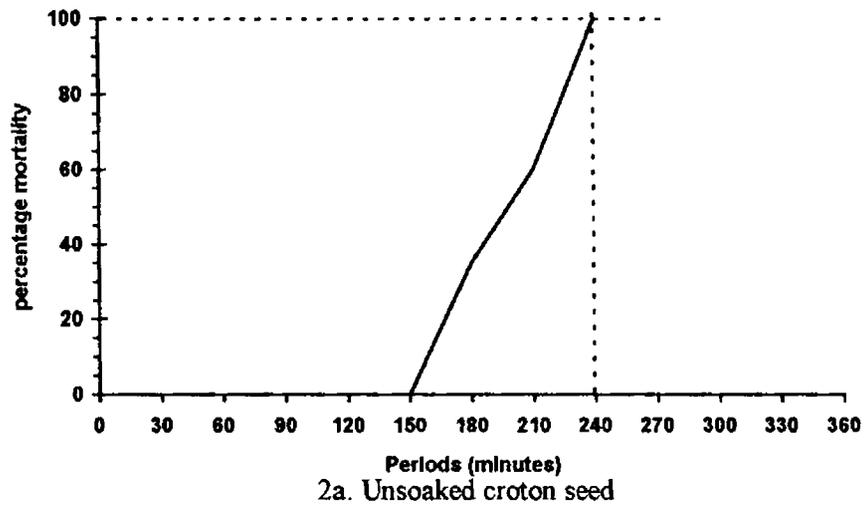


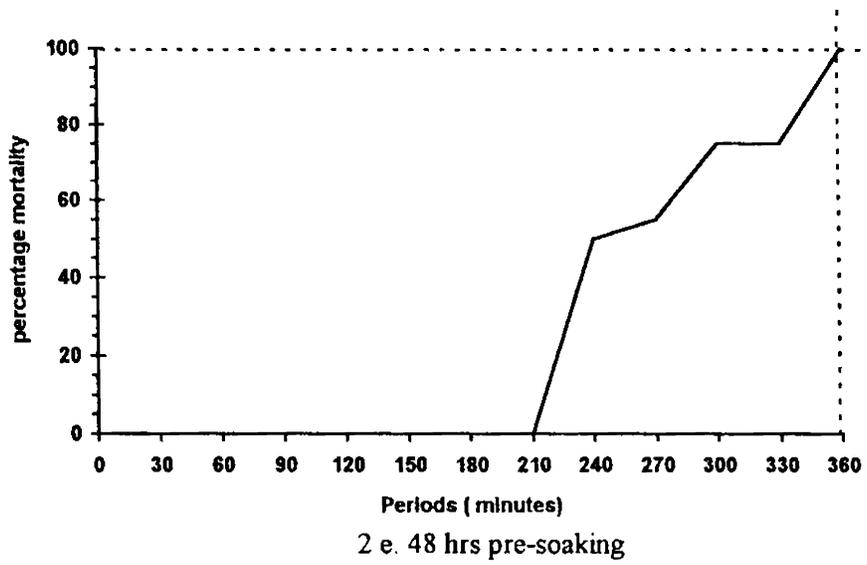
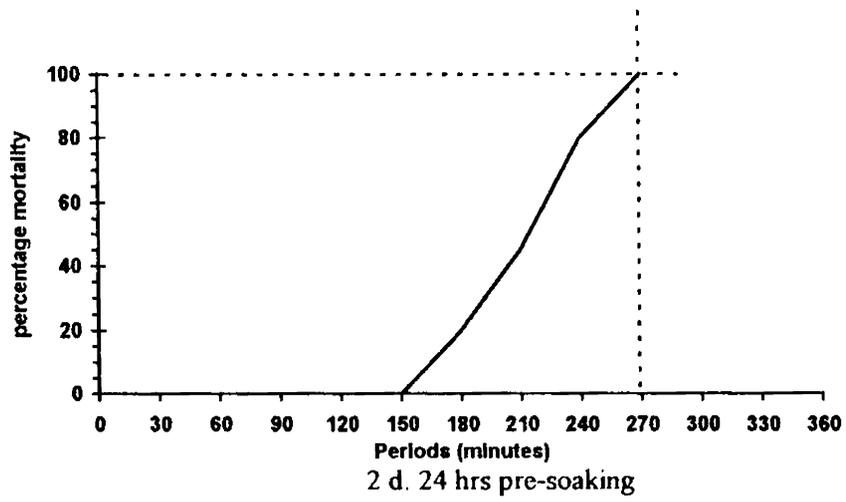
1d. 24 hrs pre-soaking



1e. 48 hrs pre-soaking

Figure 2 a-e. Tilapia mossambica: Time course of percentage mortality against a constant concentration (4 ppm) of croton seed pre soaked for different durations





test animal exposed to the different media, the highest (60%) being recorded in the medium containing the cake pre-soaked for 12 hours. Total mortality was recorded within 90 minutes of exposure of T. mossambica to the cake pre-soaked for 6, 12 and 24 hours. 75% mortality occurred during the corresponding period in the medium containing unsoaked cake and which had been pre-soaked for 48 hours.

Similar experiments were carried out to delineate the effect of pre-soaking of croton seed for varying durations (Table 1b & Fig. 2a-e). Neither the pre-soaked material nor the unsoaked one in the media elicited any mortality among the test species, T. mossambica, upto 2-5 hours. Moderate levels of mortality were observed within 3 hours in the test media which contained unsoaked croton and those which were pre-soaked for 6, 12 and 24 hours; the maximal mortality being observed in the test vessel containing croton pre-soaked for 6 hours. Absolute mortality was recorded within 4 hours in all the test media containing unsoaked croton and those which had been subjected to pre-soaking for 6 and 12 hours. When the duration of pre-soaking was enhanced to 24 hours absolute mortality occurred in 4 1/2 hours. When croton seed was pre-soaked for a longer duration (48 hours) the test media did not elicit any toxicity to T. mossambica upto 3 1/2 hours as indicated by a total lack of mortality. However, beyond 4 hours there was a gradual increase in the rate of mortality and by 6 hours, absolute mortality effected.

4.1.2. Effects of the piscicides on dissolved oxygen and pH of the test media.

The effects of different concentrations of mahua oil cake and croton seed on the dissolved oxygen and pH of the test media over a period of 96 hours are detailed out in Tables 2a-3^b, Figs. 3a-4^b. Data on the fluctuations in the dissolved oxygen level of the test media containing graded concentrations of mahua oil cake at successive intervals of time are presented in Table 2a. A concentration - dependent gradual reduction in oxygen content was noticed in all the media containing varying concentrations of the piscicide (50 - 250 ppm) upto 24 hours and thereafter the levels were found to increase steadily. The lowest values were recorded around the 24th hour. An experiment was run simultaneously to understand the source of oxygen for replenishment in the medium during the period of restoration of normalcy, by sealing the medium with liquid paraffin. The dissolved oxygen in the medium was found to gradually decrease over the period of experimentation with no sign of recovery at any stage indicating a very high biological oxygen demand during the toxically active phase of the cake. The trend in the variation of pH of the media is outlined in Table 2b. ³⁶ As can be seen in the Table, the pH decreased gradually upto about 48 hours and increased thereafter. A concentration - dependent declension in the pH was discernible; the lowest being recorded in the test media containing 250 ppm of the cake.

Table 2a. Effect of different concentrations of mahua oil cake on the dissolved oxygen (ml/ l) content of the test medium.

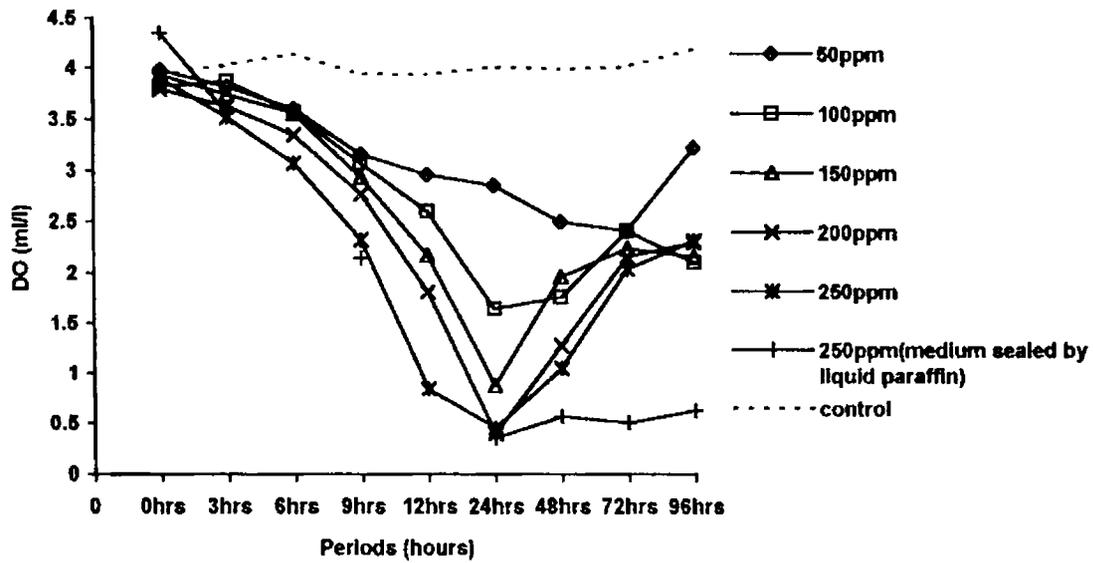
Periods of observation (hours) \ Concentrations (ppm)	0	3	6	9	12	24	48	72	96
50	3.98	3.82	3.60	3.16	2.96	2.85	2.49	2.40	3.22
100	3.82	3.87	3.57	3.07	2.60	1.64	1.75	2.40	2.09
150	3.93	3.74	3.56	2.93	2.17	0.87	1.95	2.23	2.15
200	3.79	3.63	3.35	2.77	1.81	0.39	1.27	2.15	2.29
250	3.90	3.52	3.07	2.32	0.84	0.45	1.04	2.03	2.31
250*	4.35	3.56		2.14		0.35	0.56	0.50	0.62
Control	3.94	4.03	4.14	3.94	3.93	4.01	3.98	4.01	4.18

* medium sealed by liquid paraffin

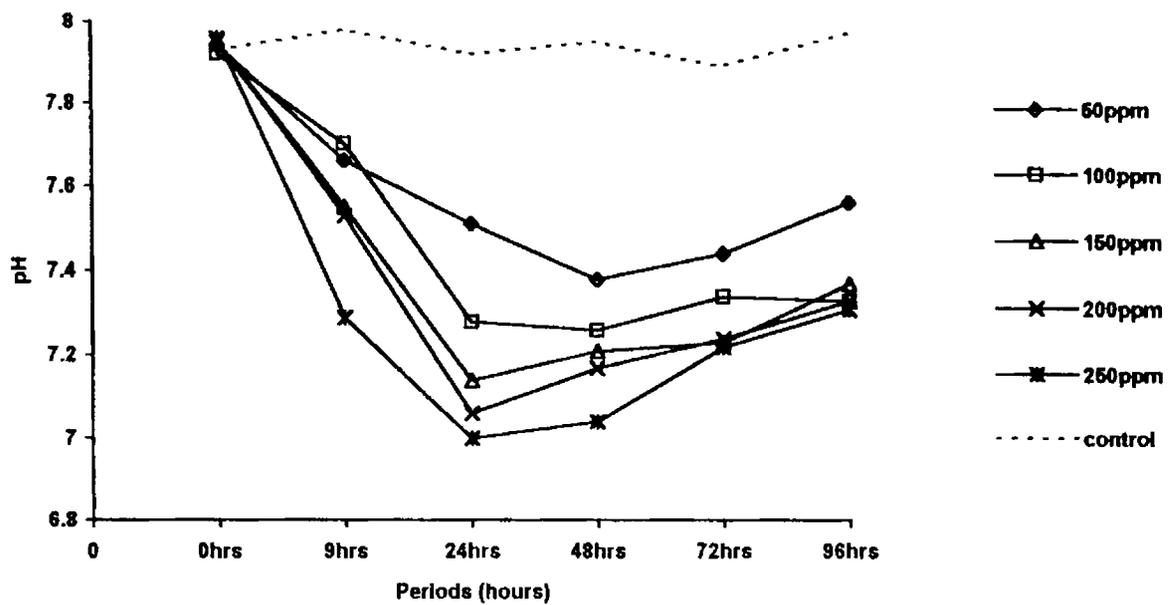
Table 2b. Effect of varying concentrations of mahua oil cake on the pH of the test medium.

Periods of observation (hours) \ concentrations (ppm)	0	9	24	48	72	96
50	7.94	7.66	7.51	7.38	7.44	7.56
100	7.92	7.70	7.28	7.26	7.34	7.33
150	7.95	7.55	7.14	7.21	7.23	7.37
200	7.95	7.53	7.06	7.17	7.24	7.33
250	7.96	7.29	7.00	7.04	7.22	7.31
Control	7.93	7.98	7.92	7.95	7.89	7.97

Figure 3a & b. Effect of different concentrations of mahua oil cake on the dissolved oxygen content and pH of the test medium



3a. Dissolved oxygen (ml/l)



3 b. pH

Table 3a: Effect of different concentrations of croton on the dissolved oxygen (ml/ l) content of the test medium

Periods of observation (hrs) \ Concentration (ppm)	0	3	6	9	12	24	48	72	96
0.5	3.71	3.54	3.12	3.48	3.43	3.51	3.71	3.79	3.93
1.0	3.65	3.34	3.20	3.4	3.20	3.54	3.71	3.62	3.82
2.0	3.51	3.23	2.75	3.3	2.9	3.09	3.43	3.34	3.51
4.0	3.48	3.06	2.81	2.78	2.64	3.0	3.45	3.06	3.71
4.0 *	3.37	3.09	2.25	2.3	2.08	1.46	0.84	0.84	0.84
Control	3.31	3.43	3.54	3.43	3.43	3.37	3.65	3.71	3.76

*medium scaled by liquid paraffin

Table 3b: Effect of different concentrations of croton seed on the pH of the test medium

Periods of observation (hrs) \ Concentration (ppm)	0	3	6	9	12	24	48	72	96
0.5	7.73	7.71	7.72	7.72	7.65	7.76	7.7	7.78	7.77
1.0	7.82	7.79	7.68	7.69	7.61	7.73	7.66	7.72	7.76
2.0	7.78	7.76	7.62	7.65	7.67	7.72	7.68	7.74	7.75
4.0	7.9	7.67	7.54	7.53	7.57	7.45	7.68	7.69	7.62
Control	7.77	7.77	7.76	7.77	7.62	7.75	7.67	7.7	7.76

Figure 4a & b. Effect of different concentrations of croton seed on the dissolved oxygen content and pH of the test media

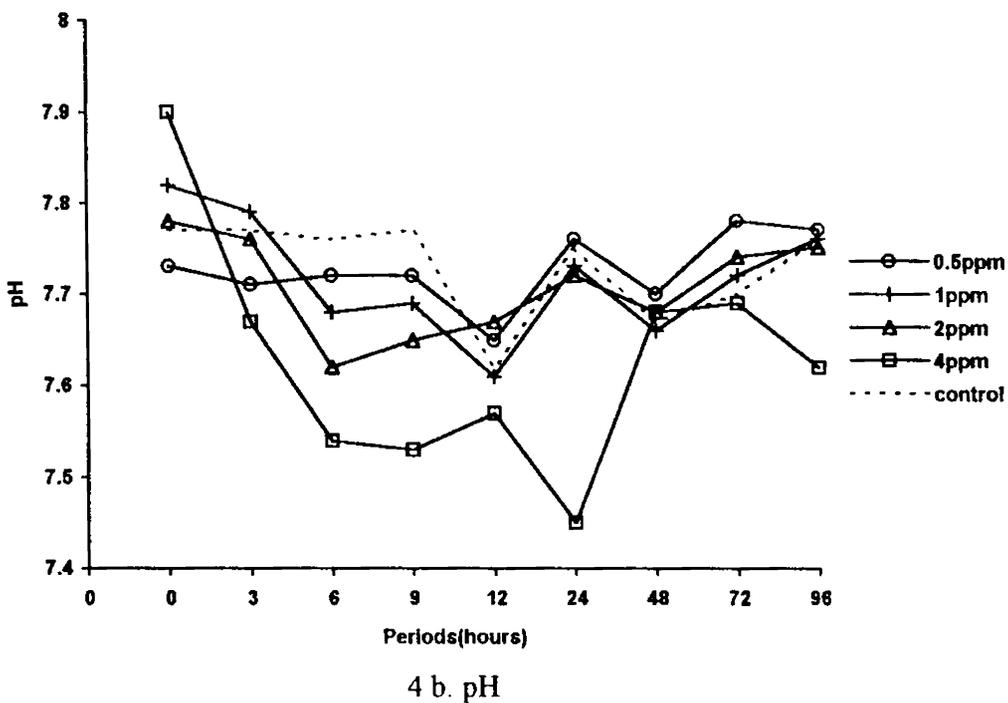
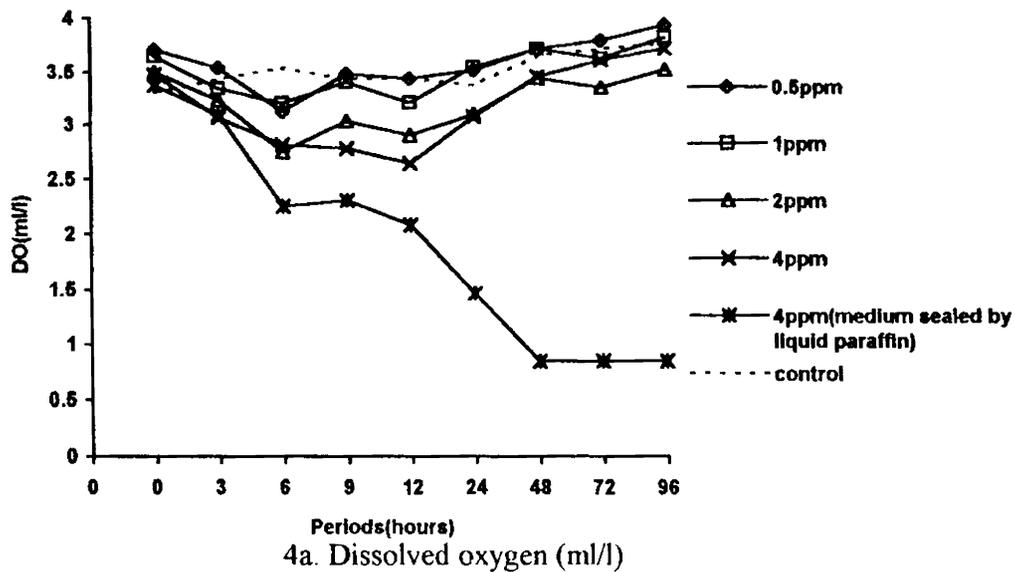


Table 3a & Fig. 4a outlines the effects of varying concentrations of aqueous suspension of croton seed on the dissolved oxygen content of the test media over a period of 96 hours. In general, the trend in variation of the oxygen content of the media containing different concentrations of the toxicant was of a dual nature. The minimal oxygen content of the test medium containing 0.5 ppm of the piscicide was 3.12 ml/l during the 6th hour. The same was true in the case of the medium with 1 ppm also. In both the cases the level of dissolved oxygen indicated a gradual fall during the early phases of sampling, to increase towards the end of the period of experimentation. A more or less similar trend was obtained in the case of 2 ppm croton - containing test media. The variation was more clear cut when 4 ppm croton was added to the ambient water; the lowest concentration, however, being recorded during the 12th hour of sampling. Sealing of the test media with liquid paraffin was found to decrease dissolved oxygen concentration very sharply, registering a minimal value of 0.84 ml/l at 48 hours. The level of oxygen content in the control registered only marginal variation when compared to that of the toxicant - containing test media. Further, the oxygen concentration in the control never decreased below the values recorded during zero hour.

The influence of different concentrations of croton on the pH of the test media is outlined in Table 3b & Fig. 4b. The pH of the medium containing 0.5 ppm of the toxicant was more or less similar at successive samplings over the 96 hour

experimental period. The same was true when the concentration of croton was increased to 1 and 2 ppm. In the test media containing 4 ppm croton, a slight reduction in pH was observed during the first quarter; registering a minimal value of 7.45. The pH was found to increase gradually thereafter. Statistical analysis revealed significant ($p < 0.01$) difference in dissolved oxygen among different concentrations and control and no significant difference at 5% level among different periods of observation. The pH also showed significant variation ($p < 0.01$) significant variation between different concentrations and periods of observation.

4.1.3. Toxic response of different test organisms to the piscicides.

4.1.3.1. Finfish

The toxic response of selected weed fishes commonly inhabiting prawn culture fields was tested by exposing them to a constant concentration (200 ppm) of mahua oil cake (Table 4a). Among the different species tested, Ambassis gymnocephalus, Megalops cyprinoides, Elops saurus and Glossogobius giurus were the most sensitive registering absolute mortality within 30 minutes of exposure. Macropodus cuppanus, Etroplus maculatus, Gambusia affinis and Aplochilus lineatus were moderately tolerant requiring about an hour before succumbing to the toxicant. Among the various fish species tested, Ophichthys microcephalus, O.

Table 4a. Finfish: Time course of percentage mortality against a constant concentration (200ppm) of mahua oil cake

Periods of observation (minutes)				
Finfish species tested	30	60	90	120
<u>Ambassis gymnocephalus</u>	100			
<u>Megalops cyprinoides</u>	100			
<u>Elops saurus</u>	100			
<u>Glossogobius giurus</u>	100			
<u>Macropodus cupanus</u>	70	100		
<u>Etroplus maculatus</u>	60	100		
<u>Gambusia affinis</u>	40	100		
<u>Aplochilus lineatus</u>	35	100		
<u>Tachysurus maculatus</u>	30	80	100	
<u>Tilapia mossambica</u>	-	60	100	
<u>Ophichthys boro</u>	-	20	80	100
<u>Ophichthys microcephalus</u>	-	-	55	100
Control	-	-	-	-

- No mortality

Table 4b. Finfish: Time course of percentage mortality against a constant concentration (4ppm) of croton seed

Finfish species tested	Periods of observation (minutes)							
	30	60	90	120	150	180	210	240
<u>Ambassis gymnocephalus</u>	60							
<u>Megalops cyprinoides</u>	30	100						
<u>Elops saurus</u>	35	100						
<u>Glossogobius giurus</u>	-	15	35	100				
<u>Macropodus cupanus</u>	15	100						
<u>Etroplus maculatus</u>	25	100						
<u>Gambusia affinis</u>	-	30	100					
<u>Aplochilus lineatus</u>	-	45	100					
<u>Tachysurus maculatus</u>	-	-	-	45	65	100		
<u>Tilapia mossambica</u>	-	-	-	-	-	45	80	100
<u>Ophichthys boro</u>	-	-	-	-	5	45	70	100
<u>Ophichthys microcephalus</u>	-	-	-	-	-	40	55	100
Control	-	-	-	-	-	-	-	-

- No mortality

boro and T. mossambica were the most tolerant, registering absolute mortality only in about 2 hours (Table 4a). The affected fishes showed stress symptoms like losing balance while swimming and gradually becoming inactive. Such inactive fishes sank to the bottom of the container, rested on their sides in a moribund state and finally died.

A similar trend in tolerance was observed when the same finfish species were exposed to 4 ppm croton seed. A striking feature of the result (Table 4b) obtained was that the rate of mortality was gradual and total mortality occurred only after a longer duration of exposure. The affected fishes came to the surface in stressed condition and displayed erratic swimming with convulsive movements. Lacerations were developed on the gills and the fishes bled through these lacerations. Mild bleeding was also noticed from the gill portion.

4.1.3.1.1 Toxic response of T. mossambica to graded concentrations of the piscicides

Individuals of T. mossambica were exposed to graded concentrations of mahua oil cake for periods upto 8 hours. The results obtained are presented in table 5a. A concentration-dependent increase in toxicity was evident. None of the individuals succumbed to death when 60 ppm of the piscicide was present in the medium. Mortality occurred after 5 hours when the concentration was 70 ppm, and only 50% of the individuals died at

Table 5a. *I. mossambica* : Time course of percentage mortality against graded concentrations of mahua oil cake

Periods of observation (minutes)	Concentration (ppm)										Control	
	60	70	75	80	90	100	200					
30	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	60	-	-	-	-	-
90	-	-	-	-	-	20	100	-	-	-	-	-
120	-	-	-	-	-	40	-	-	-	-	-	-
150	-	-	-	-	-	100	-	-	-	-	-	-
180	-	-	-	-	-	-	-	-	-	-	-	-
210	-	-	-	-	30	-	-	-	-	-	-	-
240	-	-	-	-	65	-	-	-	-	-	-	-
270	-	-	-	-	100	-	-	-	-	-	-	-
300	-	-	-	-	-	-	-	-	-	-	-	-
330	-	-	-	35	-	-	-	-	-	-	-	-
360	-	20	25	100	-	-	-	-	-	-	-	-
390	-	50	55	-	-	-	-	-	-	-	-	-
420	-	50	70	-	-	-	-	-	-	-	-	-
450	-	50	100	-	-	-	-	-	-	-	-	-

-No mortality

the end of the experiment. A more or less similar mortality rate was observed when 75 ppm of mahua oil cake was present in the medium. However, total mortality was recorded by the end of the experiment. A sudden spurt of mortality was observed among individuals of T. mossambica when exposed to 80 ppm of the piscicide. The entire lot was found dead between the 5th and 6th hour of exposure. Increased toxicity was observed as evidenced by absolute mortality was observed when subjected to higher concentrations of the cake. Four hours were required for total mortality when individuals were exposed to 90 ppm; the same being 2.5 and 1.5 hours for 100 and 200 ppm of the cake respectively.

Table 5b outlines the percentage mortality of T. mossambica following exposure to selected concentrations (0.5 - 4ppm) of croton seed. A concentration of 0.5 ppm of the material in the medium did not elicit lethality to the test species. When the concentration was doubled, half of the exposed individuals died in about 6.5 hours. Longer exposure to the same concentration did not cause any increase in the percentage mortality. Exposure to 1.5 ppm of croton seed also produced a more or less similar result. The toxicant could not induce total mortality during the period of exposure. Enhancement of the concentration to 2 ppm and above produced absolute mortality much earlier. A clear cut reduction in lethal time was observed with an increase in concentration of the toxicant. While 2 ppm

Table 5b. *T. mossambica*: Time course of percentage mortality against graded concentrations of croton seed

Concentration(ppm)	Periods of observation (minutes)							
	0.5	1	1.5	2	2.5	3	4	Control
30	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-
150	-	-	-	-	-	-	-	-
180	-	-	-	-	-	-	45	-
210	-	-	-	-	-	-	80	-
240	-	-	-	-	-	35	100	-
270	-	-	-	-	55	60		-
300	-	-	-	-	95	100		-
330	-	-	-	35	100			-
360	-	15	-	100				-
390	-	55	60					-
420	-	55	80					-
450	-	55	80					-

-No mortality

of the toxicant needed 6 hours to effect absolute mortality, 2.5 ppm of the same toxicant required only 5 hours to cause the same effect. The time for absolute mortality in the case of 3 and 4 ppm of the piscicide was about 5 hours and 4 hours respectively.

4.1.3.1.2 Effect of the piscicides on the haematological parameters of T.mossambica

Experiments were conducted to analyse the effect of 80 ppm mahua oil cake on the blood profile of T. mossambica over a period of 6 hours. The data obtained is illustrated in table 6a. It can be seen from the Table that the haemoglobin content (Hb %) of the exposed individuals decreased significantly when compared to their counterparts in the control lot. The values dropped from 11.21 to 7.98. A similar trend in variation was observed in the case of haematocrit values (Ht %) and total erythrocyte count (TEC) also. The haematocrit values fell sharply from 30.49 at the start of the experiment to a surprisingly low 20.87 at the end. In the case of the case of TEC, the value came down from $1.9 \times 10^6 / \text{mm}^3$ to $1.14 \times 10^6 / \text{mm}^3$ at the close of the experiment. The Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) of the exposed individuals on the other hand increased steadily when compared to the control animals during the experimental period.

Table 6a. Effect of mahua oil cake(80ppm) on the blood parameters of *T. mossambica* exposed over a period of 6 hours

Blood parameters	0		2		4		6	
	Exp	Con	Exp	Con	Exp	Con	Exp	Con
Haemoglobin (%)	11.21 ± 0.31	11.21 ± 0.31	9.46 ± 0.02	12.75 ± 0.37	8.4 ± 0.05	11.99 ± 0.29	7.98 ± 0.05	12.0 ± 0.1
Haematocrit (%)	30.49 ± 2.39	30.49 ± 2.39	25.74 ± 0.23	30.84 ± 0.37	24.25 ± 2.66	29.99 ± 0.64	20.86 ± 0.58	30.53 ± 0.53
Total Erythrocyte Count ($\times 10^6/\text{mm}^3$)	1.9 ± 0.05	1.9 ± 0.05	1.5 ± 0.1	2.02 ± 0.18	1.33 ± 0.05	2.11 ± 0.24	1.14 ± 0.05	2.05 ± 0.11
Mean Corpuscular Volume (MCV)	160.47	160.47	171.6	152.67	182.33	142.13	182.98	148.93
Mean Corpuscular Haemoglobin (MCH)	59	59	63.07	63.12	63.16	56.82	70	58.54

Table 6b. Effect of croton seed (2ppm) on the blood parameters of *T. mossambica* exposed over a period of 6 hours

Blood parameters	0		2		4		6	
	Exp	Con	Exp	Con	Exp	Con	Exp	Con
Haemoglobin (%)	11.98 ± 0.71	11.98 ± 0.71	9.03 ± 0.31	13.38 ± 0.1	8.62 ± 1.27	10.49 ± 1.7	8.4 ± 0.58	10.93 ± 1.36
Haematocrit (%)	28.58 ± 2.26	28.58 ± 2.26	24.51 ± 1.74	30.13 ± 4.38	22.84 ± 1.38	29.13 ± 0.57	20.98 ± 0.56	28.69 ± 1.25
Total Erythrocyte Count ($\times 10^6/\text{mm}^3$)	2.46 ± 0.21	2.46 ± 0.21	1.34 ± 0.23	2.63 ± 0.17	1.15 ± 0.09	2.14 ± 0.26	0.78 ± 0.05	2.21 ± 0.08
Mean Corpuscular Volume (MCV)	116.18	116.18	182.91	114.56	198.61	136.12	268.97	129.82
Mean Corpuscular Haemoglobin (MCH)	48.69	48.69	67.39	50.87	74.95	49.02	107.69	49.46

Table 6b outlines the data obtained on the blood parameters of individuals of T. mossambica following exposure to a constant concentration (2 ppm) of croton seed. The pattern of variation of the various blood parameters was more or less similar with that observed in the case of mahua oil cake. Statistical analysis of data revealed a significant difference ($p < 0.05$) in the values of Hb, Ht & TEC between the experimental and control samples following exposure to mahua oil cake and croton seed.

4.1.3.2 Penaeus indicus

The toxicity of mahua oil cake to the postlarvae of Penaeus indicus was put to detailed investigation. The data obtained is given in Table 7a. While 200 ppm of the cake did not effect any mortality among individuals of the prawn species during the 24 hour period of exposure, all the other concentrations tested produced total mortality. In fact, concentrations ranging from 300-500 ppm required 24 hours to cause total mortality. Higher doses of the cake (750-1250 ppm) however required only 12 hours to produce the same effect. Postlarvae of Penaeus indicus were exposed to selected concentrations of croton seed (0.25, 0.5, 1, 2, 3, 4 & 5 ppm). The calculated percentage mortality is outlined in Table 7b. The lower doses of the toxicant (0.25-1 ppm) were rather ineffective in bringing about mortality among the postlarvae.

Table 7a. *P. indicus* (postlarvae): Time course of percentage mortality against graded concentrations of mahua oil cake

Periods of observation (hours) \ Concentration (ppm)	3	6	12	24
200	-	-	-	-
300	-	-	-	75
400	-	-	-	100
500	-	-	80	100
750	-	-	100	
1000	-	-	100	
1250	-	-	100	
Control	-	-		

-No mortality

Table 7b. *P. indicus* (postlarvae): Time course of percentage mortality against graded concentrations of croton seed

Periods of observation(hours) \ Concentration (ppm)	3	6	12	24
0.25	-	-	-	-
0.5	-	-	-	-
1	-	-	-	25
2	-	40	100	
3	-	55	100	
4	-	80	100	
5	-	80	100	
Control	-	-	-	-

-No mortality

Concentrations ranging from 2 to 5 ppm on the other hand were highly toxic causing absolute mortality of the individuals in 12 hours. During the first 3 hours of exposure, in fact, no mortality was recorded in any of the concentrations tested.

4.1.3.3 Villorita cyprinoides

The toxic effect of selected concentrations of mahua oil cake on the clam Villorita cyprinoides over a duration of 96 hours is given in table 8a. None of the individuals died upto 36 hours in any of the concentrations tested. Neither 50 ppm nor 100 ppm of the cake was capable of inducing mortality among the individuals of the clam species during the 96 hour exposure period. However, higher concentrations viz. 200 and 250 ppm were lethal to the test species from 48 hours onwards, effecting 100% mortality in 72 hours.

The data on the percentage mortality of V. cyprinoides following exposure to selected concentrations of croton seed is given in Table 8b. A notable feature of the result obtained was a concentration - dependent declension in the lethal time. While 1 ppm of the piscicide was capable of causing only 50% mortality among individuals of the clam during 96 hours, 2 ppm needed only 72 hours to produce absolute mortality. Higher concentrations of 4 ppm and 5 ppm required only 60 hours and 48 hours respectively to produce the same extent of mortality.

Table 8a. *V. cyprinoides* : Time course of percentage mortality against graded concentrations of mahua oil cake

Periods of observation(hours) Concentration (ppm)	12	24	36	48	60	72	84	96
50	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-
150	-	-	-	20	30	65	80	80
200	-	-	-	20	50	100		
250	-	-	-	50	65	100		
Control	-	-	-	-	-	-	-	

-No mortality

Table 8b. *V. cyprinoides* : Time course of percentage mortality against graded concentrations of croton seed

Periods of observation(hours) Concentration (ppm)	12	24	36	48	60	72	84	96
0.5	-	-	-	-	-	-	-	-
1	-	-	-	25	40	50	50	50
2	-	-	-	50	70	100		
4	-	-	-	75	100			
5	-	0	45	100				
Control	-	-	-	-	-	-	-	-

-No mortality

4.1.4 Persistence of toxicity of different concentrations of the piscicides in the test media

The persistence of toxicity of different concentrations of mahua oil cake in the media was tested with a low tolerant fish species, Ambassis gymnocephalus as the target organism. 75 ppm of the cake effected total mortality of the test individuals on day 1 (Table 9a & Figs. 5a-e) Exposure of A. gymnocephalus to the same medium from day 2 to day 7 did not cause any mortality among the individuals. When 100 ppm of the cake was present, absolute mortality occurred among the experimental individuals on the first 2 days, about 75% mortality on day 3 and no mortality from day 4 onwards. Increased duration of persistence of toxicity was seen in the media when the concentration was increased to 150, 200 and 250 ppm. Absolute mortality was recorded upto the 4th day in these concentrations. On day 5, while marginal mortality was reported in the media containing 150 and 200 ppm, about 75% mortality was recorded in the medium containing 250 ppm of the toxicant. From day 6, the experimental media containing 150 and 200 ppm did not indicate any toxicity with reference to the test fish. However, 250 ppm of the cake continued to exert lethal effect to A. gymnocephalus.

Table 9b & Figs. 6a-f outlines the percentage mortality among A. gymnocephalus when exposed to 0.5 to 5 ppm croton seed for a period upto 7 days. A concentration of 0.5 ppm

Table 9a. *A. gymnocephalus*: Time course of percentage mortality against graded concentrations of mahua oil cake

Periods of observation(days) \ Concentration (ppm)	1	2	3	4	5	6	7
75	100	-	-	-	-	-	-
100	100	100	75	-	-	-	-
150	100	100	100	100	10	-	-
200	100	100	100	100	20	-	-
250	100	100	100	100	95	35	-
Control	-	-	-	-	-	-	-

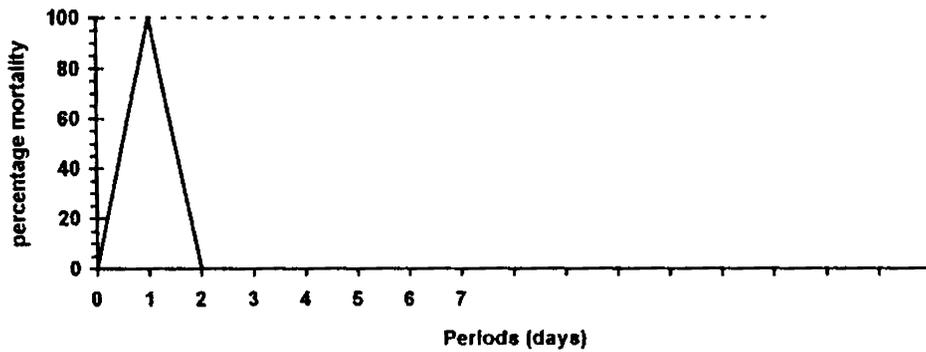
-No mortality

Table 9b. *A. gymnocephalus*: Time course of percentage mortality against graded concentrations of croton seed

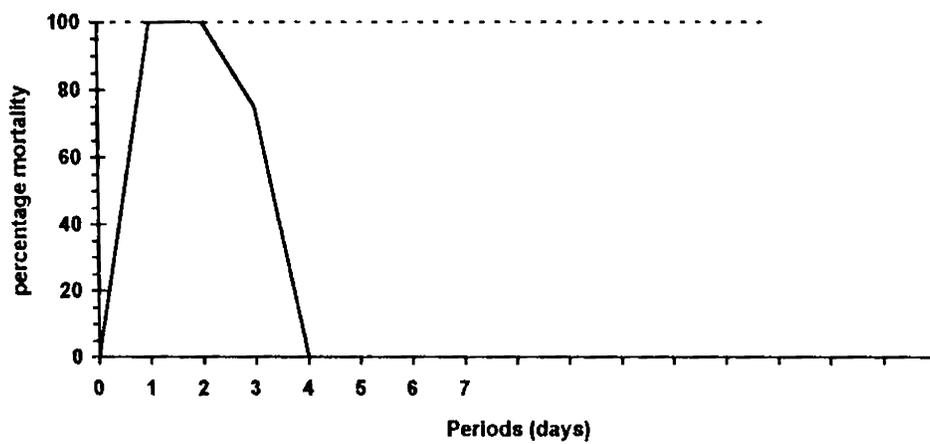
Periods of observation(days) \ Concentration (ppm)	1	2	3	4	5	6	7
0.5	100	-	-	-	-	-	-
1	100	20	-	-	-	-	-
2	100	100	40	-	-	-	-
3	100	100	75	-	-	-	-
4	100	100	100	55	-	-	-
5	100	100	100	70	35	-	-
Control	-	-	-	-	-	-	-

-No mortality

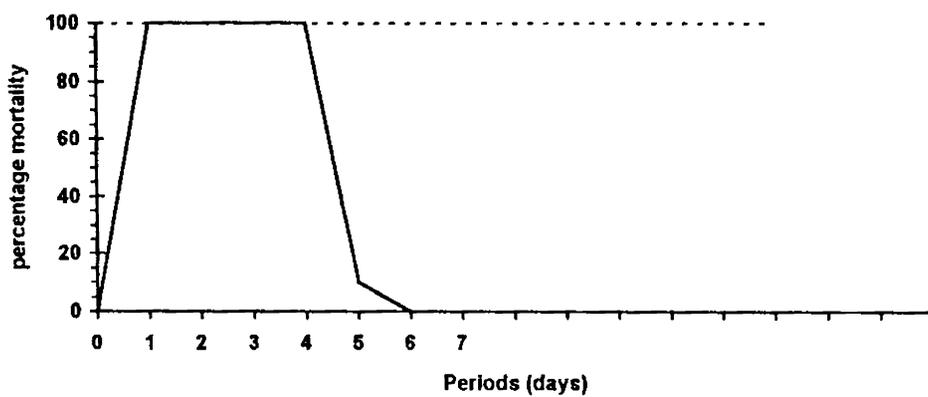
Figure 5 a-e. A gymnocephalus Time course of percentage mortality against graded concentrations of mahua oil cake.



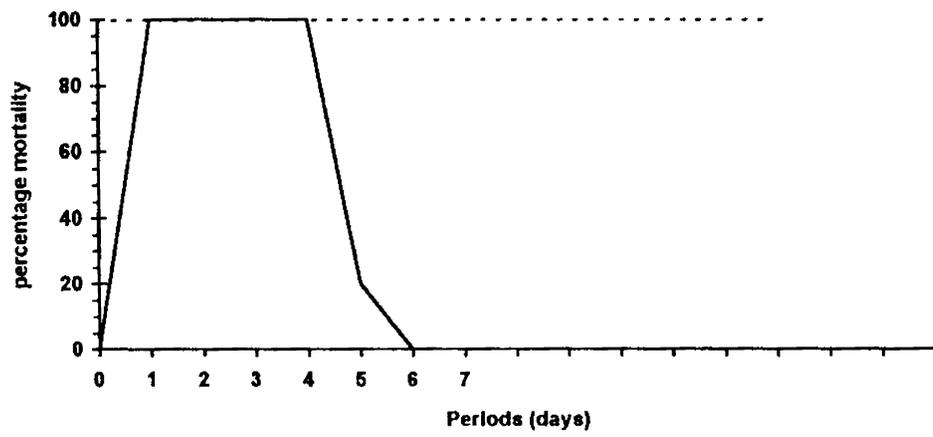
5 a. 75 ppm



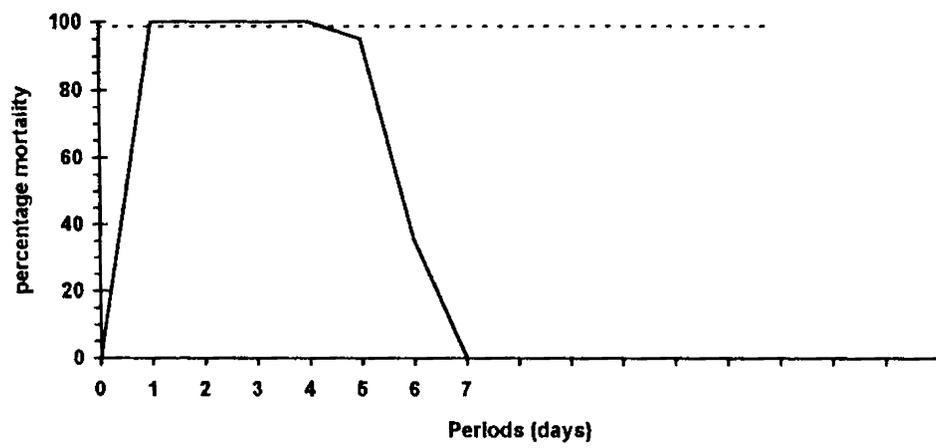
5 b. 100 ppm



5 c. 150 ppm

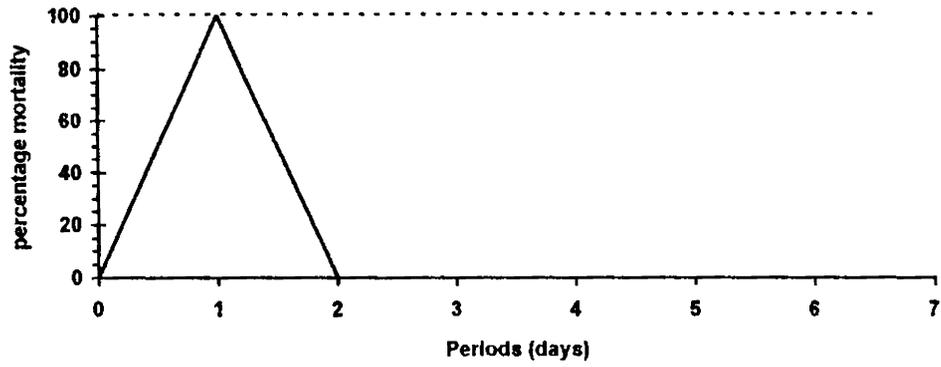


5 d. 200 ppm

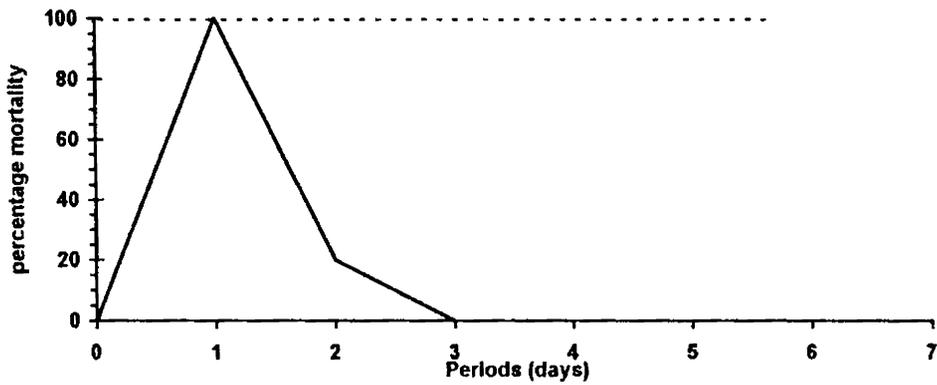


5 e. 250 ppm

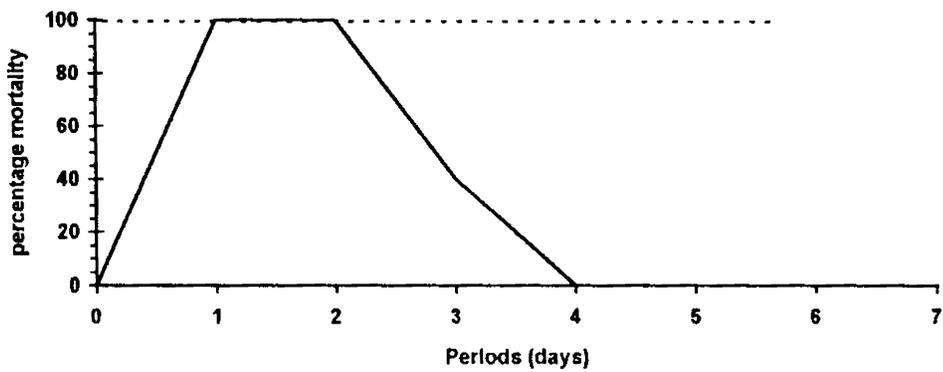
Figure 6 a-f. *A. gymnocephalus*: Time course of percentage mortality against graded concentrations of croton seed



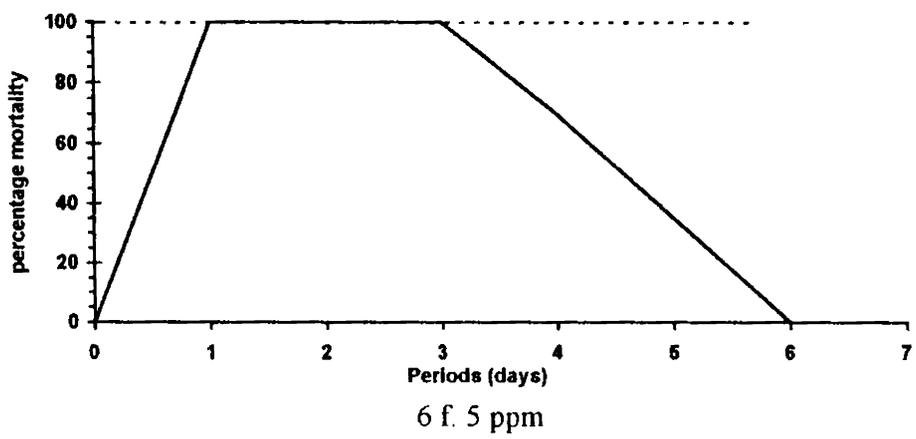
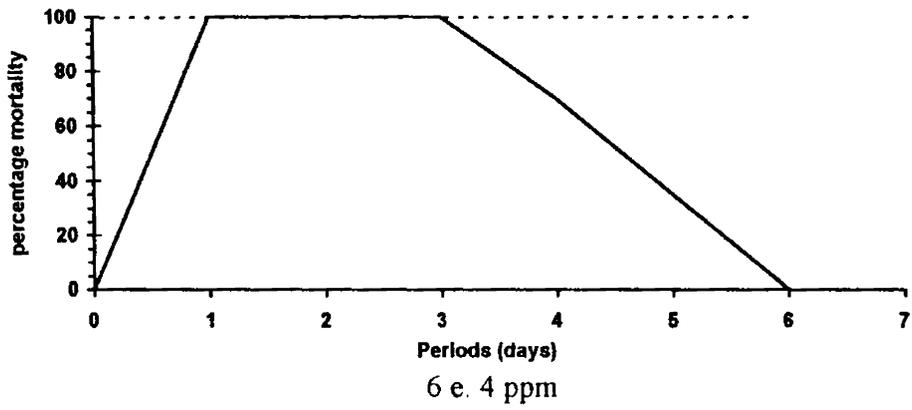
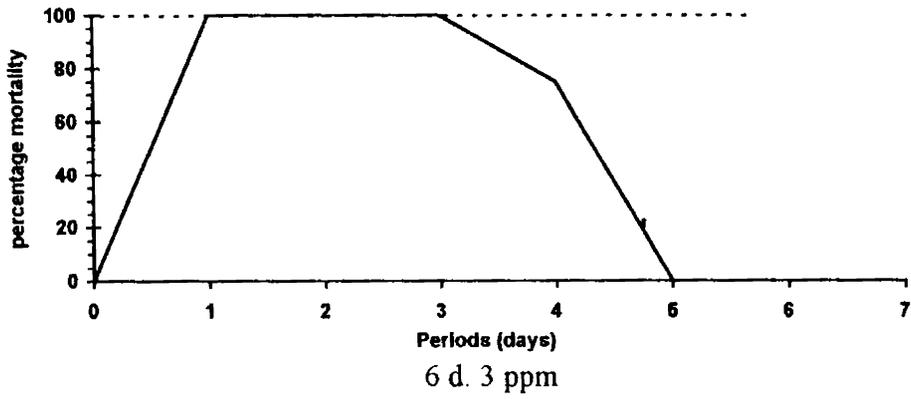
6 a. 0.5 ppm



6 b. 1 ppm



6 c. 2 ppm



was lethal to the test animal only on the first day of the experiment. The same was true in the case of 1 ppm of croton also, although marginal mortality was observed on the second day. Increase in concentration of croton to 2 ppm indicated lethality upto day 3. The same was true in the case of 3 ppm also. The toxicity of the medium containing 4 ppm was found to persist upto day 4 as evidenced by the mortality of individuals of A. gymnocephalus. The toxicity of the highest concentration of croton tested (5 ppm) was also found to persist in the medium producing mortality even upto day 5, after which duration the toxicant was found to become ineffective.

4.2 FIELD EXPERIMENTS

4.2.1 Effects of different concentrations of mahua oil cake and croton seed on the field culture system and delineation of their optimum concentration for field application.

Selected prawn culture ponds were treated with different concentrations of mahua oil cake and croton seed with a view to delineate the lowest concentration of the two piscicides, which would result in hundred percent mortality of all weed fishes and selected clam species. The field study also centred around analysing the effects of the two piscicides on the hydrographic parameters, selected prawn species, zooplankton and benthos. The data obtained is outlined in Tables 10-25.

4.2.1.1 Effect on a few hydrographic parameters

The data on the fluctuations in hydrographic parameters of the ponds treated with different concentrations of mahua oil cake is presented in Table 10.a. It can be seen from the Table that the water temperature of the experimental ponds ranged between 31.35 °C and 31.75 °C at the beginning of the experiment. During the course of study, the temperature increased gradually depicting a higher range of 32.25 °C to 32.7 °C towards the middle of the experimental period to reduce thereafter to a lower range of 31.1 °C to 32.6 °C at the end of the experiment. A more or less similar trend was observed in the case of the control pond also.

The data on the dissolved oxygen content of the pond water treated with graded concentrations of mahua oil cake (Table 10a) indicated that in the pond treated with 150 ppm of the piscicide there was a drop in dissolved oxygen content from 3.76 ml/l to 1.58 ml/l on the 2nd day of the experiment. Interestingly, the levels were found to increase gradually to reach a high level of 6.45 ml/l on the 5th day of the experiment. Since then the oxygen content maintained a more or less steady level. When the concentration was increased to 200 ppm, the variation trend was more or less identical. In fact the oxygen level dipped to an undetectably low level on the third day of the experiment increasing gradually thereafter. From day 5

onwards, the O₂ levels were found to vary considerably. In the pond treated with 250 ppm of the cake, the oxygen levels were below detectable limits on the 3rd and 4th day of the experiment. From day 5 onwards, a gradual increase in oxygen content was noticed and a remarkably high level of 7 ml/l was recorded on the final day of the experiment. The variation in oxygen level in the pond treated with 300 ppm of mahua oil cake was in line with that observed in the pond treated with 250 ppm. The only variation observed was the relative absence of oxygen for a longer duration extending from day 2 to day 7. In the pond containing 200 ppm of the cake, where the medium was diluted (8-10%) with tidal water followed by the elimination of weed fishes, the dissolved oxygen content did not at any point during the sampling period drop to an undetectably level and the lowest level of dissolved oxygen recorded was only 1.07 ml/l (Table 10.c). Indeed the pattern of fluctuation of dissolved oxygen was of a similar nature as observed in the undiluted medium, showing no significant difference between the two on statistical analysis.

Table 10a also contains the data on the pH of the pond water exposed to varying concentrations of mahua oil cake over a period of 10 days. The pH of the experimental ponds at the beginning of the period of observation was of the range 6.6 - 7.2. From day 1 onwards the pH values revealed an increasing trend with frequent fluctuation. The highest pH recorded in the

Table 10.c : Hydrographic parameters of ponds treated with 200 ppm mahua oil cake and 4 ppm croton seed and the test medium subsequently diluted with tidal water 12 hrs after treatment along with the control.

Piscicides	Mahua oil cake				Croton seed			
	Temp (°C)	O ₂ ml/l	pH	pH	Temp (°C)	O ₂ ml/l	pH	pH
Hydrographic parameters								
Periods of observation[Hrs.]								
0 Expt.	32.15	4.62	8.08		31.95	5.59		8.35
Contr.	32.2	4.78	8.1		32.2	4.78		8.1
24 Expt.	33.53	1.96	7.45		32.7	7.76		8.55
Contr.	32.2	5.8	8		32.2	5.8		8
48 Expt.	32.23	1.07	7.43		31.9	4.61		8.23
Contr.	31.8	3.39	7.65		31.8	3.39		7.65
72 Expt.	32.7	3.87	8.08		31.83	6.15		8.4
Contr.	31.75	4.22	7.65		31.75	4.22		7.65
96 Expt.	33.43	5.43	8.3		32.8	6.1		8.55
Contr.	32.25	4.09	7.65		32.25	4.09		7.65
120 Expt.	33.43	5.02	8.43		32.78	6.72		8.6
Contr.	32.85	4.57	8.2		32.85	4.57		8.2

different concentrations ranged between 7.9 and 8. The control pond also displayed a similar pattern of variation ranging in pH from 7.18 to 8.05.

Table 10.b outlines the variations in hydrographic parameters of the ponds treated with 5 different concentrations of croton seed. It is evident from the Table that the temperature of the ambient water was more or less uniform throughout the period of study in all the treatment ponds and the control except at 24 hours and 48 hours where a marginal increase was noticed. The fluctuation in oxygen content and pH of the water was also of a similar nature. The data presented in Table 10c also describes the results obtained on the hydrographic parameters of a pond treated with 4 ppm of croton seed and the medium diluted with tidal water. No pronounced variation in water temperature, pH and dissolved oxygen was observed when compared with those observed in the undiluted treatment pond containing the same concentration.

4.2.1.2 Effect on the test organisms

4.2.1.2.1 Finfish

Results of the field trials carried out to assess the toxic response of different weed fishes to selected concentrations of mahua oil cake are presented in Table 11.a. The different species under test displayed a wide range of tolerance to the different concentrations of the toxicant. While

absolute mortality occurred in the case of Ambassis gymnocephalus, Megalops cyprinoides and Elops saurus within 3 hours of treatment in the presence of 150 ppm of mahua oil cake, Etroplus maculatus Glossogobius giurus and Macropodus cupanus required 6 hours to produce the same result. Aplochilus lineatus, Gambusia affinis, Tachysurus maculatus and Tilapia mossambica on the other hand recorded varying percentages of mortality none reaching cent percent, within the 96 hour duration. In fact, 150 ppm of the toxicant was insufficient to cause any mortality among individuals of Ophichthys boro or O. microcephalus. Concentrations of 250 ppm and 300 ppm were capable of effecting absolute mortality among all the finfishes tested, within a period of 6 hours. In these concentrations O. boro and O. microcephalus depicted considerable tolerance as evidenced by the highest lethal time (LT¹⁰⁰).

Table 11.b. contains data on the toxic response of selected finfishes to graded concentrations of croton seed. Among the different species tested, only A. gymnocephalus indicated absolute mortality in the lowest concentration (2 ppm) of croton seed administered. When the concentration was increased to 3 ppm, all members of the species, A. gymnocephalus, E. maculatus, M. cyprinoides, E. saurus, M. cupanus, A. lineatus and G. affinis died within varying durations extending from 3 to 12 hours. Concentrations of 4 ppm and 5 ppm were sufficient to kill all the experimental finfishes within 3 to 6 hours.

4.2.1.2.2 Penaeus indicus

Table 11b. Time course of percentage mortality of selected test organisms against graded concentrations of croton seed

Concentration (ppm)	2						3						4						5									
	3	6	12	24	48	72	96	3	6	12	24	48	72	96	3	6	12	24	48	72	96	3	6	12	24	48	72	96
Intervals of observation (hours)																												
Test organisms																												
Finfish species:																												
<u>Ambassis</u>	30	100						100							100							100						
<u>Gymnocephalus</u>																												
<u>Etroplus maculatus</u>	30	30	80	80	80	80	80	100	100						100	100						100	100					
<u>Megalops</u>	0	25	25	25	25	25	25	100							100							100						
<u>cyprinoides</u>																												
<u>Elops saurus</u>	0	15	15	15	15	15	15	100							100							100						
<u>Glossogobius</u>	0	5	0	10	10	10	10	20	45	45	45	45	45	100							100							
<u>giurus</u>																												
<u>Macropodus</u>	0	20	40	40	40	40	40	20	40	100					100							100						
<u>cupanus</u>																												
<u>Aplocheilichthys lineatus</u>	20	50	30	30	30	30	30	30	45	100					100							100						
<u>Gambusia affinis</u>	0	5	30	30	30	30	30	20	35	100					100							100						
<u>Tachysurus</u>																												
<u>maculatus</u>																												
<u>Tilapia mossambica</u>																												
<u>Ophichthys boro</u>																												
<u>O. microcephalus</u>																												
<u>P. indicus (Prawn)</u>																												
<u>Villonia (Clam)</u>																												
<u>cyprinoides</u>																												
Control																												

- No mortality

Among individuals of the postlarvae of Penaeus indicus exposed to different concentrations of mahua oil cake, no mortality was recorded in 150 ppm during the 96 hours of observation. While 65% of the individuals died during the same period in the medium containing a higher concentration (200 ppm), of mahua oil cake, absolute mortality occurred during 48 to 96 hours following exposure to 250 ppm. Absolute mortality was found to set in 24 hours earlier in the medium containing 300 ppm of the cake.

Postlarvae of P. indicus exposed to selected concentrations of croton seed showed varying levels of tolerance as depicted by their complete survival in 2 ppm, 40% mortality in 3 ppm and absolute mortality in 4 ppm and 5 ppm (Table 11.b). None of the individuals died within 48 hours in the medium containing 3 ppm and 4 ppm of croton whereas an early (within 48 hours) onset of mortality was noticed when exposed to 5 ppm croton seed.

4.2.1.2.3 Villorita cyprinoides

No lethal effect was encountered among individuals of the clam species, Villorita cyprinoides when subjected to 150 ppm and 200 ppm of mahua oil cake under field conditions. However, all individuals of the species died within 72 to 96 hours in a higher concentration of 250 ppm (Table 11.a). The medium

containing 300 ppm cake indicated more toxicity to the clam species as evidenced by absolute mortality of the experimental individuals within 48 to 72 hours.

Whereas 2 ppm of croton seed was ineffective in causing mortality among individuals of V. cyprinoides, a concentration of 3 ppm was capable of producing 40% mortality within a period of 72 to 96 hours (Table 11.b). Hundred percent mortality was recorded within 72 hours and 48 to 72 hours in the medium containing 4 ppm and 5 ppm of croton seed, respectively.

4.2.1.2.4 Zooplankton

The influence of selected concentrations of mahua oil cake on the zooplankton density of prawn culture ponds was investigated. The data obtained on the relative abundance of various zooplankton groups inhabiting the culture ponds is given in Tables, 12a-c.

Table 12a outlines the zooplankton density of the pond treated with 150 ppm mahua oil cake. Copepods were the most dominant group among the zooplankton populations. Among copepods, calanoids were found to be represented in maximum number. Besides copepods, the plankton samples obtained at the start of the experiment contained ostracods, amphipods and hydrozoan medusae. However, at the end of the experiment, the plankton samples obtained from the treated pond were poorly

Table 12a. Zooplankton density (Nos/m³) of the pond treated with 150ppm mahua oil cake along with the control

Organisms	0	24	120	240
a.COPEPODS				
(i) Calanoids-				
Control	215	88	326	377
Experiment	80	192	64	32
(ii)Cyclopoids				
Control	167	36	90	136
Experiment	24	16	8	32
(iii)Harpacticoids				
Control	4	76	40	17
Experiment	16	56	24	6
b.OSTRACODS				
Control	-	-	-	-
Experiment	16	20	64	32
c.AMPHIPODS				
Control	-	-	-	-
Experiment	24	20	86	6
d.HYDROZOAN MEDUSAE				
Control	35	40	60	50
Experiment	16	-	-	-
e.FISH LARVAE				
Control	10	12	16	72
Experiment	-	-	-	-
f.MOSQUITO LARVAE				
Control	-	-	-	-
Experiment	-	-	56	800

- Not present

represented. The only members observed were copepods and ostracods. Interestingly, a large number of mosquito larvae were observed in the plankton sample, their presence being felt only from day 5 onwards. One point to be mentioned here is that the plankton samples of the control pond had representatives from copepods, hydrozoan medusae and fish larvae throughout.

Table 12.b. gives data on the zooplankton abundance in the pond treated with 200 ppm mahua oil cake. The plankton samples contained only copepods, ostracods and mosquito larvae; those from the control ponds containing hydrozoan medusae and fish larvae, besides copepods. In general, the copepod population dwindled in numbers following treatment with the piscicide, and by the end of the experiment their numbers reached considerably low proportions. The mosquito larvae in the treatment pond were found to assume huge proportions (12480×10^3 nos/m³) towards the end. As in the case of the medium having 150 ppm mahua oil cake the mosquito larvae were encountered in the plankton collections made at 120 hours after the treatment.

The numerical abundance and fluctuations of different groups of zooplankton in the ponds under treatment at 0, 24, 120 and 240 hours following treatment with 250 ppm and 300 ppm of mahua oil cake are charted out in Tables, 12d and 12e. In both cases, copepods formed the major zooplankton component; their numbers diminishing from the first day of treatment and reaching

Table 12b. Zooplankton density (Nos/m³) of the pond treated with 200ppm mahua oil cake along with the control

Organisms	0	24	120	240
a.COPEPODS				
(i) Calanoids-				
Control	215	88	326	377
Experiment	338	135	13	64
(ii)Cyclopoids-				
Control	167	36	90	136
Experiment	263	115	38	186
(iii)Harpacticoids				
Control	4	76	40	17
Experiment	19	141	32	-
b.OSTRACODS				
Control	-	-	-	-
Experiment	20	45	58	-
c.HYDROZOAN MEDUSAE				
Control	35	24	60	50
Experiment	-	-	-	-
d.FISH LARVAE				
Control	-	-	-	-
Experiment	-	-	-	-
e.MOSQUITO LARVAE				
Control	-	-	-	-
Experiment	-	-	1100	12480

- Not present

zero at 240 hours. The plankton sample collected at 240 hours contained only mosquito larvae, their abundance being directly proportional to the concentration of the cake applied.

Table 12.c outlines the data obtained on the relative abundance of the zooplanktons in the experimental pond containing 200 ppm mahua oil cake and subjected to subsequent dilution with tidal water after 12 hours and daily tidal exchange after 5 days. The copepod population showed an increasing trend over the 240 hour period of observation when compared with the earlier case (Table 12.b).

The fluctuations in zooplankton groups in the experimental ponds subsequent to the treatment with selected concentrations of croton seed are given in the Tables, 13a - 13e. In all the ponds under study, copepods were the major components showing no significant difference in density between the experimental and control ponds. Fish larvae found in the collections made at 0 hour were completely absent in the subsequent collections made at 24, 120 and 240 hours, in all the concentrations tested (2 ppm - 5 ppm). The presence of mosquito larvae was noted in the collections made at 240 hours from the ponds containing 4 ppm and 5 ppm croton. In fact, they were totally absent in the collections made at the corresponding hours in the ponds having 2 ppm and 3 ppm of the piscicide.

Table 12c. Zooplankton density (Nos/m³) of the pond treated with 200ppm mahua oil cake and the medium subsequently diluted with tidal water 12 hrs after treatment, along with the control

Periods of observation (hours)	0	24	120	240
Organisms				
a.COPEPODS				
(i) Calanoids-				
Control	88	88	68	51
Experiment	67	237	102	628
(ii)Cyclopoids-				
Control	13	18	26	13
Experiment	16	51	29	458
(iii)Harpacticoids				
Control	-	-	-	-
Experiment	3	9	-	-
b.HYDROZOAN MEDUSAE				
Control	-0	19	13	6
c.FISH LARVAE				
Experiment	-	-	-	-
Control	-	-	-	-
Experiment	3	-	-	-
d.MOSQUITO LARVAE				
Control	-	-	-	-
Experiment	-	-	-	-

- Not present

Table 12d. Zooplankton density (Nos/m³) of the pond treated with 250ppm mahua oil cake along with the control

Organisms	0	24	120	240
a.COPEPODS				
(i) Calanoids-				
Control	215	88	326	377
Experiment	160	96	23	-
(ii)Cyclopoids-				
Control	167	36	90	136
Experiment	290	256	20	-
(iii)Harpacticoids				
Control	4	76	40	17
Experiment	48	48	20	-
b.OSTRACODS				
Control	-	-	-	-
Experiment	58	80	88	-
c.HYDROZOAN MEDUSAE				
Control	35	24	60	50
Experiment	-	-	-	-
d.FISH LARVAE				
Control	10	12	16	72
Experiment	-	-	-	-
e.MOSQUITO LARVAE				
Control	-	-	-	-
Experiment	-	-	2880	46800

- Not present

Table 12e. Zooplankton density (Nos/m³) of the pond treated with 300ppm mahua oil cake along with the control

Organisms	0	24	120	240
a.COPEPODS				
(i) Calanoids-				
Control	215	88	326	377
Experiment	9519	8320	13	-
(ii)Cyclopoids-				
Control	167	36	90	136
Experiment	724	568	64	-
(iii)Harpacticoids				
Control	4	76	40	17
Experiment	141	110	26	-
b.OSTRACODS				
Control	-	-	-	-
Experiment	18	13	10	-
c..HYDROZOAN MEDUSAE				
Control	35	24	60	50
Experiment	-	-	-	-
d.FISH LARVAE				
Control	10	12	16	72
Experiment	-	-	-	-
e.MOSQUITO LARVAE				
Control	-	-	-	-
Experiment	-	-	3200	65000

- Not present

Table 13a. Zooplankton density (Nos/m³) of the pond treated with 2ppm croton seed along with the control

Organisms	0	24	120	240
a.COPEPODS				
(i) Calanoids-				
Control	632	320	360	112
Experiment	426	208	312	340
(ii)Cyclopoids-				
Control	72	312	624	1600
Experiment	118	66	88	312
(iii)Harpacticoids				
Control	24	12	9	16
Experiment	14	12	21	8
b.CLADOCERANS				
Control	-	-	-	-
Experiment	8	26	11	37
c.AMPHIPODS				
Control	8	12	6	9
Experiment	22	20	32	24
d.HYDROZOAN MEDUSAE				
Control	184	40	568	48
e.FISH LARVAE				
Experiment	112	64	6	-
Control	24	28	88	60
Experiment	16	-	-	-

- Not present

Table 13b. Zooplankton density (Nos/m³) of the pond treated with 3ppm croton seed along with the control

Periods of observation (hours)	0	24	120	240
Organisms				
a.COPEPODS				
(i) Calanoids-				
Control	652	320	360	112
Experiment	712	458	267	680
(ii)Cyclopoids-				
Control	72	312	624	1600
Experiment	68	24	112	126
(iii)Harpacticoids				
Control	24	12	9	16
Experiment	13	9	-	-
b.OSTRACODS				
Control	-	-	-	-
Experiment	6	4	6	16
c.CLADOCERANS				
Control	-	-	-	-
Experiment	24	10	13	48
d.AMPHIPODS				
Control	8	12	6	9
Experiment	24	9	-	-
e.HYDROZOAN MEDUSAE				
Control	1848	40	568	48
Experiment	136	24	-	-
FISH LARVAE				
Control	24	28	88	60
Experiment	35	-	-	-

- Not present

Table 13c. Zooplankton density (Nos/m³) of the pond treated with 4ppm croton seed along with the control

Organisms	Periods of observation (hours)				
	0	24	120	240	
a.COPEPODS					
(i) Calanoids-					
Control	632	320	360	112	
Experiment	2112	176	616	4728	
(ii)Cyclopoids-					
Control	72	312	624	1600	
Experiment	344	144	24	744	
(iii)Harpacticoids					
Control	24	12	9	16	
Experiment	8	64	16	24	
b.AMPHIPODS					
Control	8	12	6	9	
Experiment	8	64	-	-	
c.HYDROZOAN MEDUSAE					
Control	184	40	568	48	
d.FISH LARVAE					
Experiment	416	-	-	-	
Control	24	28	88	60	
Experiment	16	-	-	-	
e.MOSQUITO LARVAE					
Control	-	-	-	-	
Experiment	-	-	736	-	

- Not present

Table 13d. Zooplankton density (Nos/m³) of the pond treated with 4ppm croton seed and the medium subsequently diluted with tidal water 12 hours after treatment, along with the control

Periods of bservation (hours)		0	24	120	240
Organisms					
a.COPEPODS					
(i) Calanoids-	Control	83	83	36	51
	Experiment	6878	57	384	9676
(ii)Cyclopoids-	Control	13	20	26	13
	Experiment	122	12	51	400
(iii)Harpacticoids	Control	-	-	26	30
	Experiment	13	6	6	19
b.HYDROZOAN MEDUSAE	Control	-	19	13	6
	Experiment	6	3	-	-
c.FISH LARVAE	Control	-	-	-	-
	Experiment	9	-	-	-

- Not present

Table 13e. Zooplankton density (Nos/m³) of the pond treated with 5ppm croton seed along with the control

Periods of observation (hours)		0	24	120	240
Organisms					
a.COPEPODS					
(i) Calanoids-	Control	632	320	360	112
	Experiment	1104	120	312	1264
(ii)Cyclopoids-	Control	372	312	624	1600
	Experiment	768	88	144	768
(iii)Harpacticoids	Control	24	20	12	16
	Experiment	8	152	56	-
b.CLADOCERANS	Control	-	-	-	-
	Experiment	48	16	56	1408
c.HYDROZOAN MEDUSAE	Control	184	40	568	48
	Experiment	256	8	-	-
d.FISH LARVAE	Control	24	18	88	60
	Experiment	8	-	-	-
e.MOSQUITO LARVAE	Control	-	-	-	-
	Experiment	-	-	-	752

- Not present

Fluctuations in the abundance of zooplankton in the ponds treated with 4 ppm croton seed and subsequently diluted with tidal water 12 hours after the treatment and daily tidal exchange from 120 hour onwards, is illustrated in Table 13 e. Here also, copepods were the major item among the different plankton groups. The trend in variation in their numerical abundance was more or less similar to those observed earlier. A significant difference noticed here was the relatively small number of mosquito larvae in the collection.

4.2.1.2.5 Benthos

Experiments were conducted to assess the impact of different concentrations of mahua oil cake and croton seed on the macrobenthos of field culture systems. Tables, 14a - 14e illustrate the toxic response of macrobenthos to 150 ppm, 200 ppm, 250 ppm and 300 ppm of mahua oil cake under field conditions. Polychaetes were the major item in the macrobenthos samples. Other groups represented in the collection included amphipods, tanaids and sea anemones. In the pond having 150 ppm of the cake a gradual reduction in number and biomass (wet weight) of polychaetes was recorded during the samplings made at 24, 120 and 240 hours after treatment. A complete absence of sea anemones was observed beyond 120 hours. Curiously enough, amphipods and tanaids did not indicate any marked fluctuation in the numerical abundance and biomass.

Table. 14e. Density of macrobenthos in number and bio-mass (wet-weight)/m² in the pond treated with 300 ppm mahua oil cake, along with the control

Periods of observations(lhrs)	0		24		120		240	
	Number	Weight	Number	Weight	Number	Weight	Number	Weight
Macrobenthos(No&wt/m ²)								
Polychaetes	Experiment	313	5.4721	13.1726	0	0	0	0
	Control	963	25.5928	21.737	925	25.4593	863	21.2268
Amphipods	Experiment	75	0.1413	38	0.0914	0	0	0
	Control	238	0.0756	225	0.0846	200	0.0693	213
Tanaids	Experiment	200	0.4125	288	0.5653	0	0	0
	Control	325	0.751125	300	0.56	213	0.2192	225
Scaneimone	Experiment	88	0.5613	75	0.697	0	0	0
	Control	63	0.4126	75	0.3768	100	0.373	88
Bivalve mollusc	Experiment	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0

Table. 15a Density of macrobenthos in number and bio-mass (wet-weight)/m² in the pond treated with 2 ppm croton seed, along with the control

Macrobenthos(No&wt/m ²).	0		24		120		240		
	Number	Weight	Number	Weight	Number	Weight	Number	Weight	
Polychaetes	Experiment	663	7.7845	613	7.1718	46.3	6.2791	362	6.3679
	Control	500	5.9635	512	6.1049	463	5.0766	475	4.7602
Amphipods	Experiment	50	0.0323	38	0.033	62	0.0815	37	0.0228
	Control	38	0.029	38	0.027	50	0.0393	50	0.0535
Tanaids	Experiment	37	0.0089	50	0.01	25	0.00675	38	0.0079
	Control	75	0.0104	100	0.07	75	0.0124	63	0.0103
Seanemone	Experiment	38	0.11025	25	0.1	37	0.2128	25	0.1521
	Control	38	0.1391	38	0.144	50	0.2015	38	0.1463
Bivalve mollusc	Experiment	12	2.354	38	6.6688	25	4.6253	25	4.5928
	Control	25	4.7243	25	5.7785	38	7.1694	25	4.7284

Considerable reduction in number and biomass of polychaetes was observed following treatment of the pond with 200 ppm of mahua oil cake (Table 14.b). Their number and biomass were found to drop from 130 numbers and 4.7419 g/m^2 area of the pond at 0 hour, to 25 numbers and 0.3229 g/m^2 respectively, towards the end of the experiment. In fact, an increase in these values was observed at 24 hours after treatment. The variations in number and biomass of amphipods and tanaids also indicated a similar trend during the course of the experiment. Sea anemones were totally absent in the collection made beyond 24 hours treatment. Contrary to the result obtained in this experiment, the magnitude of reduction in the number and biomass of the macrobenthos was less pronounced when the water with the same concentration of the toxicant was diluted with tidal water, 12 hours after the treatment (Table 14.c). Application of 250 ppm and 300 ppm of the cake to the culture ponds led to a complete disappearance of the four major groups of organisms during the 120 and 240 hours of sampling (Tables, 14.d & 14.e). As in the previous case, an increase in the number and biomass of polychaetes was observed at 24 hours in the pond having 250 ppm concentration of the piscicide.

Experiments conducted to assess the effect of selected concentrations of croton seed (2 ppm - 5 ppm), on the macrobenthos of field culture systems revealed a concentration - dependent diminishing trend with reference to different groups of

Table. 15b Density of macrobenthos in number and bio-mass (wet-weight)/m² in the pond treated with 3 ppm croton seed, along with the control

Periods of observations(hrs)		0		24		120		240	
Macrobenthos(No&wt/m ²).		Number	Weight	Number	Weight	Number	Weight	Number	Weight
Polychaetes	Experiment	425	4.3006	350	3.5951	312	3.3021	262	2.8544
	Control	500	5.9635	512	6.1049	463	5.0766	475	4.7602
Amphipods	Experiment	75	0.0539	50	0.0378	37	0.029	37	0.03
	Control	38	0.029	38	0.027	50	0.0393	50	0.0555
Tanaids	Experiment	38	0.008	38	0.007	38	0.0066	25	0.0051
	Control	75	0.0104	100	0.07	75	0.0124	63	0.0103
Seaanemone	Experiment	25	0.1053	25	0.0901	38	0.1466	25	0.1039
	Control	38	0.1391	38	0.144	50	0.2015	38	0.1463
Bivalve mollusc	Experiment	38	7.4138	38	7.2409	0	0	0	0
	Control	25	4.7243	25	5.7785	38	7.1694	25	4.7284

Table. 15c Density of macrobenthos in number and bio-mass (wet-weight)/m² in the pond treated with 4 ppm croton seed. along with the control

Periods of observations (hrs)	0		24		120		240	
	Number	Weight	Number	Weight	Number	Weight	Number	Weight
Macrobenthos (No&wt/m ²)								
Polychaetes	275	3.3206	150	1.5288	100	1.1684	50	0.7533
Control	500	5.9635	512	6.1049	463	5.0766	475	4.7502
Amphipods	38	0.0236	38	0.0288	25	0.0293	13	0.0112
Control	38	0.029	38	0.027	50	0.0393	50	0.0555
Tanuids	50	0.0083	38	0.0074	25	0.006	25	0.0054
Control	75	0.0104	100	0.07	75	0.0124	63	0.0103
Scannemone	25	0.09	25	0.1026	12	0.0476	25	0.104
Control	38	0.1391	38	0.144	50	0.2015	38	0.1463
Bivalve mollusc	25	5.8894	38	10.5103	0	0	0	0
Control	25	4.7243	25	5.7785	38	7.1694	25	4.7284

Table 15e. Density of macrobenthos in number and bio-mass (wet-weight)/m² in the pond treated with 5 ppm croton seed, along with the control

Periods of observations(hrs)		0		24		120		240	
Macrobenthos(No&wt/m ²)		Number	Weight	Number	Weight	Number	Weight	Number	Weight
Polychaetes	Experiment	138	1.5081	125	1.3391	62	0.6724	25	0.4345
	Control	500	5.9635	512	6.1049	463	5.0766	475	4.7602
Amphipods	Experiment	38	0.0509	25	0.0214	13	0.0113	13	0.011
	Control	38	0.029	38	0.027	50	0.0393	50	0.0535
Tanaids	Experiment	63	0.0104	50	0.0086	25	0.005	0	0
	Control	75	0.0104	100	0.07	75	0.0124	63	0.0103
Scannemone	Experiment	25	0.0824	13	0.0345	25	0.0755	13	0.0349
	Control	38	0.1391	38	0.144	50	0.2015	38	0.1463
Bivalve mollusc	Experiment	25	4.7849	38	8.1811	0	0	0	0
	Control	25	4.7243	25	5.7785	38	7.1694	25	4.7284

organisms over a period of 240 hours (Tables 15a - 15e). In all the concentrations tested, except 2 ppm, the bivalve molluscs were absent in the collections made beyond 120 hours. No significant reduction in number and biomass of macrobenthos was observed when the test medium containing 4 ppm of croton seed was diluted with tidal water, 12 hours after the treatment (Table 15d), when compared with the undiluted medium having the same concentration (Table 15.c).

4.2.1.2.6 Persistence of toxicity of the piscicides in the field test media

Experiments were conducted to evaluate the persistence of toxicity of the two piscicides in the field test media with Ambassis gymnocephalus, representing the least tolerant group of finfish species tested, as the target organism. The results revealed that the duration of persistence of toxicity of the piscicides in the test media was concentration - dependent. The toxicity of different concentrations of mahua oil cake such as 150 ppm, 200 ppm, 250 ppm and 300 ppm persisted for 1, 4, 4 and 7 days respectively (Table 16.a). At the same time, the toxicity of the medium containing 200 ppm of the cake subjected to dilution with tidal water, 12 hours after treatment, persisted only for a single day.

Table 16a. A. gymnocephala : Time course of percentage mortality against graded concentrations of mahua oil cake

Periods of observation (days) \ Concentration (ppm)	1	2	3	4	5	6	7	8
150	100	-	-	-	-	-	-	-
200	100	100	100	60	-	-	-	-
250	100	100	100	75	-	-	-	-
300	100	100	100	100	100	100	55	-
Control	-	-	-	-	-	-	-	-

- No mortality

Table 16b. A. gymnocephala: Time course of percentage mortality against graded concentrations of croton seed

Periods of observation (days) \ Concentration (ppm)	1	2	3	4
2	100	-	-	-
3	100	70	-	-
4	100	80	-	-
5	100	100	100	-
Control	-	-	-	-

- No mortality

Observations on the persistence of toxicity in the test media having different concentrations of mahua oil cake with regard to the postlarvae of the penaeid prawn, Penaeus indicus are presented in Table 17a. The medium exposed to 200 ppm of the cake remained lethal to the post larvae, upto day-2. On the other hand, lethal effects of 250 and 300 ppm of the cake on the postlarvae persisted upto day-6 and day-7, respectively.

A concentration dependent persistence of toxicity of graded concentrations of croton seed under field conditions was observed with reference to the most sensitive finfish species, A. gymnocephalus (Table 16b). The medium containing 2 ppm of the toxicant resulted in absolute mortality of the fish species tested, on the first day, but did not produce any lethal effect on the species thereafter. Although absolute mortality of the fish was noticed on the first day of experiment in the medium containing 3 ppm of the piscicide, only 70% mortality was recorded on day-2. No incidence of mortality was observed from day-3 onwards in the medium. A more or less similar result was obtained when the test medium contained 4 ppm of the piscicide. The medium containing 5 ppm of the toxicant exhibited lethal effect upto day-3.

Results of the experiments involving exposure of postlarval forms of P. indicus to media containing different concentrations of croton seed are illustrated in Table 17.b. While the medium having 2 ppm of the piscicide did not have any

Table 17a. *P. indicus* (postlarvae): Time course of percentage mortality against graded concentrations of mahua oil cake

Periods of observation (days) \ Concentration (ppm)	1	2	3	4	5	6	7	8	9	10
150	-	-	-	-	-	-	-	-	-	-
200	-	45	-	-	-	-	-	-	-	-
250	-	65	95	100	100	90	-	-	-	-
300	-	90	100	100	100	100	50	-	-	-
Control	-	-	-	-	-	-	-	-	-	-

- No mortality

Table 17b. *P. indicus* (postlarvae): Time course of percentage mortality against graded concentrations of croton seed

Periods of observation (days) \ Concentration (ppm)	1	2	3	4	5
2	-	-	-	-	-
3	30	-	-	-	-
4	100	-	-	-	-
5	100	100	-	-	-
Control	-	-	-	-	-

- No mortality

lethal effect on the postlarvae, those which contained 3 and 4 ppm displayed lethal effect only on the first day. Curiously enough, the medium exposed to 5 ppm croton seed was found to exert lethal effect on the postlarvae upto day-2 only.

4.2.2. Effect on the productivity of the culture system

Field experiments were undertaken to evaluate the influence of mahua oil cake and croton on the productivity of culture systems, comprising of growth, survival and production profile of the penaeid prawn, P. indicus over a prawn culture period of 90 days.

4.2.2.1. Texture, nutrients and pH of soil

4.2.2.1.1. Soil texture

Results of the preliminary survey conducted for evaluating the texture of the bottom soil of the ponds employed for the present study are presented in Table 18. Bottom soil of the ponds under study were silty sand; sand fraction ranging from 86.89% to 89.64%, silt 7.82% to 12.69% and clay fraction 0.43 to 2.54%.

4.2.2.1.2. Soil nutrients

Table 19a. contains details of the fluctuations in soil nutrients at fortnightly intervals over a prawn culture period of 90 days, in the ponds treated with 200 ppm mahua oil

Table 18: Soil texture of ponds (piscicide-treated and Control) selected for productivity studies

Soil constituents	Mahua treated ponds	Croton treated ponds	Control pond
Sand (%)	89.02	89.64	86.89
Clay (%)	1.48	2.54	0.43
Silt (%)	9.5	7.82	12.69

Table 19.a : Soil nutrients and pH of the ponds treated with mahua oil cake (200 ppm) along with the control

Periods of samplings (days)	Nutrients	Organic carbon (%)	Phosphate (Kg/ha)	Potassium (Kg/ha)	pH
1	Exp.	0.47	90.00	386.00	7.06
	Contr.	0.39	94.00	350.00	6.91
15	Exp.	0.61	93.50	406.75	7.02
	Contr.	0.68	91.00	436.50	6.80
30	Exp.	0.42	79.00	390.00	6.85
	Contr.	0.18	84.00	456.50	6.88
45	Exp.	0.33	87.00	442.00	7.06
	Contr.	0.16	71.00	417.00	6.94
60	Exp.	0.39	82.50	338.75	6.99
	Contr.	0.05	73.00	380.00	6.89
75	Exp.	0.35	79.00	359.50	7.02
	Contr.	0.08	75.00	375.00	6.89
90	Exp.	0.30	91.00	324.25	6.88
	Contr.	0.03	81.00	349.50	6.81

Table 19.b : Soil nutrients and pH of the ponds treated with croton (4 ppm) along with the Control

Periods of sampling(days)	Nutrients	Organic carbon (%)	Phosphate (Kg/ha)	Potassium (Kg/ha)	pH
1	Exp.	0.27	66.50	485.50	6.74
	Contr.	0.39	94.00	550.00	6.91
15	Exp.	0.55	70.00	493.80	6.80
	Contr.	0.68	91.00	436.50	6.80
30	Exp.	0.34	73.50	524.80	6.79
	Contr.	0.18	84.00	456.50	6.88
45	Exp.	0.15	63.50	536.00	6.89
	Contr.	0.16	71.00	417.00	6.94
60	Exp.	0.18	64.00	520.00	6.89
	Contr.	0.05	73.00	380.00	6.89
75	Exp.	0.33	73.00	430.00	6.89
	Contr.	0.08	75.00	375.00	6.89
90	Exp.	0.23	65.50	501.00	6.94
	Contr.	0.03	81.00	349.50	6.81

cake. During the period of study, the percentage of organic carbon varied between 0.3 (90th day) and 0.61 (15th day) in the treated pond while the control pond recorded a minimum value of 0.03 and maximum value of 0.68 on the 90th and 15th day respectively (significantly lower than ($p < 0.05$) that of the treatment ponds). The phosphate content fluctuated between 79 and 93.5 Kg/ha in the experimental pond; the maximum being observed on the 15th day and the minimum on the 30th and 75th days. Observations made in the control pond revealed that the minimal value of 71 Kg/ha was indicated on 90th day and a peak of 94 Kg/ha on the 1st day. The content of potassium in the experimental pond ranged between 324.25 Kg/ha (90th day) and 442 Kg/ha (45th day); the same in the control pond being 349.5 Kg/ha on the 90th day and 456.5 Kg/ha on the 30th day.

The pattern of fluctuation of soil nutrients of ponds treated with croton seed is illustrated in Table 19.b. The minimum value of 0.15% organic carbon was observed on the 45th day of experiment and the maximum of 0.56% on the 15th day. The content of organic carbon in the control pond, ranged between 0.03% (90th day) and 0.56% (15th day), showing no significant difference from that of the treatment ponds. The phosphate levels indicated a minimum of 64 Kg/ha and maximum of 73.5 Kg/ha in the experimental pond. In the control pond it ranged between 73 Kg/ha (60th day) and 94 Kg/ha (1st day). The potassium content of the soil from the treatment pond varied between 320

Kg/ha (Day 60) and 501 Kg/ha (Day 90) whereas the control pond registered a minimal potassium content of 349.5 Kg on the 90th day, and a maximum value of 456.5 Kg/ha on the 30th day.

4.2.2.1.3. Soil pH

The soil pH of the piscicide-treated ponds selected for productivity studies maintained almost a uniform level throughout the period of study. It ranged between 6.88-7.06 and 6.74-6.94 in mahua-treated and croton treated ponds respectively. In the control pond, the pH varied between 6.8 and 6.94 (Table 19a & b).

4.2.2.2. Hydrographic parameters

During the course of study on the productivity of field culture systems treated with the optimum concentration (200 ppm) of mahua oil cake, water samples were collected regularly (11 am) at fortnightly intervals for a period of 90 days to delineate various hydrographic parameters such as depth, temperature salinity, pH and dissolved oxygen. The data obtained is presented in Table 20 a. The mean depth of the experimental pond fluctuated between 0.33m on the 60th day and 0.43m on the first day. The mean depth of the control pond ranged between 0.39m on the 60th day and 0.49m on the first day.

Table. 20a. Hydrographic parameters of the pond treated with mahua oil cake (200 ppm) along with the control

Hydrographic parameters		Depth (m)	Temp (°C)	Salinity (‰)	pH	O ₂ (ml/l)
1	Exp.	0.43	32.15	17.40	8.20	4.62
	Contr.	0.49	32.20	17.32	8.10	4.78
15	Exp.	0.40	31.43	18.91	7.68	3.66
	Contr.	0.46	31.30	19.35	7.60	3.17
30	Exp.	0.42	33.88	19.53	8.10	5.08
	Contr.	0.48	33.15	19.60	7.65	5.27
45	Exp.	0.35	33.73	18.94	8.63	7.50
	Contr.	0.41	33.40	18.91	8.65	7.11
60	Exp.	0.33	34.83	16.65	9.13	8.39
	Contr.	0.39	34.40	16.23	9.10	9.48
75	Exp.	0.41	34.78	15.56	8.20	5.22
	Contr.	0.47	34.35	15.39	8.20	5.81
90	Exp.	0.41	34.33	18.57	8.65	5.63
	Contr.	0.47	33.85	18.15	8.35	3.73

The mean water temperature of the mahua-treated ponds ranged between 31.43 C on day-15 and 35.83 C on day-60. In general, the water temperature showed an increasing trend from the beginning to the end of the period of study. The fluctuations of temperature in the control pond was also along similar lines, recording a low value of 31.3 C on the 15th day and a peak of 35.4 C on the 60th day of treatment.

The salinity of the pond water under treatment showed an increasing trend, rising gradually from of 17.4‰ on day-1 to increase upto 19.53‰ on day-30. A decreasing profile was observed thereafter reaching a minimum of 15.56‰ on the 75th day, again to rise gradually to 18.57 on the 90th day of experimentation. The salinity profile of the control pond also exhibited the same trend depicting a range of 15.39 - 19.6‰.

The pH of the mahua-treated pond water ranged between 7.7 and 9.15. The similar trend in variation of pH in the control pond was observed in the experimental pond depicting a range of 7.6-9.1.

The values of dissolved oxygen content of the mahua-treated ponds during the 90 days culture period varied between 3.66 ml/l on the 15th day and 8.39 ml/l on the 60th day. In general, the oxygen levels increased from day-1 to the concluding day. Ranging between 3.17 ml/l and 9.48 ml/l, the dissolved oxygen content of the control pond fluctuated on similar lines as observed in the experimental pond.

Table 20b. contains the data obtained on the hydrographic parameters of the ponds treated with croton seed and maintained under observation for a period of 90 days.

The mean depth of the croton-treated ponds ranged between 0.34 m (60th day) and 0.44 m (1st day) . The control pond on the other hand had a mean depth of 0.39m on day-60 and 0.49m on day-1, the trend in variation being similar to that of the experimental pond.

During the 90 days culture period the mean water temperature of the croton-treated ponds showed a peak value of 35.18^o C on the 75th day and a minimum value of 30.3^o C on the 15th day after treatment. In the control, the maximum value was recorded on the 60th day (35.4^o C) in the control and the minimum on the 15th day (31.3^o C) In general, the temperatures of both the experimental and control ponds showed an increasing trend from the beginning to the end of the culture period.

The salinity of the experimental pond treated with croton seed showed an increase from 17.4% on day-1 to 19.75% on day-30, thereafter declining to 15.64% on the 75th day to again to rise to 18.62% on the 90th day of treatment. The salinity of the control pond ranged between 15.39 and 19.6%, fluctuating in the same line as in the case of the experimental pond.

The pH of the water of the croton-treated pond fluctuated between 7.7 and 9.15 as recorded on the 15th and 60th

Table. 20b. Hydrographic parameters of the pond treated with croton seed (4ppm) along with the control

Periods of sampling(days)	Depth (m)	Temp (°C)	Salinity (‰)	pH	O ₂ (ml/l)
1	Exp.	31.98	17.40	8.35	5.59
	Contr.	32.20	17.32	8.10	4.78
15	Exp.	30.30	19.10	7.70	2.82
	Contr.	31.30	19.35	7.60	3.17
30	Exp.	33.15	19.75	8.03	6.47
	Contr.	33.15	19.60	7.65	5.27
45	Exp.	32.98	18.80	8.78	7.55
	Contr.	33.40	18.91	8.65	7.11
60	Exp.	34.18	16.81	9.15	9.31
	Contr.	34.40	16.23	9.10	9.48
75	Exp.	34.08	15.64	8.15	5.64
	Contr.	34.35	15.39	8.20	5.81
90	Exp.	33.55	18.62	8.54	6.72
	Contr.	33.85	18.15	8.35	3.73

day, respectively. The water of the control pond had a pH range of 7.6 (15th day) to 9.1 (60th day). In both the cases, the pH showed an increasing trend during the course of study.

The dissolved oxygen content of the ponds under treatment with croton varied between 2.82 ml/l as recorded on the 15th day and 9.31 ml/l as on the 15th & 60th day respectively. The control pond also depicted a similar trend with a peak of 9.48 ml/l on the 60th day and the minimum of 3.17 ml/l on the 15th day of treatment. In general, higher values of dissolved oxygen were recorded during the second half of the experimental period.

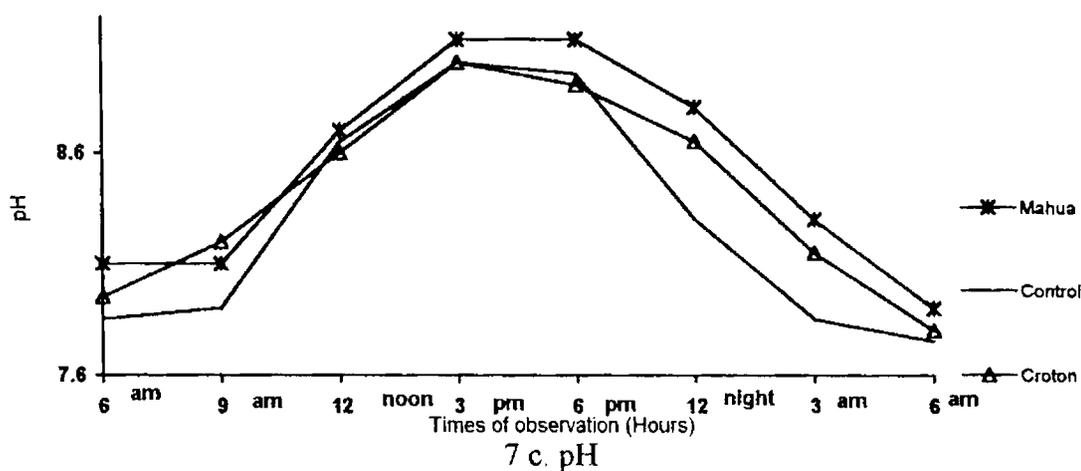
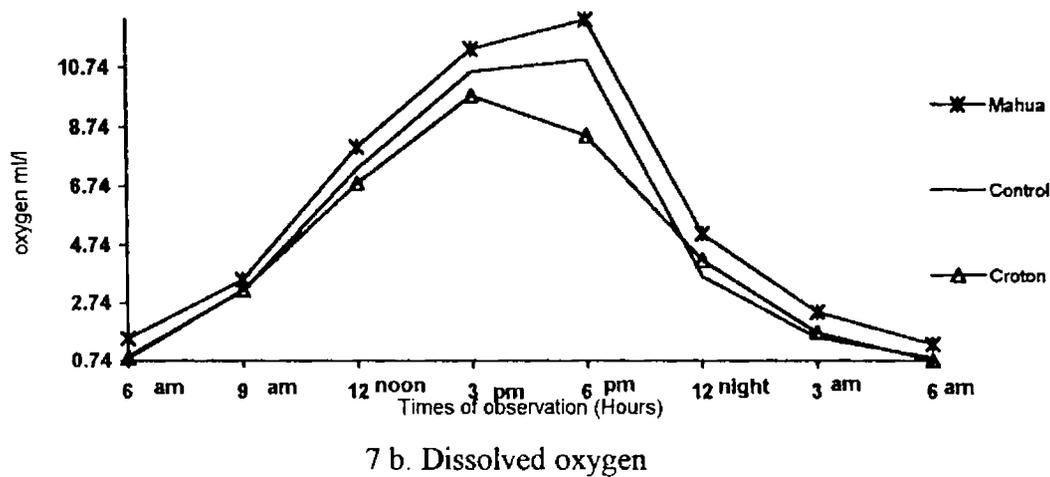
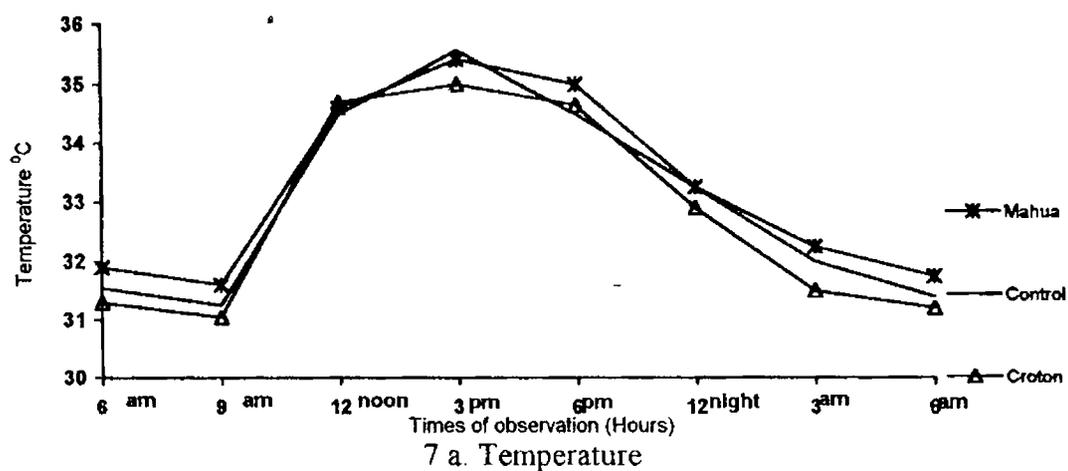
Diurnal monitoring of the hydrographic parameters of the experimental ponds revealed distinct patterns of their fluctuation (Table 20 C and Figs. 7a-c). In the ponds treated with mahua oil cake the minimum temperature (31.6 °C) was recorded at 09.00 hrs and the maximum (35.4 °C) at 15.00 hrs. The same trend was observed in the case of control pond also recording a temperature range of 31.25 - 35.55 °C. The maximum pH of 9.1 was recorded at 15.00 and 18.00 hrs and the minimum value of 7.9 at 06.00 hours, in the mahua-treated pond; the same in the control pond, being 9 (15.00 hours) and 7.75 (06.00 hours) respectively.

In the mahua-treated ponds, the dissolved oxygen content displayed a very low value of 1.52 ml/l at the start of observations (06.00 hrs) to increase gradually thereafter, registering a peak (12.34 ml/l) at 18.00 hours, followed by a

Table 20c. Diurnal variation of temperature, pH and dissolved oxygen of the experimental ponds, treated with 200 ppm MOC and 4 ppm croton along with the control.

Time of observation (Hrs.)	Ponds treated with mahua oil cake			Ponds treated with Croton seed			Control pond		
	Temp. °C	pH	O ₂ ml/l	Temp. °C	pH	O ₂ ml/l	Temp. °C	pH	O ₂ ml/l
06.00	31.90	8.10	1.52	31.30	7.95	0.84	31.55	7.85	0.89
09.00	31.60	8.10	3.56	31.05	8.20	3.19	31.25	7.90	3.17
12.00	34.60	8.70	8.06	34.70	8.60	6.83	34.50	8.65	7.33
15.00	35.40	9.10	11.35	35.00	9.00	9.78	35.55	9.00	10.59
18.00	35.00	9.10	12.34	34.65	8.90	8.44	34.50	8.95	11.00
24.00	33.25	8.80	5.13	32.90	8.65	4.22	33.25	8.30	3.67
03.00	32.25	8.30	2.44	31.50	8.15	1.72	32.00	7.85	1.55
06.00	31.75	7.90	1.31	31.20	7.80	0.75	31.40	7.75	0.85

Figure 7 a - c Diurnal variation of hydrographic parameters in ponds treated with mahua oil cake and croton seed along with the control



gradual decline to reach a minimum of 1.31 ml/l the next day morning at 06.00 hrs. The dissolved oxygen profile of the control pond also showed a similar variation pattern, recording a minimum and maximum of 0.85 ml/l and 11 ml/l respectively.

The trend in the diurnal fluctuation of hydrographic parameter in the croton-treated ponds revealed a temperature range of 31.05 °C at 09.00 hours and 35.0 °C, at 15.00 hours. The same trend was observed in the control pond also with a range of 31.25 - 35.55 °C.

From a value of 7.95 at 06.00 hours, the pH increased to 9 at 15.00 hours in the croton - treated pond. A comparable fluctuation was evinced in the control pond also where the range was 7.75 - 9.

The dissolved oxygen content of the croton-treated pond, at the beginning of the diurnal observation (06.00 hours) was a low 0.84 ml/l, gradually increasing thereafter to reach a peak value of 9.78 ml/l at 18.00 hours. From 18.00 hours onwards the value showed a declining trend reaching a minimum of 0.75 ml/l the next day morning at 06.00 hours. The trend in the control pond was also similar, recording a range of 0.85 - 11 ml/l.

4.2.2.3 Water nutrients and primary production

4.2.2.3.1. Water nutrients

Fortnightly observations on water nutrients such as

Table 21a. Ammonia-nitrogen (NH₄-N) (microgram at/l) of the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm) along with the control

Periods of observation (days)	1	15	30	45	60	75	90
Ponds							
Mahua treated ponds	20.95	22.28	17.41	18.98	17.4	22.46	22.47
Croton treated ponds	19.33	20.01	19.4	21.77	19.25	26.92	22.11
Common feeder canal	19.43	17	15.97	16.69	22.06	22.65	21.75
Control pond	20.91	20.41	17.45	18.86	17.78	23.38	20.81

Table 21b. Nitrite-nitrogen (NO₂-N) (microgram at/l) of the ponds treated with mahua oil cake (200ppm) and croton seed (4 ppm) and the feeder canal along with the control

Periods of observation(days)	1	15	30	45	60	75	90
Ponds							
Mahua treated ponds	0.28	0.25	0.18	0.23	0.29	0.15	0.25
Croton treated ponds	0.35	0.44	0.17	0.35	0.4	0.29	0.28
Control pond	0.34	0.21	0.15	0.21	0.29	0.19	0.28
Common feeder canal	0.53	0.23	0.44	0.22	0.36	0.19	0.54

ammonia - Nitrogen, nitrite Nitrogen, nitrate - Nitrogen and inorganic phosphate in the ponds treated with mahua oil cake and croton seed and in the feeder canal were made and the data obtained are presented in Tables 21 a - d.

The content of ammonia - Nitrogen in the ponds treated with mahua oil cake varied between 17.4 $\mu\text{g at/l}$, (30th and 60th day) and 22.47 $\mu\text{g at/l}$ (90th day). In croton-treated ponds it ranged between 19.25 $\mu\text{g at/l}$ and 26.92 $\mu\text{g at/l}$ on the 60th day and 75th day respectively. Water samples collected from the feeder canal had an ammonia - Nitrogen content varying from 15.97 $\mu\text{g at/l}$, on the 30th day to 33.65 $\mu\text{g at/l}$ on the 75th day. The ammonia - Nitrogen content of the control pond on the other hand fluctuated between 17.45 $\mu\text{g at/l}$, (day-30) and 23.38 $\mu\text{g at/l}$, (day-75). In general, the lowest and highest values of ammonia - Nitrogen were encountered on the 30th and 75th day of treatment in all the ponds including the feeder canal .

In the case of Nitrite - Nitrogen of mahua-treated ponds, the minimal value of 0.15 $\mu\text{g at/l}$ was recorded on the 75th day and the maximum of 0.29 $\mu\text{g at/l}$ on the 60th day (Table 21 b). The NO_2^- -N dipped from 0.28 $\mu\text{g at/l}$ (day 1) to 0.18 $\mu\text{g at/l}$ on the 30th day increasing again upto the 60th day registering the highest recorded value during the 90 days of observation.

The value of Nitrite - Nitrogen in the croton-treated ponds ranged between 0.17 and 0.44 $\mu\text{g at/l}$ with two peaks on the

Table 21c. Nitrate-nitrogen (NO₃-N) (microgram a/l) of the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm) and the feeder canal along with the control

Periods of observation(days)	1	15	30	45	60	75	90
Ponds							
Mahua treated ponds	3.84	2.22	3.71	3.04	4.7	1.69	1.49
Croton treated ponds	3.94	3.36	3.51	2.93	3.52	2.16	1.26
Control pond	4.2	1.82	3.68	2.78	3.91	1.47	1.28
Common feeder canal	6.34	2.07	5.78	5.7	15.11	1.36	3.64

Table 21d. Inorganic PO₄ (microgram a/l) of the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm) and the feeder canal along with the control

Periods of observation(days)	1	15	30	45	60	75	90
Ponds							
Mahua treated ponds	5.43	8.46	11.64	14.12	16.65	10.91	9.82
Croton treated ponds	8.53	10.03	11.06	13.33	12.82	11.41	12.29
Control pond	6.67	7.89	11.17	14.03	13.98	13.08	9.79
Common feeder canal	8.24	7.65	11.75	14.71	14.3	11.97	8.58

30th day (0.44 $\mu\text{g at/l}$) and 60th day (0.4 $\mu\text{g at/l}$) respectively.

The values of Ammonia - Nitrogen in the control pond fluctuated between 0.15 $\mu\text{g at/l}$, and 0.34 $\mu\text{g at/l}$. From day-1 onwards, a declining trend was observed registering a minimal value on the 30th day, to rise again on the 60th day (29 $\mu\text{g at/l}$). The value then dropped to 0.19 $\mu\text{g at/l}$ on the 75th day, increasing thereafter to reach a value of 0.28 $\mu\text{g at/l}$, on day 90. In the feeder canal, the fluctuation of Nitrite - Nitrogen was of an interesting nature, the values decreasing and increasing on alternate dates of sampling at fortnightly intervals. Here, the concentration ranged between 0.19 $\mu\text{g at/l}$ and 0.53 $\mu\text{g at/l}$, indicating 4 peaks, on the first (0.53 $\mu\text{g at/l}$), 30th (0.44 $\mu\text{g/l}$), 60th (0.36 $\mu\text{g at/l}$) and 90th (0.54 $\mu\text{g at/l}$) day.

Table 21 C outlines the data obtained on the fortnightly fluctuations in Nitrate - Nitrogen ($\text{NO}_3\text{-N}$) of the ponds treated with mahua oil cake and croton seed. In the mahua-treated ponds the values of $\text{NO}_3\text{-N}$ varied between 1.49 $\mu\text{g at/l}$ and 4.7 $\mu\text{g at/l}$, on the 90th day and 60th day, respectively. From the start of the experiment to the 60th day, relatively higher values of $\text{NO}_3\text{-N}$ were recorded while those observed on the 75th and 90th day have relatively lower. In the croton-treated ponds, on the other hand, a declining trend in $\text{NO}_3\text{-N}$ values was observed from day-1 to day-90; the values being 3.94 $\mu\text{g at/l}$ and 1.26 $\mu\text{g at/l}$, respectively. In the control pond, the

highest value of $\text{NO}_3 - \text{N}$ ($4.2 \mu\text{g at/l}$) was recorded on the 1st day and the lowest ($1.28 \mu\text{g at/l}$) on the 90th day. Interestingly, on the 15th day the value was found to decline sharply to $1.82 \mu\text{g at/l}$ to go up again and maintain a comparatively higher level from the 30th to the 60th day. The value then fell to a minimum on the 90th day. The $\text{NO}_3 - \text{N}$ concentration of the feeder canal was highly fluctuating, registering a high value of $15.11 \mu\text{g at/l}$ (60th day) and a low of $1.36 \mu\text{g at/l}$ (75th day). In general, the $\text{NO}_3 - \text{N}$ values recorded during the first half of the sampling period were higher in all the ponds including the feeder canal when compared to those obtained during the latter part. The fortnightly fluctuations in inorganic phosphate (PO_4) at of the ponds treated with mahua oil cake and croton seed are depicted in table 21d. The phosphate values of mahua-treated ponds varied between $5.43 \mu\text{g at/l}$, (day 1) and $16.65 \mu\text{g at//l}$ (day 60). Infact, the values recorded from the 30th to the 75th day were compratively higher. The same pattern of fluctuation of PO_4 values were observed in the case of other ponds also, including the feeder canal. In the croton-treated ponds, the PO_4 values ranged between $8.53 \mu\text{g at/l}$ (day -1) and $13.33 \mu\text{g at/l}$ (day -45). In the control pond, the lowest PO_4 values of $6.67 \mu\text{g at/l}$ was registered on the 1st day and the highest of $14.03 \mu\text{g at/l}$ on the 45th day. In the feeder canal the PO_4 values ranged between $7.65 \mu\text{g at/l}$ and $14.71 \mu\text{g at/l}$ on the 15&45 th day respectively. It is evident from the Table (Table 21d) that in all the ponds and feeder canals, covered

under the present study the highest PO₄ concentrations were recorded on the 45th day with the exception of the mahua-treated ponds where it was on the 60th day of treatment.

4.2.2.3.2. Primary production

The variations in primary production in the control and experimental ponds treated with mahua oil cake and croton seed were recorded at fortnightly intervals over a period of 90 days. Table 22 and Figs. 8a-d outlines the results obtained on the production pattern. It is evident from the Table that the gross production in the mahua-treated pond increased steadily from an initial value of 1561.55 mg C/m³/day recorded on the 1st day, reaching a peak value of 6657.11 mg C/m³/day on the 60th day to decline thereafter to a value of 4209.38 mg C/m³/day on the 90th day. The variations in gross production in the control pond also followed the same pattern, registering a higher magnitude of production on all sampling days when compared to the treatment pond. The gross production values in the control pond ranged from 1722.35 mg C/m³/day at 0 hour to 7132.36 mg C/m³/day on the 60th day, the value declining to 4488.1 mg C/m³/day on the 90th day.

In the case of net production in the treatment ponds, an increasing trend was observed from day-1 (793.28 mg C/m³/day), registering a peak value of production on the 30th day (4491.67 mg C/m³/day). Thereafter, the values were of a highly fluctuating

Table 22. Primary production in the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm) along with the control (mg C/m³/day)

Periods of observation (days)	Mahua treated pond		Croton treated pond		
	Gross pdn	Net pdn	Gross pdn	Net pdn	
0	Exp.	1561.55	793.28	3905.65	3280.32
	Contr.	1722.35	1114.88	1722.35	1114.88
15	Exp.	3125.95	2260.13	1154.11	850.45
	Contr.	2908.69	2529.92	2908.69	2529.92
30	Exp.	6256.89	4491.67	5259.94	3798.45
	Contr.	6060.36	4752.53	6060.36	4752.53
45	Exp.	5978.17	3980.69	7939.93	6131.83
	Contr.	6553.48	6081.8	6553.48	6081.8
60	Exp.	6657.11	4202.23	5288.52	2433.44
	Contr.	7132.36	4995.51	7132.36	4995.51
75	Exp.	3969.97	2547.78	2726.45	1786.66
	Contr.	4609.59	3030.18	4609.59	3030.18
90	Exp.	4209.38	3483.99	4194.06	2912.26
	Contr.	4488.1	4002.13	4488.1	4002.13

Fig. 8a Fluctuation in Primary production (Gross) in the pond treated with mahua oil cake(200ppm) over a period of 90 days along with control

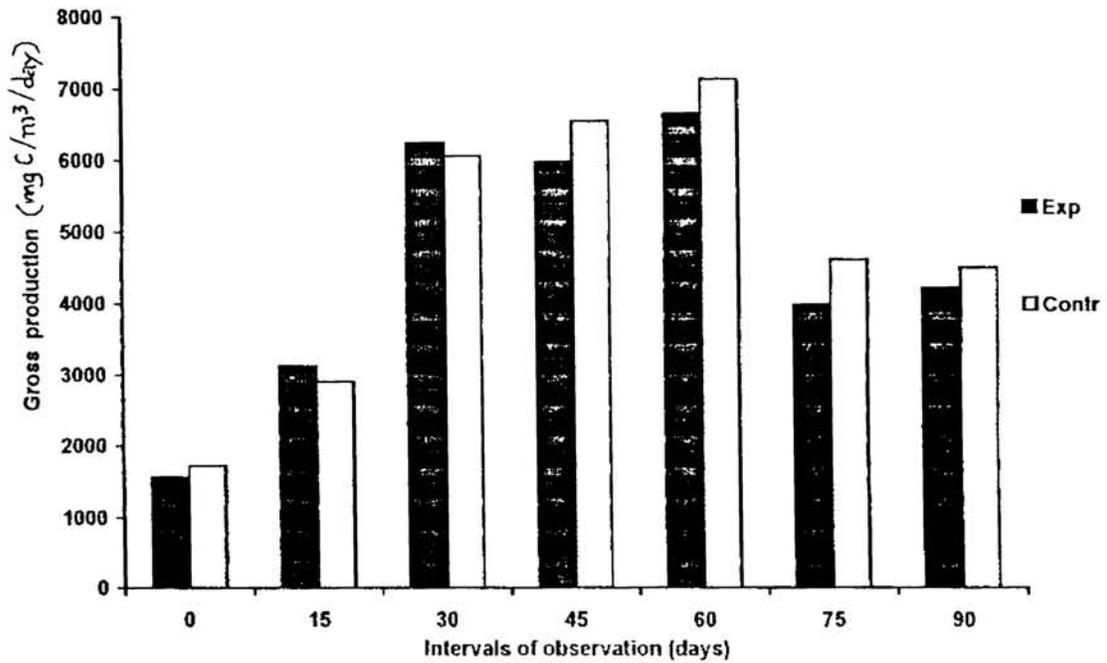


Fig. 8b Fluctuation in Primary production (Gross) in the pond treated with croton seed (4ppm) over a period of 90 days along with control

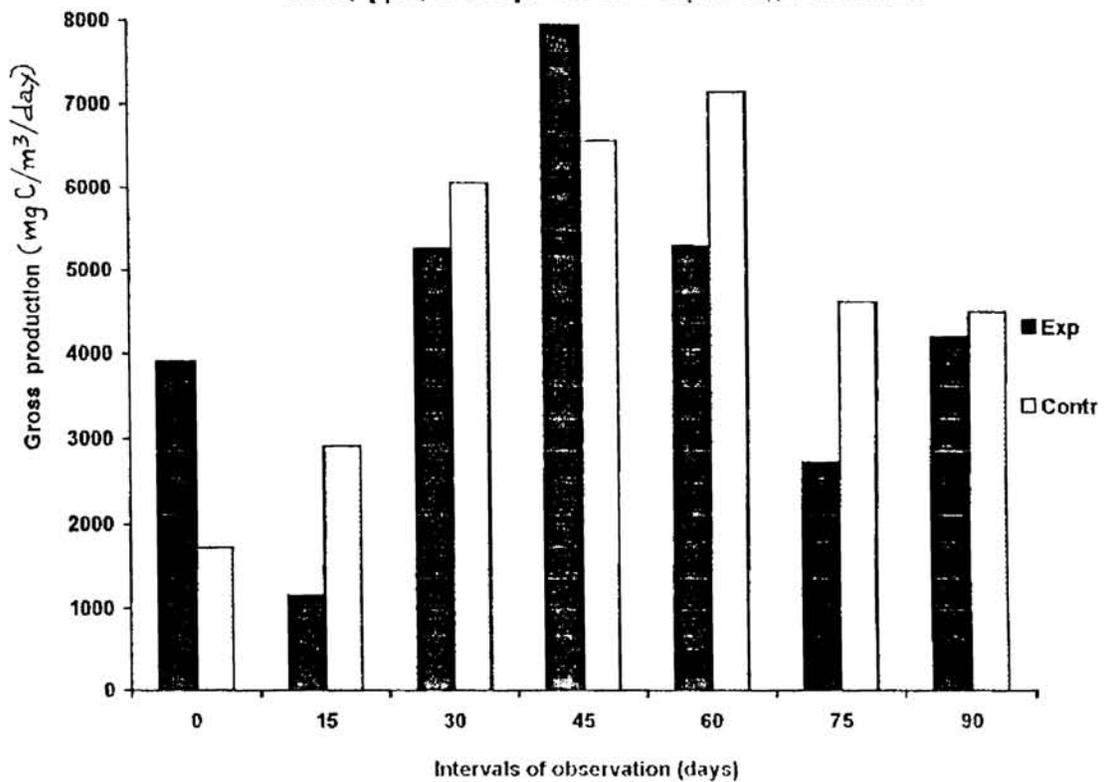


Fig. 8c. Fluctuation in Primary production (Net) in the pond treated with mahuaoil cake(200ppm) over a period of 90 days along with control

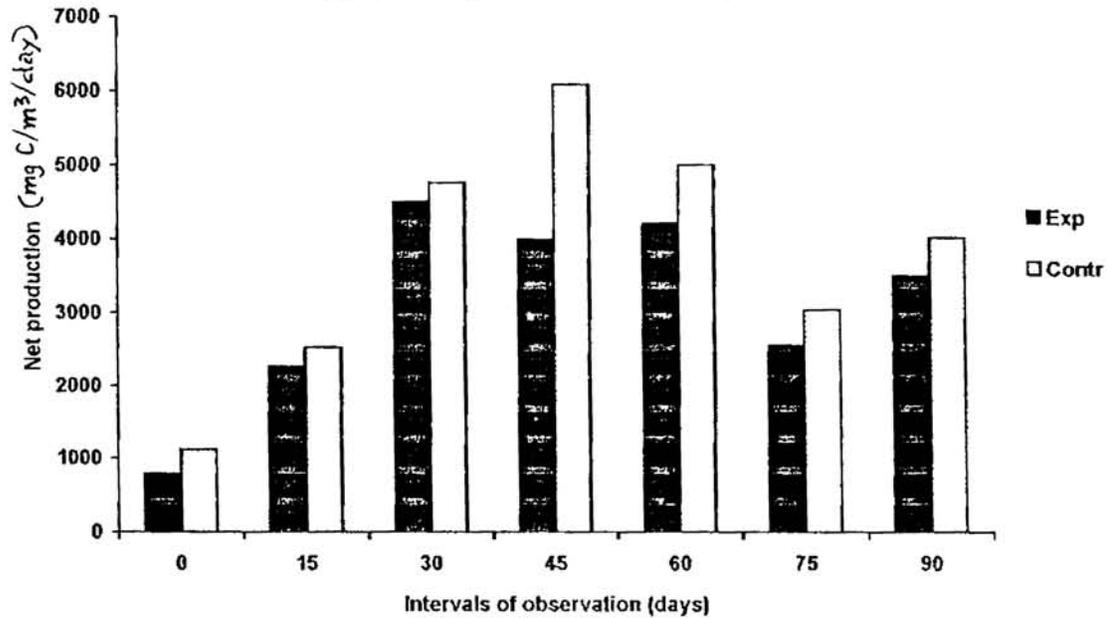
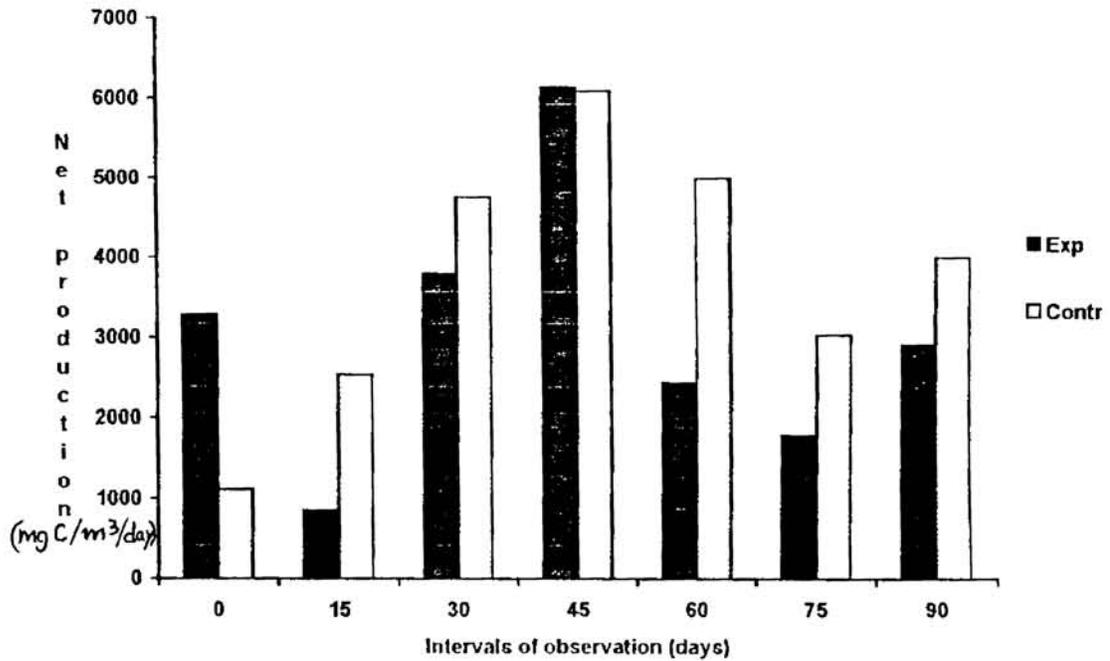


Fig. 8d Fluctuation in Primary production (Net) in the pond treated with croton seed (4ppm) over a period of 90 days along with control



nature finally, indicating a value of $3483.99 \text{ mg C/m}^3/\text{day}$ on the 90th day. In the case of the control pond the initial net production value of $1114.88 \text{ mg C/m}^3/\text{day}$ increased steadily to reach a peak value of $6081.8 \text{ mg C/m}^3/\text{day}$ on the 45th day to decrease thereafter to $3030.18 \text{ mg C/m}^3/\text{day}$ on the 75th day, and increase to $4002.13 \text{ mg C/m}^3/\text{day}$ on the 90th day. As in the case of gross production, the control pond exhibited a production magnitude, higher than that of the pond under treatment.

The quantum of primary production in the ponds treated with croton seed was less when compared to the control pond mostly, during the period of experimentation. A gross production of $3905.65 \text{ mg C/m}^3/\text{day}$ recorded before treatment steadily increased to $7939.93 \text{ mg C/m}^3/\text{day}$ (45th day) and decreased thereafter to $2726.45 \text{ mg C/m}^3/\text{day}$ on the 75th day. The gross production values in the control pond ranged between $1722.35 \text{ mg C/m}^3/\text{day}$, (day 1) to $7132.36 \text{ mg C/m}^3/\text{day}$, (day 60), the value recorded on the 90th day being $4488.1 \text{ mg C/m}^3/\text{day}$.

Though, the net production recorded at zero hour in the pond treated with croton seed was more than that that of the control pond, on all the subsequent sampling days thereafter, except on the 45th day, the magnitude was lower. Statistical analysis of the data revealed no significant difference in gross or net production among the treatment and control ponds.

4.2.2.3.3 Zooplankton

The numerical fluctuations among of different groups of zooplankton during the fortnightly sampling in the ponds under mahua oil cake treatment is illustrated in Table 23^a. Copepods formed the major constituent of the plankton samples collected from the treatment as well as control pond. Among copepods calanoids were the main item followed by cyclopoids and harpacticoids. Other groups such as hydrozoan medusae, fish larvae etc. appeared sporadically in the treatment and control ponds.

The number of copepods recorded ($87/m^3$) on the first day before treatment increased to $29865/m^3$ on the 30th day of treatment, thereafter showing a declining trend reaching a value of $6773/m^3$ on the 90th day. The increase in the number of copepods in the control pond during the course of 90 days was less pronounced as observed in the case of the ponds under treatment. This was evidenced by a gradual increase from $102/m^3$, on day-1 to a peak value of $3263/m^3$ only, on the 45th day, thereafter showing a decreasing trend ($954/m^3$ on day-90). The hydrozoan medusae appeared in small numbers on the 15th day of observation in both the treatment and control ponds, continuing to exist in the two ponds with marginal variations till the end of the period of observation. The few numbers ($3/m^3$) of fish larvae encountered in the treatment pond on the first day before treatment was absent on the 15th day, but again reappeared on the 30th day.

Table 23a. Zooplankton density (nos/m³) in the ponds treated with mahua oil cake (200 ppm) along with control

Periods of observation(days)	Copepods			Total	Hydrozoan medusae	Fish larvae	Mosquito larvae
	Calanoids	Cyclopoids	Herpetocoids				
0	Contr.	83	13	6	102	0	0
	Exp.	68	16	3	87	0	3
15	Contr.	391	6	0	397	6	0
	Exp.	4122	51	3	4179	39	0
30	Contr.	564	51	6	621	77	0
	Exp.	28407	1359	99	29865	3	32
45	Contr.	3244	19	0	3263	13	13
	Exp.	14369	468	13	14850	298	10
60	Contr.	545	103	0	648	13	0
	Exp.	5734	968	0	6702	135	3
75	Contr.	840	109	0	949	71	0
	Exp.	2952	64	0	3016	26	84
90	Contr.	788	160	6	954	19	0
	Exp.	6315	452	7	6773	10	6

Thereafter, they were present only in the treatment ponds, until the end of the culture period. Mysids present in the treatment pond @ 103 nos/m^3 was found to decline to 3 nos/m^3 on the 15th day, increasing thereafter with considerable fluctuations. The few fish larve (10 nos/m^3) present in the collections on the first day before treatment was absent in the sample collected on the 15th day and later on their presence was only felt sporadically. Mosquito larvae @ 23 nos/m^3 were noticed in the collections from the treatment ponds only (15th day).

Zooplankton samples collected from the ponds under croton seed treatment revealed a rich copepod population prior to treatment as evidenced by a density of 7014 nos/m^3 recorded on the first day, when compared with those in the control pond (Table 23.b). Here again, calanoids were the dominant item. An increased number of copepods (8418 nos/m^3), observed on the 15th day fell sharply to 1160 nos/m^3 on the 30th day in the treatment pond. Their number improved thereafter registering a peak value of 10590 nos/m^3 on the 60th day, fluctuating again to a value of 8792 nos/m^3 on the 90th day. In the control pond there was an increase in the number of copepods from 102 nos/m^3 (day-1) to 3263 nos/m^3 (day-45), fluctuating thereafter to register a density of 954 nos/m^3 on the 90th day. Mosquito larvae appeared @ 10 nos/m^3 in the collection on the 15th day from the treatment pond and they were totally absent in all subsequent samplings.

Table 23b. Zooplankton density (nos/m³) in the ponds treated with croton seed (4 ppm) along with control

Periods of observation(days)	Copepods										Hydrozoan medusae	Fish larvae	Mysids	Mosquito larvae
	Calanoids			Cyclopoids			Total	Herpetocoids	Total	Total				
	Calanoids	Cyclopoids	Herpetocoids	Calanoids	Cyclopoids	Herpetocoids								
0	Contr.	83	13	6	102	0	0	0	0	0	0	0	0	0
	Exp.	6879	122	13	7014	7	10	103	0	0	0	0	0	0
15	Contr.	391	6	0	397	6	0	0	0	0	0	0	0	0
	Exp.	7020	1343	55	8418	327	0	3	10	0	0	0	0	0
30	Contr.	564	51	6	621	77	0	0	0	0	0	0	0	0
	Exp.	1077	84	0	1160	93	0	173	0	0	0	0	0	0
45	Contr.	3244	19	0	3263	13	0	0	0	0	0	0	0	0
	Exp.	5369	378	0	5747	3	0	237	0	0	0	0	0	0
60	Contr.	545	103	0	648	13	0	6	0	0	0	0	0	0
	Exp.	9215	1362	13	10590	0	0	295	0	0	0	0	0	0
75	Contr.	840	109	0	949	71	0	0	0	0	0	0	0	0
	Exp.	7279	221	0	7500	10	0	170	0	0	0	0	0	0
90	Contr.	788	160	6	954	19	0	0	0	0	0	0	0	0
	Exp.	7914	846	32	8792	80	23	0	0	0	0	0	0	0

4.2.2.3.4 Macrobenthos

As part of the studies on the effect of mahua oil cake and croton seed on the productivity of prawn culture systems, fortnightly samples of macrobenthos were collected to understand their abundance and variations during a prawn culture period of 3 months and the data are presented in tables, 24 a and 24 b.

Table 24 a contains data on the variations of macrobenthos in number and biomass (wet weight) in ponds treated with the optimum concentration of mahua oil cake (200 ppm) delineated during the present study. Among the various components of the macrobenthos, polychaetes constituted the dominant item. Within the first fortnight after the application of mahua oil cake, their number and biomass registered a considerable reduction, following the same trend thereafter to be completely absent from day 60 onwards. Though, the declining trend was noticed among the polychaete population from the control pond also, their continued presence in the latter pond was observed upto the 75th day of treatment.

Amphipods and tanaids were uniformly represented in the collections from the treatment and control ponds upto the 30th day, a gradually declining trend being observed in the case of amphipods and a sharp reduction among tanaids. Sea anemones were recorded in the collection upto the 45th and 60th day in the treatment and control ponds respectively.

The fortnightly variations in numerical abundance and biomass of macrobenthos in ponds treated with croton seed are presented in table 24.b. Among the different organisms polychaetes were the most abundant group. From an initial value of 625 nos/m² (day 1) their number dwindled to 225 nos/m² on the 30th day, disappearing completely thereafter, unlike in the control pond where polychaetes were present in the collections upto the 75th day. Tanaids and amphipods were recorded only upto the 30th day in both treatment and control ponds. Sea anemones revealed an increasing trend with frequent fluctuations from zero hour onwards, disappearing altogether from the collections from the 75th day. Although bivalves were present in the collections from the pond under treatment at zero hour, they were totally absent in all subsequent samplings.

4.2.2.3.5. Growth, Survival and Production of *Penaeus indicus* in ponds treated with mahua oil cake and croton seed.

Subsequent to the elimination of undesirable organisms from the culture ponds by treating with the optimum concentrations of mahua oil cake and croton seed. (200 ppm and 4 ppm, respectively), seeds of the Penaeid prawn, *Penaeus indicus* were stocked in the ponds on the fifth day following application of the piscicides and their daily rate of growth in length and weight, survival and production profile were followed upto a period of 85 days (Tables, 25a-c).

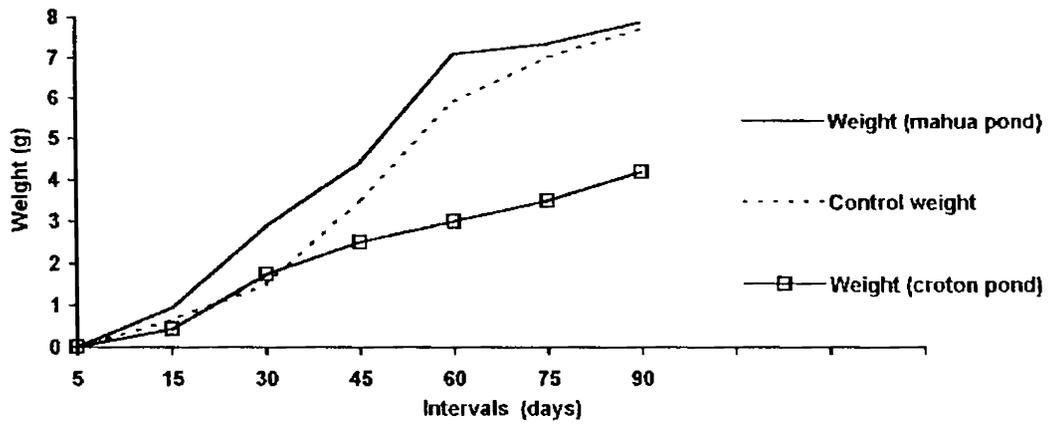
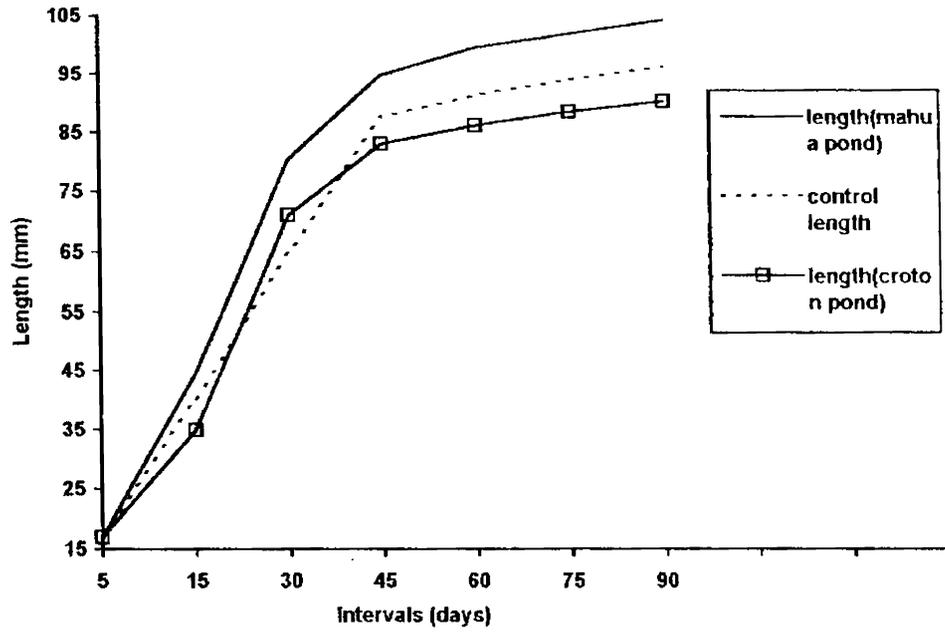
Table 25 a & Fig.9 contains details of the growth

Table 25a. Penaeus Indicus: Rate of growth in the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm), along with the control

Periods (days) of observation after treatment.	5*		15		30		45		60		75		90	
	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)	L (mm)	W (g)
Ponds treated with mahua oil cake	17	0.0193	44.47	0.95	80.32	2.88	94.55	4.41	99.39	7.08	101.57	7.33	103.88	7.85
Ponds treated with croton seed	17	0.0193	34.98	0.44	71	1.75	82.85	2.5	85.94	3	88.2	3.49	89.95	4.18
Control pond	17	0.0193	40.25	0.67	64.85	1.5	87.4	3.52	91	5.9	93.69	7	96	7.7

L: mean length W: Mean weight *; Prawn seeds stocked on the 5th day after the pesticide application.

Fig. 9. *Penaeus indicus*: Growth in length and weight in mahua and croton treated ponds and control



pattern of P. indicus at regular intervals, cultured in ponds treated with mohua oil cake and croton seed. The mean length and weight of P. indicus seeds at the time of their stocking in the ponds were 17 mm and 0.0193 g respectively. The prawns reared in mahua-treated ponds had a mean length and weight of 103.88 mm and 7.85 g respectively at the time of harvesting on the 85th day of stocking (or 90th day of piscicide application).

Prawns harvested from the croton-treated ponds only had a mean length of 89.95 mm and weighing 4.18 g. The rate of growth of prawns from the control pond was almost identical with that recorded from the mahua-treated ponds as evidenced by a mean weight of 7.7 g; the mean length, however, being less than that recorded from the mahua-treated ponds.

Table 25.b illustrates the rate of growth (increase in length and weight per day) of prawns at regular intervals, cultured in ponds treated with the piscicides. In the mahua - treated ponds prawns registered the maximum growth in length, during the first 45 days as revealed by an increase in length from 0.95 mm/day to 2.75 mm/day, to decline thereafter to a minimum of 0.15 mm/day during the 61-90 days period. The maximum rate of growth in weight was recorded during 16th-60th day of treatment.

In general, ponds treated with croton seed displayed the lowest growth rate in length and weight. During the 85 days culture period, the maximum rate of growth was recorded during

Table 25b. Penaeus Indicus: Rate of growth (Length & Weight/day) in the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm) along with control.

Periods (Days)	5--15		16--30		31--45		46--60		61--75		76--90	
	Length (mm/day)	Wt. (g/day)										
Ponds treated with mahua oil cake	2.75	0.09	2.39	0.13	0.95	0.11	0.32	0.18	0.15	0.02	0.15	0.03
Ponds treated with croton seed	1.79	0.05	2.4	0.08	0.79	0.05	0.21	0.03	0.15	0.05	0.12	0.03
Control pond	2.33	0.07	1.64	0.03	1.5	0.13	0.24	0.16	0.18	0.07	0.15	0.05

Table 25c. Penaeus Indicus: Survival and Production in the ponds treated with mahua oil cake (200 ppm) and croton seed (4 ppm) from the 5th to 90th day of piscicide treatment. along with control

Pond systems	Total area of culture ponds (m ²)	Stocking rate of seeds (nos./m ²)	Number of seeds stocked	Total production (Kg)	No. of prawns retrieved	Percentage of survival
Ponds treated with mahua oil cake	595	5	2975	19.77 (332.27 kg/ha)	2518	84.65
Ponds treated with croton seed	603	5	3015	10.88 (180.43 kg/ha)	2602	86.3
Control pond	447	5	2235	6.73 (150.56 kg/ha)	874	39.11

the 5th-30th day of treatment, recording a value of 1.79-2.4 mm/day.

As is evident from the Table (Table 25.b) the maximum growth rate in the control pond was 1.5 to 2.33 mm/day during 5th - 45th day. The minimum growth registered was 0.15 mm/day and the growth rate in weight ranged between 0.03 and 0.16 g/day. In general, the growth pattern revealed a gradually decreasing trend towards the end of the culture period in the treatment and control ponds as well.

Details of the extent of culture ponds, stocking rate of prawn seeds, prawn production, percentage of survival, and the quantity of miscellaneous catch from the different ponds comprising of different species of fin fishes and prawns are illustrated in table 25.c Total water area deployed for prawn culture after treatment with mahua oil cake, croton seed and also the control pond, ranged between 447 m^2 and 603 m^2 . Prawn seeds were stocked @ 5 nos/ m^2 area. The percentage of survival of prawns cultured in mahua - treated, croton - treated and the control pond were 84.65, 86.30 and 39.11 respectively. The production values were 332.27 kg/ha, 180.43 kg/ha and 150.56 kg/ha, in the order mentioned above. At the time of harvesting, the control pond yielded a miscellaneous catch of 2.34 kg comprising of different species of undesirable finfishes and prawns. A similar catch weighing 0.19 to 0.22 Kg was realized from the piscicide-treated ponds also, along with the cultured prawns.

5 DISCUSSION

Over the past two decades there has been a general tendency to intensify the traditional / extensive shrimp culture and to shift from an uncontrolled "Polyculture" to "monoculture" of shrimp by replacing trapping of wild juveniles, with stocking (Csavas, 1993). Prawn farmers have long been aware of the fact that production from the pond is adversely affected by pests. To obtain the maximum survival and growth of the prawn seeds stocked in the culture ponds, it is absolutely essential that all undesirable fishes or weed fishes including predators and competitors be eliminated from the culture system, before stocking with the desirable species; necessitating the application of suitable toxicants having piscicidal effects.

Piscicides may broadly be classified into inorganic chemicals and toxins of plants origin. Increasingly widespread use of chlorinated hydrocarbon insecticides as piscicides is becoming a source of real danger to fishes of economic importance and other aquatic and wild life (Choudhuri, 1975) Their highly toxic and persistent nature poses a threat to shrimp health and product quality in particular and human health in general and so their use should be discouraged (Apud et al 1989) Baticados (1986) found that the use of organotin molluscicide by Philippine shrimp farmers caused soft shell syndrome in cultured shrimps emphasizing the potential of some of those compounds to affect adversely the shrimp culture itself, as well as the external

environment. The environmental impact of shrimp culture has become a problem of increasing concern in many tropical and subtropical countries (Ong, 1982; Chua et al. 1989; Pullin^{et al.}, 1989). It would therefore be sensible to look for piscicides of plant origin which at certain concentrations would destroy pest organisms inhabiting culture systems and at the same time be naturally degradable and eco-friendly. Accordingly, two piscicides of plant origin, namely mahua oil cake and croton seed were employed during the present study, which involved laboratory and field investigations.

A striking feature of the results obtained during the laboratory experiments was that pre-soaking of the two piscicides was beneficial with regard to their toxic potency (Tables 1 a & b and figures^{1a-e & 2a-e}) Mahua oil cake exhibited maximum toxicity under a pre-soaking period of 12 hours. Further, the toxicity was found to diminish with an increase in pre-soaking time of 48 hours similar to that observed in the case of unsoaked material. These findings seem to suggest that saponin, the active piscicidal principle (Lakshman, 1983) can readily leach out when subjected to pre-soaking, gradually losing its toxicity beyond 12 hours of soaking due to progressive degradation. The observed increase in the lethal time in the case of unsoaked cake indicates the relevance of pre-soaking in the leaching process of the active principle.

In the case of croton seed, pre-soaking for 6 hours yielded the best result (table 1.b & Figs. ^{2a-e}) Pre-soaking for more than 12 hour on the other hand reduced the toxic potency of the material. Pre-soaking, it may be pointed out here, serves a dual purpose with regard to croton seed. Since the material is commercially available in the form of seeds, it is necessary that they be finely ground before application, for which pre-soaking would be beneficial; in fact, pre-soaking (for not more than 6 hours) enhances the toxicity of the seeds as well.

The effect of piscicides on the hydrographic parameters of the test media in general was evidenced by a concentration - bound gradual reduction in oxygen content in all the media containing graded concentrations of the two toxicants. Similar observations have been made under laboratory conditions with mahua oil cake (Banerjee et al. 1979), tea seed cake (De et al. 1987 and Minsalan and Chiu, 1986) and temru fruit, *Diospyros cordifolia* Roxb. (Sharma and Simlot, 1971). Since mahua oil cake and croton seed are organic and biodegradable, a temporary depletion of dissolved oxygen in the medium is always likely, due to the ongoing biodegradation process occurring in the test medium.

In an attempt to understand the source of oxygen for replenishment in the medium, the surface of the test medium containing 250 ppm and 4 ppm of mahua oil cake and croton seed respectively, was sealed with liquid paraffin. Interestingly, no

revival of oxygen content was observed following its decline contrary to what occurred in the media maintained without paraffin sealing (Tables, 2a & 3a figures^{3a & 4a}) indicating that the source of oxygen for replacement was atmospheric air.

Experimental observations on the relative tolerance of weed fishes to the two piscicides suggested that the finfishes could be classified into 3 groups, viz; least tolerant, medium tolerant and most tolerant. Ambassis gymnocephalus, Megalops cyprinoides, Elops saurus while Glossogobius giurus constituted the least tolerant group and Macropodus cupanus, Etroplus maculatus, Gambusia affinis, Aplochilus lineatus and Tachysurus maculatus. were the medium tolerant ones. Tilapia mossambica, Ophichthys boro and O. microcephalus were the most sturdy species.

The highly tolerant nature of T. mossambica has been observed earlier by Terazki et al. (1980) following exposure to tea seed cake which also contains saponin, the piscicidal element of mahua oil cake.

With respect to croton seed, the least tolerant group of finfishes included A. gymnocephalus, E. saurus, M. cyprinoides, E. maculatus and M. cupanus. A. lineatus, G. affinis and G. giurus represented the medium tolerant group whereas T. maculatus, T. mossambica, O. boro and O. microcephalus were the most tolerant finfishes tested. A similar response of T. mossambica to croton seed treatment has also been reported by Babu (1965).

Finfishes exposed to mahua oil cake showed various stress symptoms like losing balance while swimming, gradually becoming inactive, reaching the bottom of the container, lying on their sides in a state of coma and finally succumbing to death. Studies on the blood profile of T. mossambica exposed to the lethal concentration of mahua oil cake over a period of 6 hours revealed a significant reduction in the total Erythrocyte Count, Haemoglobin content and Haematocrit values. Similar findings have been made by Roy et al. (1989) in the air breathing climbing perch, Anabas testudineus (Bloch) and Sumit Home Chaudhuri et al. (1986) in the air breathing catfish Heteropneustes fossilis and the carp, Cyprinus carpio when exposed to lethal concentration of saponin. Sumit Home Chaudhuri et al. (1986) further suggested that the haemolytic action of saponin would be manifested in several ways:

- i) The viscosity of blood would rise.
- ii) Loss of haemoglobin would critically reduce the oxygen - carrying capacity of the blood, thus producing anoxia and acidosis
- iii) Possibility of haemoglobinuria conditions where the haemoglobin would be excreted through urine, as free urine has easy access into the glomerular membrane of kidney tubules as a result of which a serious disturbance in the kidney functioning will be caused (iv) acute anaemia, which is a distinct possibility. All these deleterious effects

depend upon the number of RBC haemolysed and the rapidity of haemolysis. He further opined that the gradual shrinkage and ultimate disintegration of RBC may contribute to the poor RBC count and haematocrit values.

In the case of croton seed treatment, the stressed fishes strived to come to the surface by jerking movements. Falling of scales and mild bleeding from the gill portion were also observed.

Haematological studies on T. mossambica exposed to the lethal concentration of croton over a period of 6 hours also indicated a decline in the RBC count, haemoglobin and haematocrit values. The observed increase in Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) could be explained as an adaptation on part of the test individuals to compensate for the fall in RBC production and make up the reduced oxygen supply.

The piscicidal property of croton seed has been attributed to its content of the toxalbumin, crotin as cited by Babu (1965). However, according to Chopra et al; (1956) seeds of croton contain 2 toxic proteins, croton globulin and croton albumin, which are essentially blood poisons (Chopra et al. 1949). Babu (1965) is of the view that this poison directly or indirectly affects the respiratory system of fishes.

Postlarval forms of the penaeid prawn Penaeus indicus displayed high degrees of tolerance to mahua oil cake as evidenced by their ability to withstand very high concentrations of mahua oil cake (750-125ppm) upto 12 hours. In fact, concentrations ranging from 300 ppm - 500 ppm required 24 hours to produce absolute mortality among them. The dissolved oxygen levels which dipped to surprisingly low levels in the medium containing higher concentrations of the cake (Table 2a & Fig.^{3a}) might have possibly enhanced the sensitivity of prawn larvae exposed to these concentrations.

Lakshman (1983) observed high degree of tolerance among shrimps to mahua oil cake and reported an LD 95 value as high as 5800 ppm of the cake. Terazaki et al. (1980) also observed the remarkably high tolerance of shrimps to relatively very high concentrations of tea seed cake (contains saponin), exceeding 10ppm that were lethal to all finfishes. Although, concentrations, less than 30 ppm of the seed cake was harmless to the postlarval shrimps, 60% of the individuals could survive in a medium containing 40 ppm of the toxicant for more than 12 hours.

The tolerance of postlarvae of P. indicus to croton seed was more than that of T. mossambica, amply illustrated by an increased duration of time needed for producing absolute mortality. Since, the hydrographic parameters of the test media (Table 3a & Fig.^{4a}) containing lethal doses of croton did not drop

below minimal threshold levels (Suseelan, 1978), they do not seem to have any role in enhancing the sensitivity of individuals to the toxicant. Bhuyan (1968) observed that freshwater prawns could survive in media containing various concentrations of Croton upto 5.33 ppm, sufficient to kill a variety of freshwater fishes including the hardiest ones.

The black clam, Villorita cyprinoides var. cochinensis representing the bivalve molluscs, which often cause much havoc to shrimp culture operations (Saji Chacko and Thomas, 1993) seemed to be more tolerant to mahua oil cake than finfishes; absolute mortality occurring only above a concentration of 200 ppm within 72 hours under laboratory conditions. The time required for effecting absolute mortality among individuals of the species could not be shortened even by higher concentration of 250 ppm.

Lakshman (1983) worked out the LD 95 values of some common gastropod molluscs inhabiting freshwater systems and was found to range from 1300 to 3260 kg of mahua oil cake. As in the case of postlarvae of prawns, the toxicity of the cake as well as the prolonged state of low oxygen content of the medium might probably have exerted a cumulative effect in enhancing the toxicity.

The present study also revealed that the clam V. cyprinoides is almost equally sensitive to croton seed treatment,

as finfishes, except that the former takes more time to reach absolute mortality under the same toxicant concentration.

Persistence of toxicity of mahua oil cake in the test media appeared to be concentration dependent, with regard to the finfish, A. gymnocephalus, representing the least tolerant group. Toxicity of the test media containing concentrations of 75 ppm to 250 ppm of mahua oil cake persisted for periods ranging from 1 to 6 days (Table 9a) under laboratory conditions. Studies by Bhatia (1970) showed that toxicity of the medium containing 100 ppm to 500 ppm of mahua oil cake retained their toxicity to fishes for 48 to 192 hours under laboratory conditions. In the case of croton seed, concentrations ranging from 0.5 ppm to 5 ppm in the media exhibited toxicity to A. gymnocephalus for periods of 1 to 5 days. The toxicity of the media containing 1 to 4 ppm of the piscicide continued upto 3 days (Babu 1965).

Among the two piscicides, only mahua oil cake had profound effect on the dissolved oxygen content of the test media under field trial. This may perhaps be due to the relatively higher organic load (150 to 3.00 mg/l) provided by mahua oil cake on the ecosystem when compared to croton seed (2 to 5 mg/l). Enhanced consumption of oxygen by the contained microorganisms for degradation of the organic matter might have lead to the depletion of dissolved oxygen. Banerjee et al; (1979) has observed that mahua oil cake applied @ 200 mg/l (the optimum

concentration delineated during the present study) led to a BOD load between 34 and 120 mg/l during the initial 10 days under field conditions.

Another observation made during the field study was that immediately after the application of mahua oil cake the pond water turned dark and turbid probably due to the tannin content of the cake, (Nadkarni, 1954) leading to a failure of penetration of sunlight and the progression of photosynthesis. When BOD exceeds supply, a drop in dissolved oxygen level even to undetectably low levels can result as observed during the present study, when the medium contained 200 ppm of MOC and above. Since the range and duration of oxygen depletion appeared to be concentration-dependent, the situation calls for a strict compliance with the optimum quantity of the cake that can be used for field application. The attempt made during the present investigation to minimise the depletion of oxygen by way of diluting the test medium with tidal water (after 12 hours following treatment and elimination of weed fishes), yielded promising results. In this case, the dissolved oxygen content did not at any point during the sampling period drop below the minimal threshold level.

The minimum (optimum) concentration of mahua oil cake capable of effecting absolute mortality among the 12 species of weed fishes tested under field condition was 200 ppm. As observed during the laboratory experiments, the least tolerant

group of finfishes included A. gymnocephalus. The snake eels O. boro and O. microcephalus were the most tolerant species tested. O. boro often burrows into the bunds of paddy fields and salt pans causing considerable damage to the bunds (Samuel, 1962). A concentration of 200 ppm MOC was insufficient to destroy the clam species, V. cyprinoides whose presence in farms seriously affects the growth of prawns (Saji Chacko and Thomas, 1991). Higher concentrations of 250 ppm and 300 ppm on the other hand could kill the clams within 96 hours and 72 hours respectively following treatment. Postlarval forms of P. indicus exhibited 65% mortality within 96 hours in the medium under 200 ppm, whereas absolute mortality was effected within 48 - 96 hours in the higher concentrations of 250 and 300 ppm. In short, the prawn and clam species proved to be more tolerant to the cake than finfishes. It seems that the low level of dissolved oxygen in the media containing 250 ppm and 300 ppm of the mahua oil cake would have enhanced the sensitivity of the two animals an observation made during the laboratory studies.

In the case of croton seed 4 ppm was found to be the minimum (optimum) concentration required for the complete elimination of weed organisms including different species of fin fishes (within 6 hrs) and the clam V. cyprinoides (within 72 hrs.). A significant finding made during the studies with croton seed was that the optimum concentration required for the elimination of finfishes is applicable to the clam species also.

Another observation made in this regard is that the lethal time of the clam could be cut short to 48 hours by enhancing the concentration to 5 ppm.

Postlarvae of P. indicus were more sensitive to croton seed than to mahua oil cake. The optimum field dose of 4 ppm of croton seed delineated during the course of the present study for total elimination of weed fishes was sufficient to effect absolute mortality of the postlarvae also. Interestingly, 4 ppm and 5 ppm of croton seed had same effect on their lethal time (6-12 hrs). Since the hydrographic profile of the field test medium were within normal levels (Table 10b & Fig) throughout the period of experimentation in all the concentrations, the possibility of their role in influencing the sensitivity of test organisms to the toxicant can be ruled out.

The dominance of copepode in the plankton collection appears to be a routine phenomenon as reported earlier by many workers (Menon et al., 1971; Pillai et al. 1975; Madhupratap & Haridas, 1975, Madhupratap, 1979; Jose et al. 1988 and Sheeba, 1992). The effect of mahua oil cake on the numerical abundance of zooplankton is clearly visible in the collections made from ponds exposed to different concentrations of the piscicide. One significant observation common to all the concentrations tested was the disappearance of fish larvae from the ponds from the 24th hour onwards. This points to the fact that concentrations ranging from 150 ppm to 300 ppm had a lethal effect on fish

larvae. The gradual reduction in the number of zooplankton in ponds exposed to concentrations of 200 ppm and above can be correlated with the pattern of decline in the dissolved oxygen content of the respective ponds. The test media which were characterised by a complete lack of zooplankton at 240th hour after treatment happened to be those ponds where the dissolved oxygen content had gone down to undetectably low levels. Therefore, it is reasonable to assume that the lethal effect of mahua oil cake on the zooplankton population is only a secondary or indirect effect. The presence of mosquito larvae in the treatment ponds from 120th hour onwards and their absence in the control pond can be correlated with the absence of finfishes following eradication especially A. lineatus, G. affinis, M. cupanus etc., which are well-known larvivorous fishes. Mosquitos prefer to breed in stagnant or slow moving shallow water either sunlit or shaded, clear or turbid and rich in organic material or polluted. Brackishwater bodies like lagoons, estuaries, canals and marshes are ideal habitats for the breeding of mosquitos (Menon, 1991). The observed direct relationship between the numerical abundance of mosquito larvae and the quantity of mahua oil cake applied can explain the preference of mosquitos for habitats rich in organic matter. It may also be taken into account the fact that the treatment ponds were maintained as closed systems throughout the period of experimentation. Above all the treatment ponds were cleared of all fishes including the larvivorous species, thus forming an ideal habitat for the breeding of mosquitos. The comparatively low incidence of

mosquito larve in the pond where the medium was diluted with tidal water after 12 hours of treatment (200ppm mahua oil cake & 4 ppm croton seed) and daily water exchange allowed from 120th hour onwards, further supports this view.

All the concentrations of croton seed ranging from 2 ppm to 5 ppm were lethal to the larval forms of finfishes encountered in the plankton collection. Hydrozoan medusae were also fully susceptible to the different concentrations. Copepods suffered only marginal reduction, especially during the initial stages of treatment; but recovered towards the lag phase of the experiment. The hydrographic parameters, which were within the normal levels in the treatment ponds, did not appear to have any influence on the sensitivity of the zooplankton to the toxicant. Mosquito larvae were not recorded from ponds in which weedfishes were destroyed only partially. Their occurrence in the treatment ponds, though only in small numbers was concentration-dependent. The comparatively low organic load received by the experimental ponds might perhaps be the reason for the lower number of mosquito larvae encountered in the plankton collection.

Bhuyan (1968) while investigating into the use of Croton tiglium seed as a fish poison in ponds observed that although zooplanktons exposed to croton seed were killed, they were found to reappear about a week after treatment. Dilution of the test medium with tidal water following treatment and

elimination of weedfishes can restrict the incidence of mosquito breeding to a great extent as observed during the present study.

The macrobenthic communities of experimental ponds, especially the polychaetes which constituted the most dominant group, suffered varying degrees of reduction in terms of their number and biomass when exposed to graded concentrations of mahua oil cake. The increase in the number and biomass of polychaetes observed at 24th hour of treatment can be attributed to their vertical migration to the upper layers of the sediment, finally to wriggle out of the burroughs, when stressed. This is further supported by the fact that polychaetes were found lying dead at the sediment surface in large numbers during the course of experimentation. From the pattern of their decline during the period of study it can be reiterated with certainty that the extend of damage is maximum between the 24th and 120th hour after treatment. The complete absence of macrobenthos at the 120th hour of sampling in concentrations of 250 ppm and above is indicative of the magnitude of ambient environmental regredation in the event of application of excessive concentrations of piscicides. Apart from the direct toxicity of the piscicide, the accumulation of particulate organic matter at the bottom of the pond can cause an increase in the BOD of the sediment. Enhanced consumption of oxygen by the macroorganisms of the sediment can result in a sudden depletion of oxygen in the waters overlying

the sediment. (Tsutsumi and Kikuchi 1983, Gowen et al. 1988). The situation can become worse within the sediment also (falling to zero even) due to an imbalance between the supply and consumption of oxygen. As a result, the delicate balance between the oxidation and reduction processes occurring in the sediment changes and the latter become the dominant pathway for the turnover of organic material. Of these, sulphate reduction is likely to be the most important reaction pathway resulting in the release of hydrogen sulphide. In confirmation to this, smell of hydrogen sulphide was experienced in sediment samples collected from the ponds exposed to concentrations of 250 ppm and 300 ppm of mahua oil cake. The accumulation of particulate waste together with the changes in physical structure of the sediment, reduced levels of oxygen and the presence of hydrogen sulphide result in significant changes in the ecology and community structure of the benthic macrofauna. In extreme cases, the macrofauna can disappear altogether (Gowen and Rosenthal, 1993). Dilution of the test medium following treatment, carried out during the course of the present study was much rewarding. Infact, through this trial it has been possible to demonstrate that only minimal damage would occur to the benthic macrofauna especially the polychaetes when dilution of the medium is effected following piscicide treatment.

Among the different groups of benthic organisms, polychaetes and bivalve molluses were more susceptible to croton seed treatment than others as evidenced by a gradual reduction

of the former in all the concentrations and a complete elimination of the latter in concentrations of 3 ppm and above. Further, the ecological damage caused by an excessive (5 ppm) concentration of croton seed to the macrobenthic communities was relatively less serious when compared to mahua oil cake treatment.

Regarding the persistence of toxicity of different concentrations of mahua oil cake under field condition, a concentration bound increase, in the duration of persistence of toxicity was observed. This points to the consequences of overloading the culture systems with organic materials, and a delay in their biodegradation and recycling. As evidenced by the response of A. gymnocephalus, the toxicity of the medium containing 200 ppm of mahua oil cake which persisted for 4 days could be reduced to single day by diluting the test medium with tidal water 12 hours after treatment. Though the test medium remained lethal to the postlarvae of P. indicus for 2 days under the same concentration, dilution of the medium 12 hours after treatment rendered the medium non-toxic to them. When the medium was treated with the optimum concentration (4 ppm) of croton seed, the lethal effect on fish life and P. indicus postlarvae persisted up to 48 hours and 24 hours respectively. When the test medium containing the same concentration was subjected to dilution, the period of lethal effect on A. gymnocephalus and P. indicus postlarvae did not prolong beyond 24 hours.

The ponds selected for post-treatment (treated with mahua oil cake and croton seed) studies on primary, secondary and tertiary production had bottom soils composed of sand, silt and clay, a condition cited ideal for the growth of P. indicus (Unnithan, 1985). The values of the different soil fractions recorded during the present investigation are in agreement with those reported earlier (Easwara Prasad, 1982 and Preetha, 1994). The nutrient status of the sediments of the canal systems in Vypeen Island, of which the present study area formed a part, has been regarded as low productive with regard to the organic carbon content (Easwara Prasad, 1982). Further, the relatively low organic carbon values recorded during the present study are in line with the earlier observation. It also falls within the limits specified by Tang and Chen (1967) while classifying the algal pasture soils of milk fish ponds of Taiwan, based on the percentage of organic carbon, as low (<1.5%), medium (1.6-3.5%) and high (>3.6%).

The comparatively high organic carbon recorded from the mahua-treated pond (0.42%), almost two times that of the control pond (0.22%) is indicative of the nutrient enrichment occurring through the mahua oil cake treatment. While there was a gradual decline in the organic content of the control pond during the course of study, it almost maintained a steady profile in the treatment pond. In the case of other parameters, no significant variations were observed between the treatment and control ponds.

Among the routine hydrographic parameters of the culture systems under study, the mean values of only the dissolved oxygen content of the mahua-treated pond exhibited noticeable difference from that of the control pond; probably an impact of the enrichment with organic matter.

During the 3 months culture period, the salinity of the ponds, in general, showed a declining trend with only marginal variation due to the intermittent pre-monsoon rain.

Studies on the diurnal variation of hydrographic parameters of the ponds under observation revealed a direct correlation among temperature, dissolved oxygen and pH in all ponds. Curiously enough, among the different experimental ponds an increase in mean oxygen concentration was recorded only in the mahua-treated pond. Moreover, the maximum oxygen level recorded during the 24 hour period of observation was also in the same pond.

Among the different water bodies, the common feeder canal consistently recorded higher mean values of nitrite-Nitrogen ($\text{NO}_2\text{-N}$) and nitrate-Nitrogen ($\text{NO}_3\text{-N}$) when compared to the control as well as treatment ponds. While inorganic phosphate was more or less uniform in all the ponds including the feeder canal, mean values of ammonia - Nitrogen ($\text{NH}_4\text{-N}$) were relatively higher in the treatment ponds.

In the case of gross and net primary production the control pond recorded relatively high values. The gradually increasing trend in primary production pattern observed in the mahua-treated ponds and the control upto the 60th day may be considered as a natural phenomena associated with the blocking of free flowing canals by newly erected earthen bunds and sluice gates while preparing the ponds for experimentation. On the other hand, the ponds deployed for croton treatment were already existing and were characterised by a luxuriant growth of phytoplankton. The observed sharp decline in the quantum of primary production in these ponds upto the 15th day of treatment might probably be due to the impact of croton seed application and the resultant temporary destruction of phytoplankton.

The relatively higher 'load' of nitrate - Nitrogen and inorganic phosphate in the ponds brought in by the common feeder canal during the 30th-60th day period might have possibly been the source of nutrients for the higher rate of primary production recorded from them during the corresponding periods. The sharp increase in primary production in the mahua-treated ponds within the first fifteen days of treatment could probably be discerned as a result of the nutrient provided by the mahua oil cake.

The increase in the numerical abundance of zooplankton from the 15th day onwards in all the ponds in general, can be directly correlated with the increase in primary production

during the corresponding period. Further, the higher number of zooplankton density observed in the treatment ponds during the entire period of study could probably be attributed to the relatively low level of grazing pressure, in the absence of weed fishes.

The decreasing trend in the number and biomass of macrobenthos in both treatment and control ponds from the start of the experiment suggests the pivotal role played by certain hydrographic parameters on their recruitment and distribution. Panikkar (1951) reported that ambient water temperature plays an important role in the abundance of marine fauna in the tropical brackishwaters. Crisp and Southward (1958) considered temperature as the most important factor affecting all the stages in the life history of organisms and that the trends of distribution are mainly due to temperature differences. But SriKrishnadhas et al (1981) commenting on the distribution pattern of polychaetes in the intertidal region of Vellar estuary expressed the view that temperature do not seem to play a major role in their distribution since the variations observed were in narrow limits (2° C). Salinity, on the other hand had an important role in limiting their distribution and density in the intertidal region. During the present study, covering a prawn culture period of 3 months, starting from the last week of February, a steady decline in the benthic communities composed of polychaetes, amphipods, tanaids and sea anemones, was observed

from the very first day onwards in the control and treatment ponds. In fact, the rate of reduction in the treated ponds was considerably higher, probably due to the toxic effect of the piscicides. Also, in the treatment ponds, macrobenthos were not represented beyond the 45th day of sampling (1st week of April) except for a few anemones on the 60th day. In the control pond only polychaetes were represented upto 75th day (1st week of May). It should be noted here that during the period of observation the salinity fluctuated between 15.39 ppt and 19.6 ppt because of intermittent pre-monsoon rain and water bottom and the water temperature between 30.3 C - 34.83 C. The present study revealed that macrobenthic communities can decline even at the initial temperature range of 31.98 C -- 32.15 C.

In fact, the macrobenthic organisms probably could not survive in temperatures beyond 34 C. The common feeder canal which was nearly 1.5m deep with a water temperature less than 32 C supported a rich and varied fauna of macrobenthos including several species of polychaetes even when there was none in the collections from the culture ponds where the temperature was above 34 C. Therefore, temperature seems to be the limiting factor especially in the case of polychaetes. It is generally observed that macrobenthos, especially polychaetes are poorly represented in the collections made during the S.W.monsoon period in the area because of the prevailing freshwater conditions. Here salinity seems to be the limiting factor. Again recolonisation starts by the beginning of post-monsoon period and

thereafter the population steadily increases through recruitment, recording the maximum during December - March (Gopalakrishna Pillai, 1977). Nair et al. (1988), while investigating into the environmental conditions of some paddy-cum-prawn culture fields of Cochin backwaters have come across a declining tendency of benthic biomass from February and attributed this to the effect of predation, Gopalakrishna Pillai (1977) indicated that the seasonal increase in benthic fauna in the area is due primarily to recruitment. This might possibly be the reason for the absence of any trace of replenishment of the benthic fauna during the period of their decline as observed in the present study. Sreekrishnadhas and Ramamoorthi (1975) reported that the abundance of polychaete larvae encountered in Porto Novo Waters during September-October may be considered as an indication of their active breeding. It can therefore be inferred from the present study that the declining trend observed in the case of benthic communities from the start of the experiment is only natural phenomenon governed primarily by the ambient temperature and secondarily by salinity and that their further recolonisation is influenced to a large extent by their breeding periodicity and salinity. In the present case, the sharp reduction trend and premature disappearance of the benthic fauna in the treatment ponds may be a direct effect of the piscicides applied, probably due to the destruction of their younger individuals during the time of application of the piscicides, preventing their succession.

Although comparable rates of survival were recorded from the mahua-treated and croton-treated ponds, the relatively better performance in terms of growth registered by P. indicus in mahua-treated and control ponds may will be related to the abundance of macrobenthos, the main source of food (Panikkar and Menon, 1956; Hall, 1962; Kurup, 1978 and Thomas, 1978). The poor percentage of survival observed in the control pond could be attributed to the predation/competition pressure exerted on the cultured prawns by the assemblage of weed organisms as evidenced by the harvest data. The mortality among individuals of P. indicus recorded from the treatment ponds can be related to the fact that postlarvae and juveniles of different species of prawns including P. indicus tolerate temperatures around 35^o C, but with varying survival rate (Kuttiyamma, 1981). Kuju (1978) brought to light the direct relationship between food requirement and the rate of growth of prawns at different stages. Slower growth rate due to the non-availability of proper food at particular stages in P. indicus has been reported by Sampath and Menon (1975) and Nandakumar (1982) in P. semisulcatus. The gradual reduction in abundance of macrobenthos upto the 45th day of sampling and their absence thereafter in the case of treatment ponds is clearly reflected in the growth rate of prawns. The continued presence of macrobenthos, especially the polychaetes upto the 75th day in the control pond evidently had beneficial effects on the growth of the prawns.

6 SUMMARY

To obtain the maximum survival and growth of prawn seeds stocked in culture systems, it is absolutely essential that all undesirable organisms including predators and competitors be eliminated from the culture systems, before stocking with the desirable species. Since, the elimination of weed organisms by a complete drainage of the field is dependent on the topographical aspects of the locality, it becomes necessary that piscicides be applied in the field.

Piscicides are either inorganic chemical pesticides or toxins of plant origin. The highly toxic and persistent nature of inorganic chemical pesticides with its residual effect poses a threat to shrimp health and product quality in particular and human health in general and so their use should be discouraged. In these circumstances it would be advisable to look for piscicides of plant origin which at certain concentrations would destroy pest organisms inhabiting the culture system and at the same time be naturally degradable and eco-friendly. For the present study two popular piscicides of plant origin namely, mahua oil cake, a product from the perennial tree, Bassia latifolia and the seeds of the croton plant, Croton tiglium were used for detailed investigations on their effect on field culture systems.

Prior to application of the two piscicides in the field, laboratory experiments were conducted on the following aspects (i) refining the method of preparation of the piscicides for application, (ii) sensitivity of different organisms including finfishes, prawn and clam species, (iii) haematological effects of the piscicides on selected fish and (iv) progressive degradation and persistence of toxicity of selected concentrations of the two piscicides with respect to finfish and cultivable prawn seed.

Pre-soaking of mahua oil cake in water for about 12 hours before application was beneficial, whereas pre-soaking for an increased duration of 48 hours reduced its toxicity. In the case of croton seed which needs grinding before use, a pre-soaking time of 6 hours was effective and was found to decrease its toxicity thereafter.

A concentration-dependent gradual reduction in dissolved oxygen content was observed in the test media following application of the piscicides; reversing to normalcy in due course. Based on the observed lethal time, the common weed fishes selected for the study could be classified into least tolerant, medium tolerant and most tolerant groups. Finfishes such as Ambassis gymnocephalus, Megalops cyprinoides and Elops saurus represented the least tolerant ones common to the two piscicides, whereas Tachysurus maculatus, Tilapia

mossambica, Ophichthys boro and O. microcephalus exhibited maximum tolerance. T. mossambica representing the most tolerant group of finfishes required 80 ppm and 2 ppm of mahua oil cake and croton seed, respectively for total mortality in 6 hours under laboratory conditions.

Postlarval forms of the penaeid prawn Penaeus indicus displayed high degree of tolerance to mahua oil cake registered total mortality in 24 hours in 400 ppm concentration. The dissolved oxygen content dipping to below tolerable limits in the test media at higher concentrations might probably had enhanced the sensitivity of the prawn larvae exposed to these concentrations. P. indicus postlarvae were more tolerant to croton seed also than the finfishes, reaching absolute mortality in 12 hours in 2 ppm.

A concentration of 200 ppm and above of MOC were effective in bringing about total mortality of the clam Villorita cyprinoides var. cochinensis within 72 hours, whereas a concentration of 2 ppm of croton seed was sufficient to produce the same effect among their counter parts during the same period of time. The lethal time could be reduced to 60 hours and 48 hours by applying 4 ppm and 5 ppm of croton seed respectively.

T. mossambica exposed to lethal concentrations of mahua oil cake and croton seed caused a gradual reduction in

the haematological parameters such as percentage of haemoglobin and haematocrit and the total erythrocyte count. The persistence of toxicity of the piscicides in the test media was found to be concentration-dependent.

The toxicity of the test media containing 75 ppm to 250 ppm of mahua oil cake persisted for periods ranging from 1 to 6 days, with regard to Ambassis gymnocephalus, representing the least tolerant group. In the case of croton seed, the toxicity of 0.5 ppm to 5 ppm persisted for periods of 1 to 5 days with regard to the same fish.

The field experiments were centred around two objectives -(i) delineation of the optimum concentration of the two piscicides for field application and observations on the effect of graded concentrations of the piscicides on the hydrography, zooplankton, macrobenthos, selected finfishes, prawn and clam species (ii) post-treatment studies on the effect of the piscicides on the productivity of the culture systems covering the hydrography, water and soil nutrients, primary production, zooplankton and macrobenthos and also the growth, survival and production of prawn, for a period of 3 months.

Between the two piscicides, only mahua oil cake had profound effect on the dissolved oxygen content of the field culture system subject to treatment. Concentrations of mahua oil cake of 200 ppm and above resulted in a reduction in the

dissolved oxygen content to undetectably low level following application. The time taken for the revival of the oxygen content was concentration dependent.

Based on the observations made during the course of the field experiments, concentrations of 200 ppm and 4 ppm were chosen as the optimum dose for the eradication of undesirable finfishes in the case of mahua oil cake and croton seed respectively. Dilution (8-10%) of the medium with tidal water, 12 hours after treatment was beneficial in minimising the reduction in dissolved oxygen and the number and biomass of macrobenthos in the case of mahua oil cake treatment.

The clam, V. cyprinoides could be completely destroyed only by a higher concentration of 250 ppm of mahua oil cake whereas, 4 ppm of croton seed delineated as the optimum for destroying weed fishes was applicable in the case of clams also for total eradication. In the case of mahua oil cake, concentrations of 200 ppm and above were toxic to P. indicus postlarvae beyond 24 hrs. of treatment, during which the dissolved oxygen dipped to undetectably low level. Concentrations of croton seed, 3 ppm and above were found to be toxic to the postlarvae.

With regard to the zooplankton and macrobenthos of the field culture systems under treatment, both the piscicides exerted a concentration-dependent lethal effect.

The toxicity of different concentrations of mahua oil cake, 150 ppm, 200 ppm, 250 ppm and 300 ppm persisted in the culture systems for 1,4,4 and 7 days respectively with regard to the least tolerant finfish A. gymnocephalus. The media containing 2 ppm, 3 ppm, 4 ppm and 5 ppm of croton seed, remained toxic to the same species of fish for durations of 1,2,2 and 3 days respectively.

The medium treated with optimum dose (200 ppm) of mahua oil cake and croton seed (4 ppm) retained their toxic effect on P. indicus postlarvae upto day-2 and day-1 respectively.

The bottom soil of the ponds selected for the post-treatment studies with reference to the productivity of the culture systems was silty-sand with comparatively low organic carbon content. Between the two piscicides, mahua oil cake was found to enrich the soil nutrient status of the pond marginally.

Diurnal variation in temperature, dissolved oxygen and pH of the pond water under experiment showed a direct correlation among the different parameters. In the case of gross and net primary production, the control pond recorded higher values indicating the minimal influence of the piscicides. Moreover, the water of the common feeder canal was also equally rich in nutrients as those in the ponds under experimentation.

The magnitude of reduction of macrobenthic organisms during the course of the 3 months culture period was more intense in the treatment ponds, in comparison with the control indicating the direct/indirect toxic effect of the piscicides.

The prawn, P. indicus attained maximum growth in length and weight (103.88mm : 7.85 g) in the mahua-treated ponds when compared with those cultured in the control (96mm : 7.7g) and croton-treated (89.95 mm : 4.18 g) ponds. Between the treatment ponds, the increased growth noticed in the mahua-treated pond could be indicative of the comparatively higher benthos biomass, whereas the predation/competition pressure within the control pond would have resulted in the lower growth of the prawns cultured there. The increased growth noticed in the control pond compared to the croton-treated pond might be due to the continued presence of higher magnitude of macrobenthos biomass upto the 75th day in the former pond.

The percentage of survival of prawns cultured in mahua-treated, croton-treated and the control pond were 84.65, 86.30 and 39.11 respectively. The production values were 332.27 Kg/ha, 180.43 Kg/ha and 150.56 Kg/ha, in the order mentioned above.

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Addenda

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