INDIVIDUAL AND COMBINED LETHAL TOXICITY OF PESTICIDE COMBINATIONS ON THE JUVENILES OF ROHU *LABEO ROHITA* (HAM.)

By

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THESIS

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DEPARTMENT OF FISHERY BIOLOGY

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PANANGAD, COCHIN

Dedicated

To

My Family

DECLARATION

I hereby declare that this thesis entitled **"INDIVIDUAL AND COMBINED LETHAL TOXICITY OF PESTICIDE COMBINATIONS ON THE JUVENILES OF ROHU *LABEO ROHITA* (HAM.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or society.

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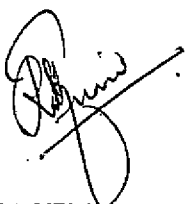

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Introduction

1.INTRODUCTION

The exploitable volume of earth's total resource of water is only 0.003 %. Freshwater is relatively scarce and is likely to become more so with the impact of global warming and population growth (Mason, 2002). Population increase, rapid industrialisation, intensive terrestrial farming and widespread health protection programmes have accelerated large-scale production and utilization of varied types of synthetic organic biocides. An array of wide spectrum of chemicals and their combinations are now being used as insecticides, herbicides, fungicides, nematicides, rodenticides and molluscicides. Many of the pesticides used are highly toxic and remain in the environment for a long time, causing pollution. Moreover, due to repeated application of pesticides, their toxic residues in environment and biota have reached alarming concentrations. Unfortunately many of these chemicals are also mutagenic (genetic damage), carcinogenic (cancer causing) or teratogenic (causing malformation) to human beings and many non-target organisms.

In our modern life pesticide has become a necessity. Even under optimal condition our capacity of food production is inadequate to prevent starvation of millions of people. Pests damage food and fibres during every stage of production, transportation and storage. The pesticides contribute directly to our health through the control of certain vector-born diseases; they contribute directly to the economy by increased production of food and fibres and through the protection of many food and other materials during storage.

Worldwide, pests cause great economic loss, being the causative agents for many diseases in human beings and animals. The pesticide revolution has started with the advent of DDT (Dichloro Diphenyl

Trichloroethane) during early 1940's (Potty, 2003). Man's relentless attempt to evolve newer, more effective and more economical pesticides for application and public health, especially for disease control have led to discovery of hundreds of chemicals for pest management. Agricultural chemicals were introduced to Indian market soon after IInd world war. Application of organochlorines such as DDT and HCH (Hexachlorocyclohexane) started during late 1940's and early 1950's followed rapidly by organophosphates and carbamates in 1960's and 1970's respectively. In 1963 Rachel Carson's book 'Silent spring' made people aware of potential dangers of pollution from pesticides.

Pesticide industry in India is the 4th largest in the world and 2nd largest in Asia pacific region only after China. Estimates of total market value vary between Rs 3,800 and Rs 4,100 crores (Anon., 2002). In India the demand for pesticide in the years 1983 and 1987 was 72,000 and 1,00,000 tonnes, respectively (Sharma, 1987). The average consumption of pesticide in our country has increased from 3.2 g.ha^{-1} in 1954-55 to 336 g.ha^{-1} in 1980 (Chottaraj, 1987). In USA and the European countries, its consumption is still higher and it ranges from 1490-1870 g.ha^{-1}. It is an irony that the use of pesticides in industrialised nations is showing a declining trend while their wide scale application, often indiscriminately, is alarmingly increasing in developing countries in the face of expanding food needs of ever growing populations and rapid shrinking of per capita availability of cultivable land (Potty, 2003).

Pesticides may be classified into five groups based on their structure viz. organochlorines, organophosphates, carbamates, pyrethroids and others. Organochlorine pesticides are hydrocarbons that contain chlorine atom and most of them are highly persistent, carcinogenic and mutagenic. They accumulate in adipose tissues of animals and are hazardous. DDT, HCH or BHC, endosulfan, dicofol,

methoxychlor, heptachlor, aldrin, dieldrin and endrin belong to this group. Most of them are banned (Pasha, 2003).

The organophosphate pesticides are esters of phosphoric or thiophosphoric acid. They are much safer when compared to the organochlorine pesticides in that they degrade much faster in the environment and do not accumulate in animals. But they are cholinesterase inhibitors. Most of the organophosphates are insecticides, like malathion, methyl parathion, guthion, fenitrothion, chlorpyrifos, quinalphos, monocrotophos, regalone, disulfoton, phosalone, phosphamidon etc.

The carbamates are derivatives of carbamic, thiocarbamic or dithiocarbamic acid. All the three-pesticides viz., insecticides, herbicides and fungicides come under this group, They are also much safer when compared to the organochlorine compounds.

The pyrethroids are relatively newer pesticides. They are synthetic structural analogues of naturally occurring pyrethrum extract from the plant *Chrysanthemum cinerariaefolium*, especially the flowers. It is commonly used as an insecticide and is much safer compared to organochlorine and organophosphate compounds. Being esters, they degrade in environment and are used in small quantities due to their high toxicity to insects. Pesticides that do not belong to this group are triazine herbicides such as atrazine, simazine, plant growth regulators such as 2,4-D (2,4-dichlorophenoxyacetic acid), dicamba, gibberilic acid, indole-3-acetic acid etc. (Pasha, 2003).

Among these, the first three groups are commonly used. It is seen that insecticides alone account for 80% and the organochlorines alone share about 40%. Next to it is the organophosphates. The organochlorines and heavy metals are placed in 'Black list' while the less dangerous chemicals like the organophosphates and herbicides make up the 'Grey list' in the European Union and the intention is to

eliminate the 'Black list' chemicals from the environment (Mason, 2002).

According to the Pesticides Manufacturers and Formulators Association of India (PMFAI), there are around 55 basic producers and over 300 pesticide formulators. Besides, there are a number of small-scale players. Around 200-odd generic products are manufactured in India. The producers manufacture the technical grade pesticide while the formulators convert them to usable form (Anon., 2002).

The toxicity of a given pesticide is not specific to the insects or the weeds it is designed to control. For this reason there is potential hazard associated with migration of organic chemicals from the place of exposure to non-target organisms. These migration pathways may be quite complex, occurring aerially at the time of application through volatilization, at the soil surface, through leaching to underground waterways or through run off to surface waters. The major source of contamination other than direct application to water is agricultural run off, sewage and effluents from industries manufacturing pesticides or using them in the process (Li, 1977).

It is estimated that generally less than 35% of the pesticide used in aerial spraying reaches the target, the remaining being carried away into the atmosphere. Pesticides lost in the atmosphere in the vapour phase generally come back to terrestrial system with the rain water (Hindin *et al.*, 1966).

The seasonal utilisation of paddy field for fish culture is quite common in Kerala and West Bengal. In the recent years, with the advent of high yielding varieties of paddy, the use of pesticide has become very popular. Therefore an assessment of environmental hazards due to toxic substances is an important challenge to toxicologists and ecotoxicologists. In the acute test of chemical to fish, death still represents equivocal end point in toxicology.

Kuttanad, the rice bowl of Kerala, is a region where there is overdose application of pesticide during punja cultivation period. According to the data compiled by Kuttanad Water Balance Study Project (KWBSP), 485 tonnes of pesticides were applied in Kuttanad area on an annual basis, of which 370 tonnes were used for punja crop alone (KWBSP, 1990). The estuarine, brackish and freshwater network of the state and the prime agricultural lands like Kuttanad and 'Kol'lands are so interconnected that the leaching of these chemicals into the water bodies is inevitable. Drastic measures to cut down the use of chemicals by means of Integrated Pest Management practices and more scientific and discriminate use of pesticides are the need of the hour.

In natural aquatic systems, fishes are exposed simultaneously to more than one biocide or contaminant because some chemicals are applied continuously and are highly persistent or others are applied as combinations to increase efficacy or reduce cost (Marking, 1977). The study of combined toxicity of insecticides like malathion, methyl parathion, endosulfan and the weedicide 2,4-D which are sequentially or even simultaneously used in paddy fields of Kuttanad and other plantation areas have not received any attention except may be in the preliminary study of Nair *et al.* (2000). Many theories have been put forth regarding the interaction among toxicants of similar and dissimilar chemical nature that may either aggravate or alleviate the toxicity of individual pollutant. Although herbicides in general are moderately toxic to fish, the possibility of enhanced toxicity (synergism) with an insecticide has not been taken into consideration. Similarly reduced toxicity (antagonism) of insecticide due to the herbicide is also to be considered. Hence systematic studies with biocides are required to know the intricacies of joint toxicity at work in areas like Kuttanad water bodies. An attempt is made in the present study to understand the

individual and combined toxicity of malathion, methyl parathion, endosulfan and 2,4-D on the juveniles of rohu (*Labeo rohita* (Ham)), a species used widely for freshwater aquaculture in the state. It is found to be a suitable test animal for toxicity monitoring (Ashraf *et al.*, 1992; Nair and Sherief, 1998; Mercy *et al.*, 2000; Ramani *et al.*, 2002 a, b).

Review of Literature

2. REVIEW OF LITERATURE

2.1 TEST ANIMALS

In short term toxicity tests, the selection of test organisms is of prime importance. Adelman and Smith (1976) listed the major criteria for the selection of appropriate fish species.

1. Must have a constant response to a broad range of toxicants, tested under similar conditions.
2. Must be available in large numbers.
3. Must be easy to handle (with low acclimation mortality).
4. Must be easy to transport (or available at the experimental site).

The different life history stages of rohu (*L. rohita*) have been found to be most suitable for toxicity monitoring and experimentation by various workers. Ashraf *et al.* (1992) worked with rohu adults for *in situ* toxicity monitoring. Sherief *et al.* (1996) and Nair and Sherief (1998) studied the bioaccumulation and chronic sublethal toxicity of phenol with rohu juveniles. Mercy *et al.* (2000) evaluated a six day rohu embryo-larval test for estimating the Maximum Allowable Toxicant Concentration (MATC) of monocrotophos under tropical conditions. Ramani *et al.* (2002 a, b) used rohu juveniles for evaluating the sublethal toxicity of monocrotophos and the resultant biochemical changes in the fish.

Some of the commonly used fishes in aquatic toxicology studies are the **salmonids** (Rainbow trout- *Salmo gairdneri*, Brown trout- *Salmo trutta*, Atlantic salmon- *Salmo salar*); **cyprinids** (Goldfish- *Carassius auratus*, Common carp- *Cyprinus carpio*, Zebrafish- *Brachydanio rerio*, Fathead minnow- *Pimephales promelas*, Sheepshead minnow-

Cyprinodon variegatus); **cyprinodontoids** (Mosquitofish- *Gambusia affinis*, Paddyfish or Medaka- *Oryzias latipes*, Mummichog- *Fundulus heteroclitus*, Guppy- *Poecilia reticulata*); **centrarchids** (Large mouth bass- *Micropterus salmoides*, Bluegill sunfish- *Lepomis macrochirus*) etc. In India the airbreathing fishes like the channids, anabantids and catfishes have been tested for their toxicant tolerance.

2.2 ACUTE LETHAL TOXICITY

Sprague (1973) states 'Acute lethal toxicity would be considered that which causes severe and rapid damage to the organism by the fastest acting mechanism of poisoning, fatal to the organism'. According to Alabaster and Lloyd (1982) acute toxicity is typically associated with breakdown of tissues and physiological systems (nervous, respiratory, muscular) at rates, which exceed rates of repair or adaptation leading to death of the organism. Hence much work on acute lethal toxicity testing (lethal levels that would kill 50% of the test fish in 24-96 hrs) ensued and voluminous literature on acute toxicity of pesticides to fishes accrued (Murty, 1986 a, b).

2.2.1 Acute individual lethal toxicity

Some of the major review and literature on toxicity of pesticide to fish are those of Henderson *et al.* (1959), Pickering *et al.* (1962), Johnson (1968, 1973), Alabaster (1969), Holden (1973), Toor and Kaur (1974), Grant (1976), Johnson and Finley (1980), McKim (1985), Fujimura *et al.* (1991) and Kristensen (1994). Johnson and Finley (1980) in the excellent compilation, summed up the result of 1578 tests conducted at the Columbia National Fisheries Research Laboratory, Missouri over a period of 14 years, with 271 chemicals and 58 test species (28 species of fish and 30 species of invertebrates).

Macek *et al.* (1978) considered 15 types of toxicity tests including full and partial life-cycle tests, embryo-larval tests, bioaccumulation tests, histological studies, biochemical tests etc., for comparative evaluation. The highest rated test overall in terms of their utility for use in assessing the hazard to aquatic environments with specific chemicals was the acute lethality test. The acute lethal tests are simple, short-term (24-96 hrs), cost effective, easily reproducible and more dramatic (Stephan and Mount, 1973).

Stephan (1977) reviewed the different methods for calculating an LC_{50}. When partial kills (between 0 and 100% mortality) occur in the different toxicant test concentrations the probit estimation method of Finney (1971) gives the appropriate LC_{50} estimate. Since the LC_{50} is the median of a population, some measure of the dispersion of the population should also be reported. The upper and lower 95% confidence limits (ULC and LLC) are the most preferred measures of dispersion because they are in the same units as the LC_{50} and are therefore, most easily understood and used by applied toxicologists (Stephan, 1977).

2.2.2 Acute combined lethal toxicity

The concentration addition (simple similar action) model of Sprague and Ramsey (1965) for describing the joint effects of toxicants on aquatic organisms is appropriate for water pollution control. The concept was first proposed by Gaddum (1948) in pharamacology. In this model the contribution of each component in the mixture is expressed as a proportion of the aqueous concentration producing a given response in a specified time (e.g. 48-h LC_{50}). In this scheme the combined or joint action is defined as additive, less than additive, more than additive or antagonistic (Fig.1).

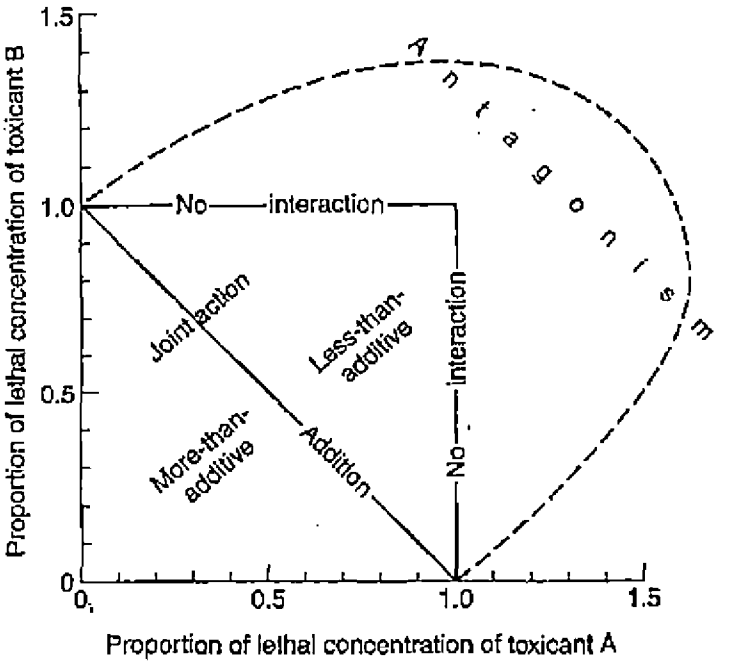

Fig. 1. Terms used to describe the combined effect of two pollutants (from Sprague 1970).

Sprague and Ramsey (1965) used the 'toxic unit' method of concentration addition model to predict the toxicity of copper and zinc mixture to Atlantic salmon (*Salmo salar*). This simple system of 'adding up' different toxicants is based on the concept that their lethal actions are similar and simply additive. Unlikely, as it might seem, this simple rule has been found to govern the combined lethal action of many pairs and mixtures of quite dissimilar toxicants also (Sprague, 1973). Marking (1977) derived the 'additive index' values based on the toxic unit concept to represent additive, greater than additive and less than additive effects by zero, positive and negative values, respectively. A clear cut linear representation of combined toxicity can be arrived at by this method and the method is followed in the present study. Broderius (1991) reviewed the methodologies in vogue and described a new model (quantitative structure activity relationship – QSAR) for similar and dissimilar chemicals, needing more experimentation and computation. Polloth and Mangelsdorf (1997) discussed the reliability of the QSAR approach in assessing toxicity.

Calamari and Alabaster (1980) reviewed the theoretical models in evaluating the effect of toxicant-mixtures in the aquatic environment. Alabaster and Lloyd (1982) set apart a chapter to review the toxicity of mixtures of toxicants, which included a section on pesticides. Murty's (1986 b) exhaustive review on toxicity of pesticides to fish included a chapter on joint action of pesticide mixtures. In this chapter he discusses in detail the controversies surrounding the terms 'synergism', 'potentiation' and 'antagonism' in the light of aquatic toxicology.

The problems of toxicity of mixtures of pesticides have been recognized quite early and laboratory studies have been conducted leading to a number of publications. Notable among them are those of Ferguson and Bringham (1966), Bender (1969), Krieger and Lee (1973), Macek (1975), Marking and Dawson (1975), Marking and Mauck

(1975), Statham (1975), Statham and Lech (1976), Fabacher *et al.* (1976), Verma *et al.* (1980), Ware (1980), Hermens and Leewangh (1982), Woodward (1982), Hermens *et al.* (1985), Gill *et al.* (1991), Arnold and Braunbeck (1994), Gupta *et al.* (1994) and Denton *et al.* (2003).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL LABORATORY

The experiment was conducted in the Post Graduate laboratory of the Dept. of Fishery Biology of the College of Fisheries, Panangad, which has concrete flooring, water supply and proper drainage facility to remove pesticide contaminated water to minimize the risk of hazards. There were provisions for lighting and adequate ventilation.

3.2 EXPERIMENTAL TANK

The experimental tanks for the acute toxicity studies were pre-conditioned plastic troughs with nine-litre capacity (Plate 1).

3.3 STORAGE TANK

Water drawn from open well was stored in large fibreglass reinforced plastic tanks and was well aerated. The water was stored for a period of 12 hrs before use.

3.4 EXPERIMENTAL ANIMAL

Juveniles of rohu (*Labeo rohita*) were obtained from the carp hatchery of the College of Fisheries, Panangad. The average size was 48.39 ± 3.9 mm and 956.47 ± 268.24 mg. They were acclimated in well water (dilution water) in the laboratory condition for 10-15 days prior to the start of the experiment. During this period they were fed *ad libitum*, once a day, on a pelleted carp feed and was kept in well-aerated water. Remains of feed and faecal matter were siphoned out regularly to avoid stress.

Plate 1. Experimental set up for 48-h LC_{50} test.

3.5 PESTICIDES USED FOR THE EXPERIMENT

Four pesticides were used for the experimental purpose, which included three insecticides and an herbicide. The insecticides were malathion and methyl parathion (organophosphates) and endosulfan (a chlorinated hydrocarbon). The herbicide used was 2,4-D.

3.5.1 Malathion

Malathion is an organophosphorus pesticide belonging to the group of dimethoxy compounds. The organophosphorus compounds are poisons with a neuro paralytic and enzymatic action. The basis of their toxicity lies in the capacity of their selective effect on enzyme of nerve tissue –cholinesterase- that leads to excessive accumulation of acetylcholine in the organism, giving rise to complex poisoning symptoms. Organophosphorus compounds also inhibit other enzymes – esterase, protease, and peroxidase and slightly increase the activity of catalase. Malathion is O,O-dimethyl-S-(1,2-dicarbethoxyethyl) phosphorodithioate. Empirical formula is $C_{10}H_{19}O_6PS_2$ and has a molecular weight of 330. It is soluble in water; Solubility at room temperature is up to 145 ppm. It is a non-systemic insecticide and acaricide. Its active ingredient is 50 % EC malathion. Malathion is formulated as 25-86% emulsifiable concentrates. In addition to field application it is also used for control of mosquitoes, flies, household insect, animal ectoparasite and human head and body lice. It is a product of Excel Industries Ltd., Mumbai.

3.5.2 Methyl parathion

Methyl parathion is an organophosphorus pesticide belonging to the group of dimethoxy compound. It is O,O-dimethyl O-(4-nitrophenyl) phosphorothioate. Empirical formula is $C_8H_{10}NO_5PS$ and has a molecular weight of 263.33. It is soluble in water at 25°C and solubility

is up to the extent of 55-60 ppm. It is a non-systemic contact and stomach poison with some fumigant action. It is a product of Bayer (India) Ltd., Mumbai. Trade name is metacid 50 and active ingredient is 50 % EC methyl parathion. It is formulated as emulsifiable concentrate. It is used in agricultural system including nurseries and greenhouses.

3.5.3 Endosulfan

It is a chlorinated hydrocarbon insecticide, belonging to the group cyclodiene and related compounds. It is a mixture of two stereo isomers, of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide. It has the empirical formula $C_9H_6Cl_6O_3S$ and a molecular weight of 406.95. Endosulfan is moderately soluble in most organic solvents but highly insoluble in water. It is a broad-spectrum insecticide and acaricide. It is a product of Excel Industries Ltd., Mumbai. Its trade name is endocel and active ingredient is 35% EC endosulfan. It is formulated as an emulsifiable concentrate. Endosulfan has been used against wide variety of agricultural pests but not against those of livestock, stored product or the household.

3.5.4 2,4-D

2,4-D is a chlorophenoxy herbicide. It is 2,4-dichlorophenoxyacetic acid. Empirical formula is $C_8H_6Cl_2O_3$ and has a molecular weight of 221.04. It is soluble in water; at 25°C solubility in water is 620 ppm. It is a product of Syngenta Crop Protection Ltd., Chennai. Its trade name is fernoxone. It contains 80% sodium salt of 2,4-D.It is mainly used against broad-leaved weeds.

3.6 PREPARATION OF STOCK SOLUTION

The stock solution was prepared using the formula

$$\text{Vol. of commercial formulation} = \frac{\text{Required vol. of stock solution} \times \text{Desired strength of stock solution}}{\text{Strength of commercial formulation}}$$

The stock solution prepared was subjected to active agitation (manually) for a period of 10 minutes before dosing. The stock solution was freshly prepared everyday.

3.7 EXPERIMENTAL PROCEDURE

3.7.1 Lethal toxicity

Preliminary 48-h exploratory tests were conducted before fixing desired concentration for finding out LC_{50} values of the pesticides. Based on the results of exploratory tests six concentrations of malathion, seven concentrations of methyl parathion, six concentrations of endosulfan and six concentrations of 2,4-D were selected for the final experiments. The 48-h acute lethal-toxicity test (48-h LC_{50}) was carried out by the static bioassay method (Sprague, 1973) with toxicant replenishment at every 12 hrs interval. The tests were carried out in plastic troughs, in triplicate with seven litres of water and stocking ten fishes each. The average animal load factor was around 1.366 $g.l^{-1}$. A control was also kept for each replicate. From the acclimated stock ten healthy fishes were selected randomly and they were starved for 24 hrs prior to the experiment. Mortality during 48-h exposure was recorded for each treatment. The 48-h LC_{50} values and their 95% confidence limits were calculated by linear regression analysis after probit transformation of mean mortality and Log_{10} transformation of the test concentrations (Finney, 1971), using SPSS software. The lower limit was termed as

LLC_{50} (Lower lethal concentration) and the upper limit was termed as ULC_{50} (Upper lethal concentration).

3.7.1.1 Concentrations of malathion

Based on the exploratory test six concentrations of malathion were selected for the final experiment. The concentration ranges from 5.0 $mg.l^{-1}$ (no mortality) to 10.0 $mg.l^{-1}$ (100% mortality). The seven treatments were 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 $mg.l^{-1}$ and a control.

3.7.1.2 Concentrations of methyl parathion

Based on the exploratory tests seven concentrations of methyl parathion ranging from 6.0 $mg.l^{-1}$ (no mortality) to 9.0 $mg.l^{-1}$ (100% mortality) were selected for the final experiment. The eight treatments were 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 $mg.l^{-1}$ and a control.

3.7.1.3 Concentrations of endosulfan

Based on the exploratory tests six concentrations of endosulfan ranging from 0.001 $mg.l^{-1}$ (no mortality) to 0.025 $mg.l^{-1}$ (100% mortality) were selected for the final experiment. The seven treatments were 0.001, 0.0025, 0.005, 0.0075, 0.01 and 0.025 $mg.l^{-1}$ and a control.

3.7.1.4 Concentrations of 2,4-D

Based on the exploratory tests six concentrations of 2,4-D ranging from 850 $mg.l^{-1}$ (no mortality) to 1100 $mg.l^{-1}$ (100% mortality) were selected for the final test. The seven treatments were 850, 900, 950, 1000, 1050, 1100 $mg.l^{-1}$ and a control.

3.7.2 Combined toxicity

The exploratory tests for combined toxicity were carried out with 1:1 ratio of the individual 48-h LC_{50} values and their proportionate decreasing combinations based on the method of Marking (1977). Six concentrations each of the five pesticide combinations were selected for the final LC_{50} study and the experiment was carried out for 48 hrs in troughs with seven litres of water and ten fishes each. The tests were carried out in triplicate with toxicant replenishment at every 12-hrs interval. Mortality was recorded at six hours intervals. The 48-h LC_{50} values and their 95% confidence limits were calculated by linear regression analysis after probit transformation of mean mortality and Log_{10} transformation of the test concentrations (SPSS software).

3.7.2.1 Concentrations of 2,4-D- endosulfan combination

Based on the exploratory test conducted six concentrations, ranging from 190 $mg.l^{-1}$ 2,4-D and 0.001.mg l^{-1} endosulfan (no mortality) to 570 $mg.l^{-1}$ 2,4-D and 0.003 $mg.l^{-1}$ endosulfan (100% mortality) were selected for the final experiment. The seven treatments were 190.0 + 0.001, 285.0 + 0.0015, 380.0 + 0.002, 450.0 + 0.0025, 500.0 + 0.00275, 570.0 + 0.003 mg l^{-1} and a control.

3.7.2.2 Concentrations of 2,4-D- malathion combination

Based on the exploratory tests six concentrations, ranging from 380 mg l^{-1} 2,4-D and 3.2 mg l^{-1} malathion (no mortality) to 620 mg l^{-1} 2,4-D and 5.2 mg l^{-1} malathion (100% mortality) were selected for the final experiment. The seven treatments were 380.0 + 3.2, 430.0 + 3.6, 476.0 + 4.0, 520.0 + 4.4, 570.0 + 4.8, 520.0 + 5.2 $mg.l^{-1}$ and a control.

3.7.2.3 Concentrations of 2,4-D- methyl parathion combination

Based on the exploratory tests six combinations ranging from 190 $mg.l^{-1}$ 2,4-D and 1.5 $mg.l^{-1}$ methyl parathion (no mortality) to 665 $mg.l^{-1}$ 2,4-D and 5.25 $mg.l^{-1}$ methyl parathion (100% mortality) were selected for the final experiment. The seven treatments were 190.0 + 1.5, 285.0 + 2.25, 380.0 + 3.0, 475.0 + 3.75, 570.0 + 4.5, 665.0 +5.25 $mg.l^{-1}$ and a control.

3.7.2.4 Concentrations of endosulfan-malathion combination

Based on the exploratory tests six concentrations ranging from 0.00025 $mg.l^{-1}$ endosulfan and 0.4 $mg.l^{-1}$ malathion (no mortality) to 0.0015 $mg.l^{-1}$ endosulfan and 2.4 $mg.l^{-1}$ malathion (100% mortality) were selected for the final experiment. The seven treatments were 0.00025 + 0.4, 0.0005 + 0.8, 0.00075 + 1.2, 0.001 + 1.6, 0.00125 + 2.0, 0.0015 + 2.4 $mg.l^{-1}$ and a control.

3.7.2.5 Concentrations of malathion- methyl parathion combination

Based on the exploratory tests six concentrations ranging from 1.6 $mg.l^{-1}$ malathion and 1.5 $mg.l^{-1}$ methyl parathion (no mortality) to 5.6 $mg.l^{-1}$ malathion and 5.25 $mg.l^{-1}$ methyl parathion (100% mortality) were selected for the final experiment. The seven treatments were 1.6 + 1.5, 2.4 + 2.25, 3.2 + 3.0, 4.0 + 3.75, 4.8 + 4.5, 5.6 + 5.25 $mg.l^{-1}$ and a control.

3.7.2.6 Sum of biological activity

Sum of biological activity was calculated based on 'toxic units' as defined by Sprague and Ramsey (1965) as:

$$S = Am/Ai + Bm/Bi$$

Where,

A and B are toxicants,

i and m are toxicities (48-h LC_{50}) of the individual toxicants and mixtures respectively,

S is sum of biological activity.

If the sum of biological activity of chemical A and B is 1.0, the toxicity is simply additive. Sums that are less than 1.0 indicate greater than additive toxicity and the sums greater than 1.0 indicate less than additive toxicity. The sums could function as an index of additive toxicity, except that values greater than 1.0 are not linear with values less than 1.0. Hence the "additive index" values of Marking (1977) were calculated.

3.7.2.7 Additive index

The "additive index" values of Marking (1977) were calculated as:

$$\text{Additive index} = (1/S) - 1 \text{ for } S \leq 1$$

$$\text{and Additive index} = S(-1) + 1 \text{ for } S \geq 1$$

The significance of deviation from 0 is determined by substituting values from 95% confidence limits for the different LC_{50} values in the formula to establish a range for additive indices. The range is derived by selecting values of 95% confidence limit yielding deviation from the additive index. The lower limits of individual toxicant (Ai and Bi) and upper limit of the mixtures (Am and Bm) are substituted for LC_{50} to determine lower limits of the index. The upper limits of individual toxicant (Ai and Bi) and lower limit of the mixtures (Am and Bm) are substituted for LC_{50} to determine upper limits of the index. Whenever an index range overlaps 0, additive toxicity is assumed. The

computation methodologies used in the present study follow the concentration addition (Simple similar action) model.

The 'magnification factor' is arrived at by the addition of '1' to the additive index value. 'Additive index' value of '0' means a magnification factor of 'x1' (strictly or simply additive), magnification factor below '1' indicate 'less than additive toxicity' and above '1' indicates 'more than additive toxicity'.

According to Broderius (1991) "With concentration addition the toxicants act independently but produce similar effects so that one component can be expressed in terms of the other. This is accompanied through adjusting for differences in their respective potencies by expressing each component in the mixtures as a proportion of the aqueous concentration producing a given response in a specific time"(here the 48-h LC_{50}). The concentration addition mixture model has been the most studied and cited concept (Broderius, 1991).

3.7.3 Inoculation of stock solution

Stock solution after agitation was inoculated into the experimental troughs containing dilution water by using 0.1 ml, 1ml, 2ml, 5ml or 10 ml graduated glass pipettes with suction bulb for pipetting the solution and to avoid hazard by sucking through mouth. After inoculation of pesticide, it is thoroughly mixed with a glass rod.

3.7.4 Layout of experiment

CRD (Completely randomized design) was used to conduct the experiment. Four pesticides individually and in combinations were used at different concentrations and for each set a control was kept. Each treatment was marked as T1, T2,...,Tn to represent treatment. Each experiment was carried out in triplicate.

3.7.5 Source of water

Well water was used for acclimation of fishes and for running the test (dilution water). Freshwater was filtered using nylon cloth and aerated to saturation prior to use. The dissolved oxygen, pH and temperature were in range of 8.0-8.5 mg l^{-1}, 7.0-7.5 and 27 ± 1°C respectively, in the aerated well water.

3.7.6 Determination of mortality

Mortality of fish was determined at regular intervals by the response of fish to gentle touch in the opercle with a glass rod. Those fishes that failed to show any movement were considered dead and were removed.

3.8 PHYSICO-CHEMICAL PARAMETERS

Water quality parameters and behavior of fish in the tank were observed. Physico-chemical parameters (D.O., pH and Temperature) were measured every 12 hrs, just prior to and immediately after dosing (renewal).

3.8.1 Dissolved oxygen

Modified standard 'Winkler's' method was used for determination of dissolved oxygen (Strickland and Parsons, 1972).

3.8.2 pH

pH was measured using pHmeter (Elico digital pH meter).

3.8.3 Temperature

Temperature was measured using thermometer with an accuracy of 0.1°C.

3.8.4 Behaviour

Locomotory and general behaviour of fish were recorded at regular intervals.

Results

4. RESULTS

Acute lethal toxicity test and combined lethal toxicity test were conducted on juveniles of rohu (*L. rohita*). The physico-chemical parameters during the experiment, observations recorded and the results obtained after analysis are presented below.

4.1 PHYSICO-CHEMICAL PARAMETERS

The physico-chemical parameters were obtained at regular intervals and the range of each parameter is given in Table 1.

The range of temperature during the experiment was 27 ± 1°C, pH ranged from 7.2 to 7.8 and the dissolved oxygen from 5.9 to 8.2 $mg.l^{-1}$. The dissolved oxygen level never dipped below 70% of the air saturation value in any of the experimental treatments.

Table 1. The range of physico-chemical parameters obtained during the experiment.

Physico-chemical parameters	Temperature	PH	Dissolved oxygen
Range	27 ± 1°C	7.2 to 7.8	5.9 to 8.2 $mg.l^{-1}$

4.2 INDIVIDUAL LETHAL TOXICITY

4.2.1 Malathion

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of malathion is presented in Table 2 and the results of probit analysis in Table 3. The concentration response curve based on probit analysis is represented in Fig. 2.

The calculated 48-h LC_{50} value and its range was 7.885 $mg.l^{-1}$ (7.279 to 8.607 $mg.l^{-1}$).

Table 2. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of malathion.

Treatment	Test conc. ($mg.l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	5.0	0	10	0	03.33 ± 4.71
T2	6.0	10	20	10	13.33 ± 4.71
T3	7.0	20	30	20	23.33 ± 4.71
T4	8.0	40	50	40	43.33 ± 4.71
T5	9.0	60	80	70	70.00 ± 8.16
T6	10.0	90	100	100	96.60 ± 4.71
T7	Control	0	0	0	0

Table 3. Result of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of malathion.

Exposure period (hrs)	LC_{50} ($mg.l^{-1}$)	95% confidence limit ($mg.l^{-1}$)		Slope (b)	Intercept (a)
		LLC_{50}	ULC_{50}		
48	7.885	7.279	8.607	11.005	-9.869
Regression equation: Probit Y= -9.869 + 11.005 log X					

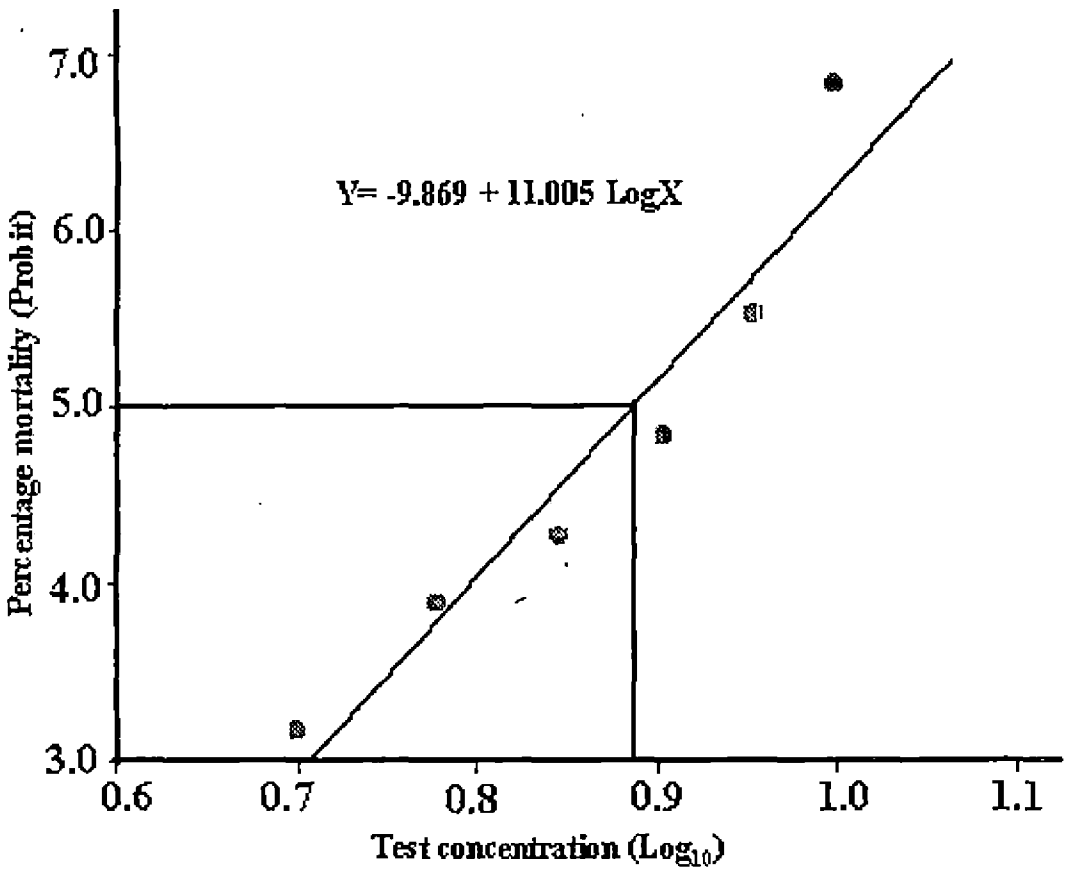

Fig. 2. 48-h LC_{50} value of malathion to juveniles of rohu (*L. rohita*).

4.2.2 Methyl parathion

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of methyl parathion is presented in Table 4 and the results of probit analysis in Table 5. The concentration response curve based on probit analysis is represented in Fig. 3.

The calculated 48-h LC_{50} value and its range was 7.340 $mg.l^{-1}$ (7.246 to 7.433 $mg.l^{-1}$).

Table 4. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of methyl parathion.

Treatment	Test conc. ($mg.l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	6.0	0	0	10	03.33 ± 4.71
T2	6.5	20	0	20	13.33 ± 9.43
T3	7.0	40	20	40	33.33 ± 9.43
T4	7.5	70	50	50	56.66 ± 9.43
T5	8.0	90	70	70	76.66 ± 9.43
T6	8.5	100	80	90	90.00 ± 8.16
T7	9.0	100	100	100	100 ± 00
T8	Control	0	0	0	

Table 5. Result of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of methyl parathion.

Exposure period (hrs)	LC_{50} ($mg.l^{-1}$)	95% confidence limit ($mg.l^{-1}$)		Slope (b)	Intercept (a)
		LLC_{50}	ULC_{50}		
48	7.340	7.246	7.433	21.686	-18.773
Regression equation: Probit Y= -18.773 + 21.686 log X					

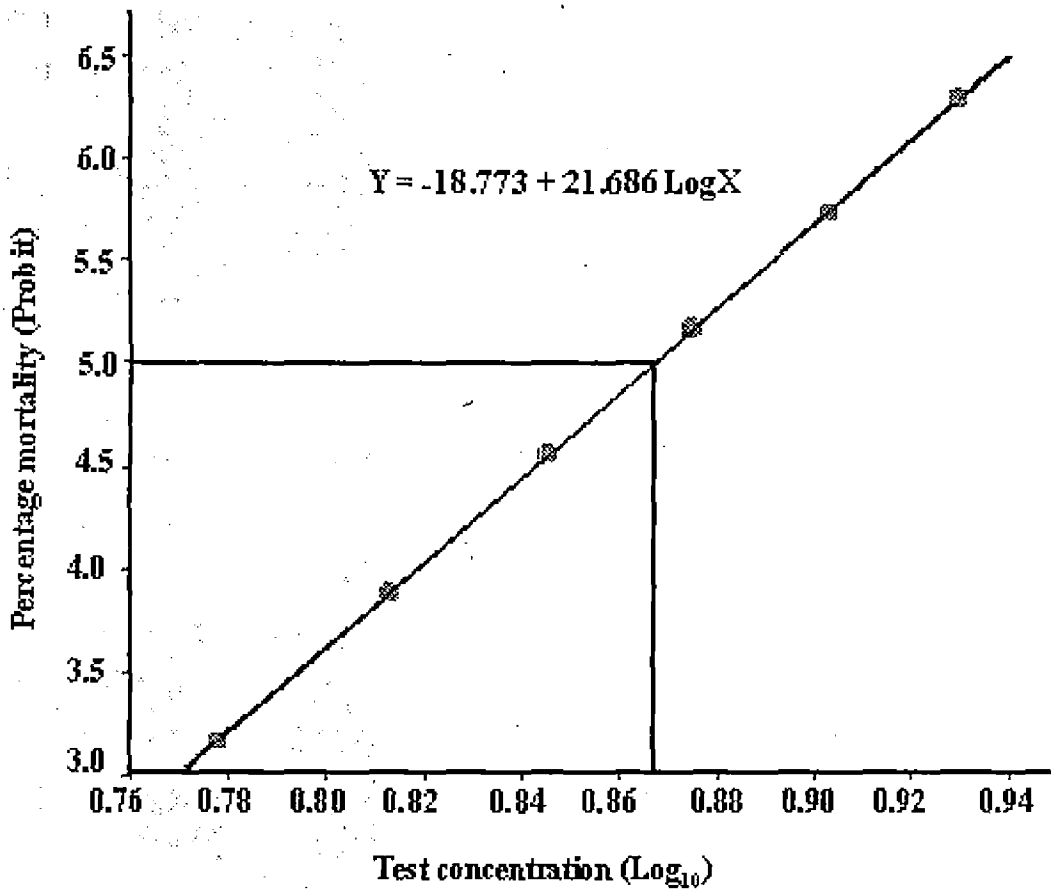

Fig. 3. 48-h LC_{50} value of methyl parathion to juveniles of rohu (*L. rohita*).

4.2.3 Endosulfan

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of endosulfan is presented in Table 6 and the results of probit analysis in Table 7. The concentration response curve based on probit analysis is represented in Fig. 4.

The calculated 48-h LC_{50} value and its range was 0.00355 $mg.l^{-1}$ (0.00251 to 0.00472 $mg.l^{-1}$).

Table 6. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of endosulfan.

Treatment	Test conc. ($mg\ l^{-1}$)	R_1	R_2	R_3	Mean±SD
T1	0.0010	10	20	0	10.00 ± 8.16
T2	0.0025	30	40	30	33.33 ± 4.71
T3	0.0050	50	70	50	56.66 ± 9.43
T4	0.0075	80	80	70	76.66 ± 4.71
T5	0.0100	100	100	90	96.66 ± 4.71
T6	0.0250	100	100	100	100.00 ± 0
T7	Control	0	0	0	0

Table 7. Result of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of endosulfan.

Exposure period (hrs)	LC_{50} ($mg.l^{-1}$)	95% confidence limit ($mg.l^{-1}$)		Slope (b)	Intercept (a)
		LLC_{50}	ULC_{50}		
48	0.00355	0.00251	0.00472	2.744	6.724
Regression equation: Probit Y= 6.724 + 2.744 log X					

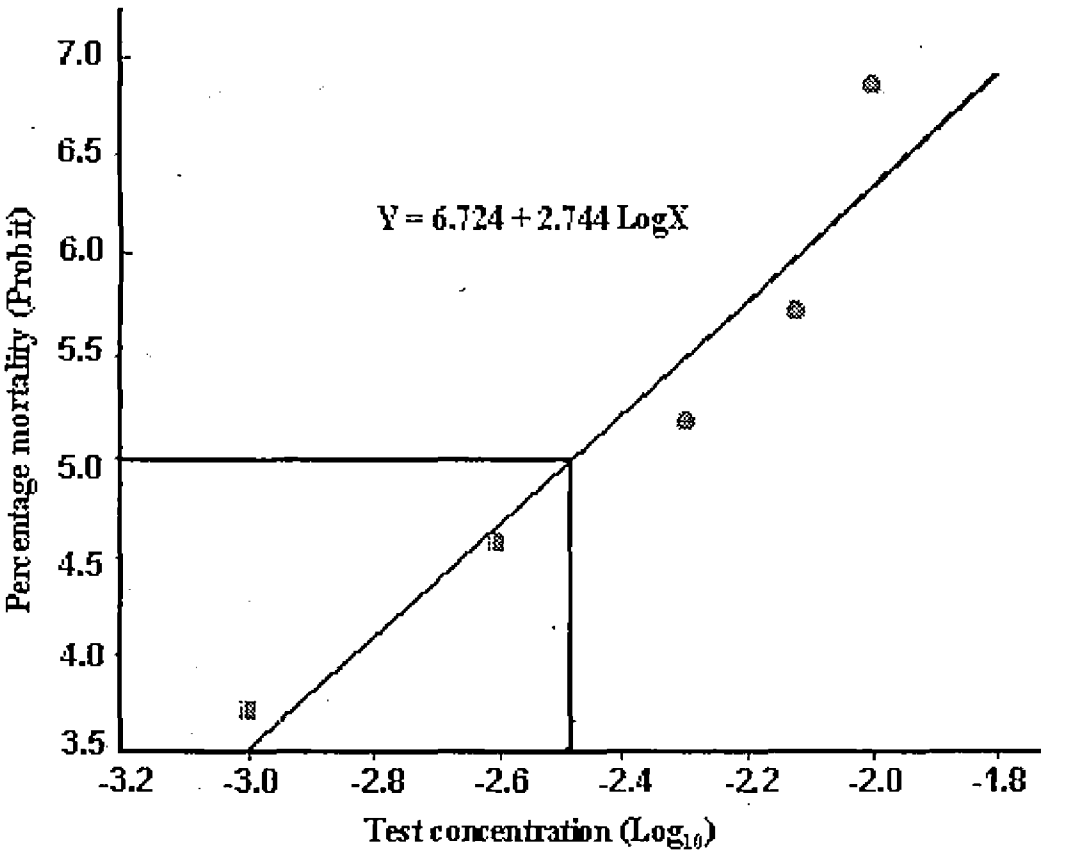

Fig. 4. 48-h LC_{50} value of endosulfan to juveniles of rohu (*L. rohita*).

4.2.4 2,4-D

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of 2,4-D is presented in Table 8 and the results of probit analysis in Table 9. The concentration response curve based on probit analysis is represented in Fig. 5.

The calculated 48-h LC_{50} value and its range was 962.434 $mg.l^{-1}$ (954.016 to 970.806 $mg.l^{-1}$).

Table 8. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D.

Treatment	Test conc. ($mg.l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	850.0	10	0	0	03.33 ± 4.71
T2	900.0	20	10	20	16.66 ± 4.71
T3	950.0	50	20	60	43.33 ± 16.99
T4	1000.0	70	50	80	66.66 ± 12.47
T5	1050.0	90	80	100	90.00 ± 8.16
T6	1100.0	100	100	100	100.00 ± 00
T7	Control	0	0	0	

Table 9. Result of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D.

Exposure period (hrs)	LC_{50} ($mg.l^{-1}$)	95% confidence limit ($mg.l^{-1}$)		Slope (b)	Intercept (a)
		LLC_{50}	ULC_{50}		
48	962.434	954.016	970.806	34.861	104.002
Regression equation : Probit Y= -104.002 + 34.861 log X					

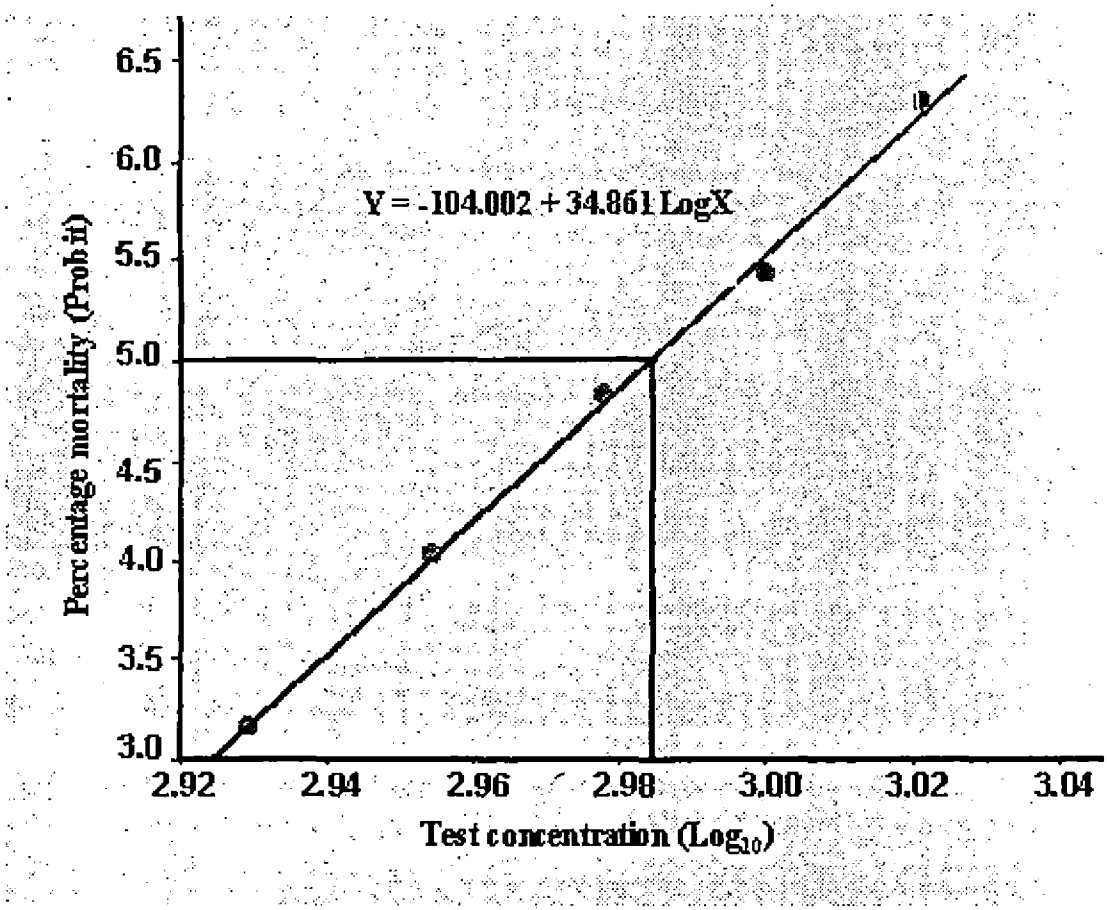

Fig. 5. 48-h LC_{50} value of 2,4-D to juveniles of rohu (*L. rohita*).

4.3 COMBINED LETHAL TOXICITY

4.3.1 2,4-D-endosulfan combination

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of 2,4-D-endosulfan combination is compiled in Table 10 and the results of probit analysis in Table 11. The concentration response curves based on probit analysis for the individual toxicants in mixture are represented graphically in Fig. 6a, Fig. 6b.

The calculated 48-h LC_{50} value of 2,4-D in the mixture was 420.167 $mg.l^{-1}$ (408.060 to 431.809 $mg.l^{-1}$). The calculated 48-h LC_{50} value of endosulfan in the mixture was 0.00226 $mg.l^{-1}$ (0.00208 to 0.00243 $mg.l^{-1}$).

The results of 48-h LC_{50} values and their 95% confidence limits (individually and in combination), range of the sum of biological activity and the range of additive index values are given in Table 12.The sum of biological activity and its range are represented in Fig. 7.

The calculated value of sum of biological index was 1.073 and the range was 0.861 to 1.421. The calculated value of additive index was – 0.073 and the range was – 0.042 to + 0.161.

Table 10. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D and endosulfan.

Treatment	Test conc. (2,4-D and endosulfan) ($mg.l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	190.0 + 0.001	0	0	0	0 ± 0
T2	285.0 + 0.0015	0	10	0	03.33 ± 4.71
T3	380.0 + 0.002	30	40	30	33.33 ± 4.71
T4	450.0 + 0.0025	50	70	50	56.66 ± 9.43
T5	500.0 + 0.00275	70	90	80	80.00 ± 8.16
T6	570.0 + 0.003	90	100	100	96.60 ± 4.71
T7	Control	0	0	0	0 ± 0

Table 11. Results of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D and endosulfan.

Exposure period (hrs)	Toxicant	LC_{50} ($mg\ l^{-1}$)	95% confidence limit ($mg\ l^{-1}$)		Slope (b)	Intercept (a)
			LLC_{50}	ULC_{50}		
48	2,4-D	420.167	408.060	431.809	11.455	-30.052
	Regression equation : Probit Y= -30.052 + 11.455 log X					
	Endosulfan	0.00226	0.00208	0.00243	10.532	27.866
	Regression equation : Probit Y= 27.866 + 10.532 log X					

Table 12. Range of biological activity (S) and the range of additive index for 2,4-D- endosulfan combination in rohu (*L. rohita*) juveniles.

Toxicant	48-h LC_{50} 95% confidence limits($mg.l^{-1}$)		'S' value (range)	Additive index (range)
	Individually	In combination		
2,4-D	962.434 (954.016 to 970.806)	420.167 (408.060 to 431.809)	1.073 (0.861 to 1.421)	-0.073 (-0.042 to 0.1614)
Endosulfan	.00355 (0.00251 to 0.00472)	0.00226 (0.00208 to 0.00243)		

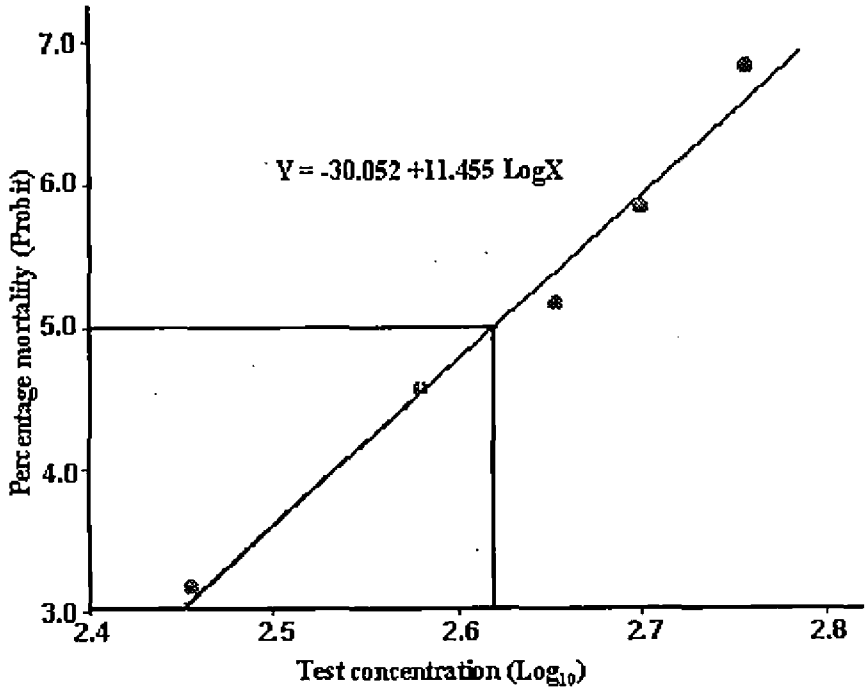

Fig. 6a. 48-h LC_{50} value of 2,4-D to juveniles of rohu (*L. rohita*) in the 2,4-D-endosulfan combination.

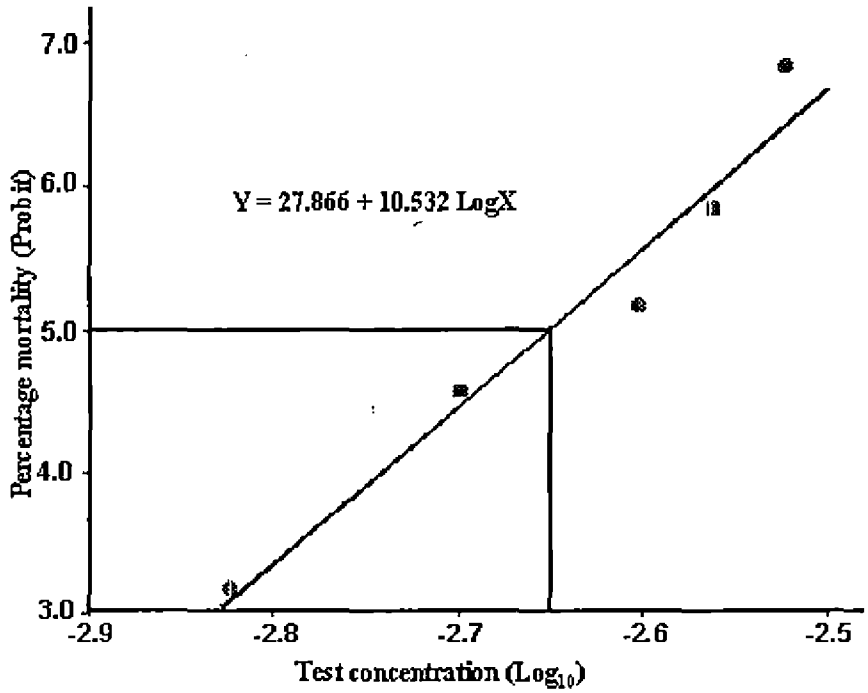

Fig. 6b. 48-h LC_{50} value of endosulfan to juveniles of rohu (*L. rohita*) in the 2,4-D-endosulfan combination.

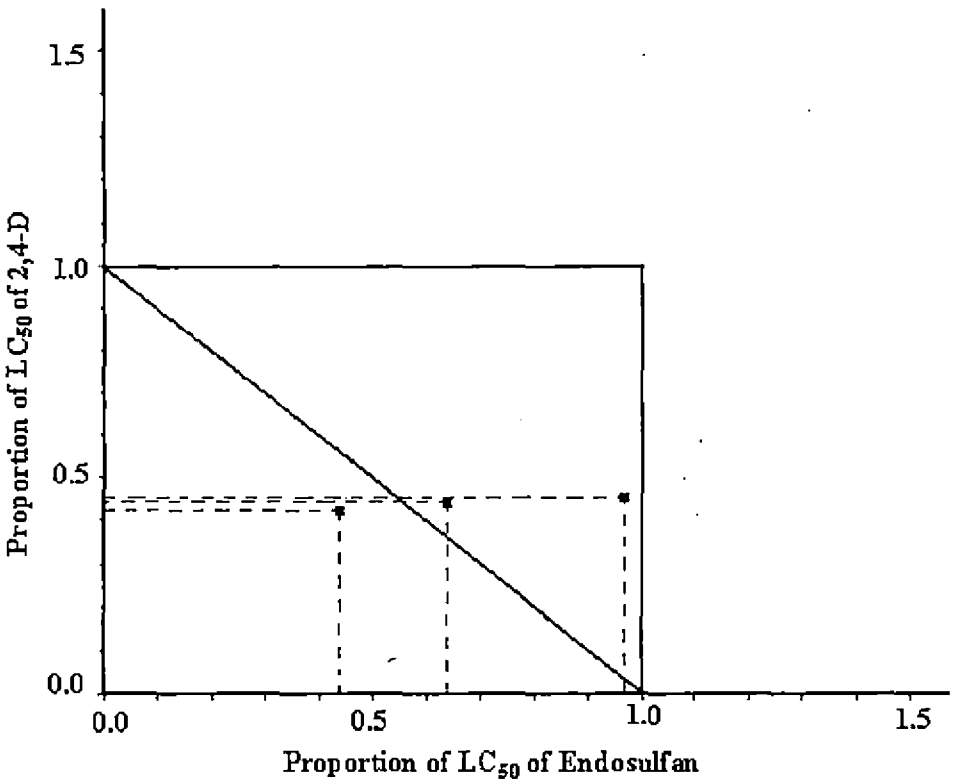

Fig. 7. The sum of biological activity and its range for the 2,4-D – endosulfan combination.

4.3.2 2,4-D-malathion combination

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of 2,4-D-malathion combination is compiled in Table 13 and the results of probit analysis in Table 14. The concentration response curves based on probit analysis for the individual toxicants in mixture are represented graphically in Fig. 8a, Fig. 8b.

The calculated 48-h LC_{50} value of 2,4-D in the mixture was 483.484 $mg.l^{-1}$ (474.955 to 491.941 $mg.l^{-1}$). The calculated 48-h LC_{50} value of malathion in the mixture was 4.069 $mg.l^{-1}$ (3.997 to 4.141 $mg.l^{-1}$).

The results of 48-h LC_{50} values and their 95% confidence limits (individually and in combination), range of the sum of biological activity and the range of additive index values are given in Table 11.The sum of biological activity and its range are represented in Fig. 9.

The calculated value of sum of biological index was 1.018 and the range was 0.954 to 1.089. The calculated value of additive index was – 0.018 and the range was – 0.0895 to + 0.0487.

Table 13. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D and malathion.

Treatments	Test conc. (2,4-D and malathion) ($mg\ l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	380.0 + 3.2	10	0	0	03.33 ± 4.71
T2	430.0 + 3.6	30	20	20	23.33 ± 4.71
T3	476.0 + 4.0	60	40	30	43.33 ± 12.47
T4	520.0 + 4.4	80	70	50	66.66 ± 12.47
T5	570.0 + 4.8	100	90	80	90.00 ± 8.16
T6	620.0 + 5.2	100	100	90	96.60 ± 4.71
T7	Control	0	0	0	0 ± 0

Table 14. Results of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D and malathion.

Exposure period (hrs)	Toxicant	LC_{50} ($mg.l^{-1}$)	95% confidence limit ($mg.l^{-1}$)		Slope (b)	Intercept (a)
			LLC_{50}	ULC_{50}		
48	2,4-D	483.484	474.955	491.941	16.631	-44.644
	Regression equation : Probit Y= -44.644 + 16.631 log X					
	Malathion	4.069	3.997	4.141	16.441	-10.021
	Regression equation : Probit Y= -10.021 + 16.441 log X					

Table 15. Range of biological activity (S) and the range of additive index for 2,4-D- malathion combination in rohu (*L. rohita*) juveniles.

Toxicant	48 h LC_{50} 95% confidence limits ($mg.l^{-1}$)		'S' value (range)	Additive index (range)
	Individually	In combination		
2,4-D	962.434 (954.016 to 970.806)	483.484 (474.955 to 491.941)	1.018 (0.954 to 1.089)	-0.018 (-0.0895 to 0.0487)
Malathion	7.885 (7.279 to 8.607)	4.069 (3.997 to 4.141)		

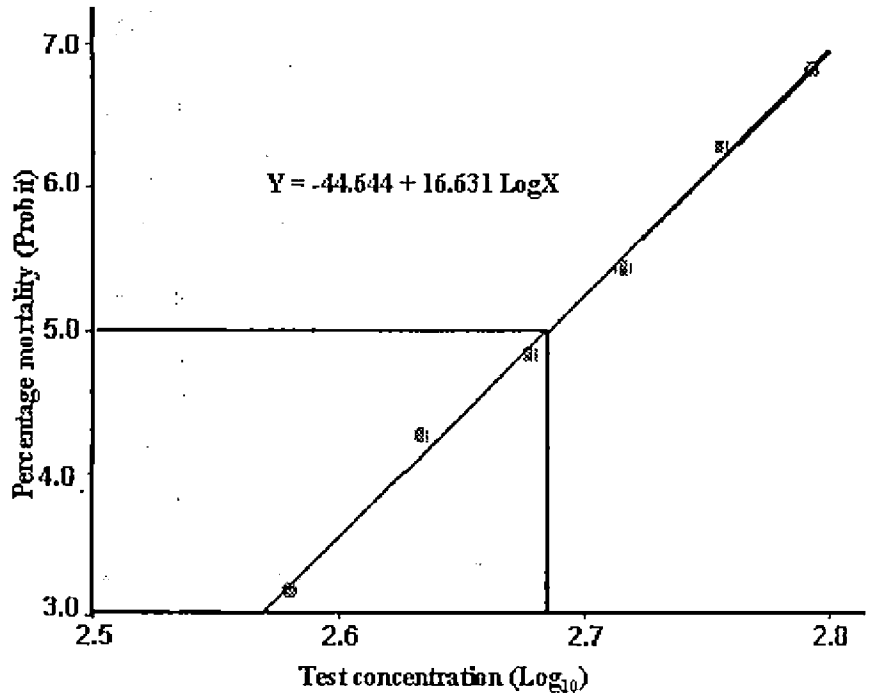

Fig. 8a. 48-h LC_{50} value of 2,4-D to juveniles of rohu (*L. rohita*) in the 2,4-D-malathion combination.

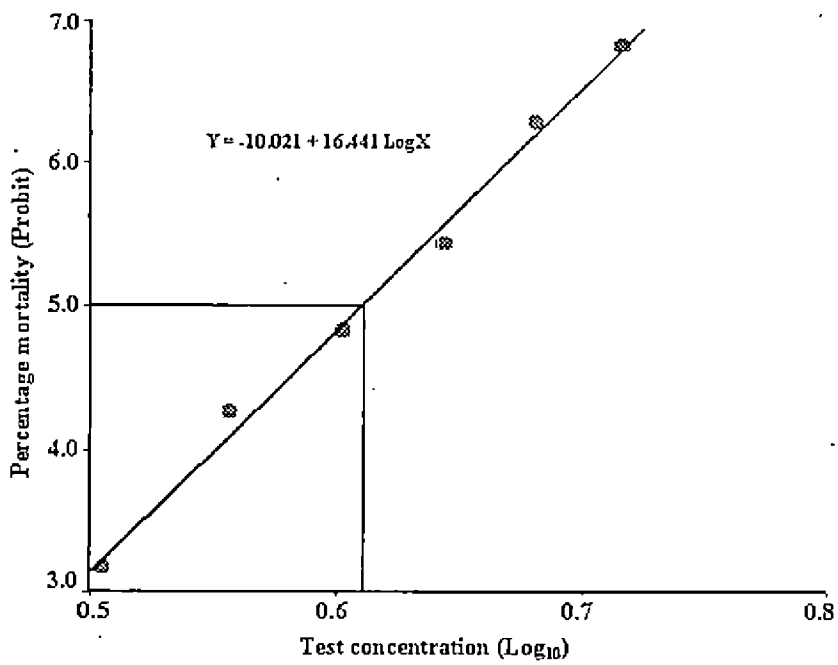

Fig. 8b. 48-h LC_{50} value of malathion to juveniles of rohu (*L. rohita*) in the 2,4-D-malathion combination.

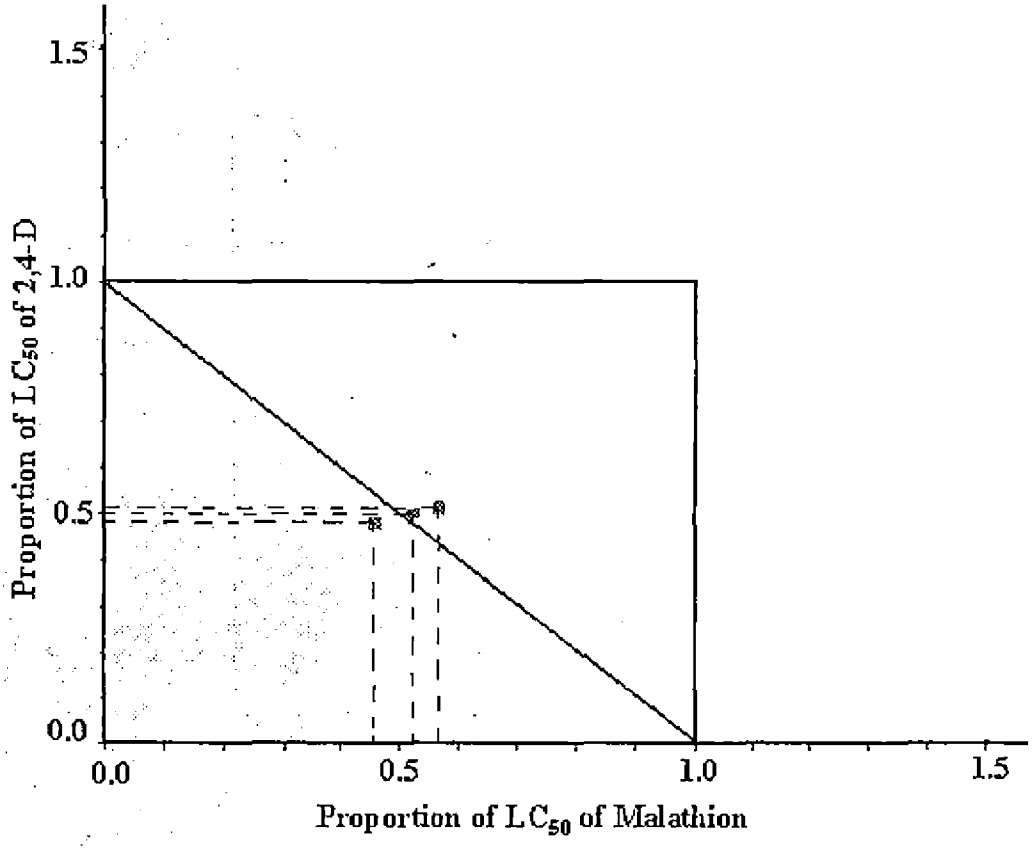

Fig. 9.The sum of biological activity and its range for the 2,4-D malathion combination.

4.3.3 2,4-D-methyl parathion combination

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of 2,4-D-methyl parathion combination is compiled in Table 16 and the results of probit analysis in Table 17. The concentration response curves based on probit analysis for the individual toxicants in mixture are represented graphically in Fig. 10a, Fig. 10b.

The calculated 48-h LC_{50} value of 2,4-D in the mixture was 389.884 $mg.l^{-1}$ (342.547 to 438.521 $mg.l^{-1}$). The calculated 48-h LC_{50} value of methyl parathion in the mixture was 3.078 $mg.l^{-1}$ (2.704 to 3.462 $mg.l^{-1}$).

The results of 48-h LC_{50} values and their 95% confidence limits (individually and in combination), range of the sum of biological activity and the range of additive index values are given in Table 18.The sum of biological activity and its range are represented in Fig. 11.

The calculated value of sum of biological index was 0.824 and the range was 0.717 to 1.024. The calculated value of additive index was 0.218 and the range was – 0.24 to + 0.395.

Table 16. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D and methyl parathion.

Treatment	Test conc. (2,4-D and methyl parathion)($mg\ l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	190.0 + 1.50	10	0	10	06.66 ± 4.71
T2	285.0 + 2.25	30	10	20	20.00 ± 8.16
T3	380.0 + 3.00	50	30	40	40.00 ± 8.16
T4	475.0 + 3.75	70	50	70	63.33 ± 9.47
T5	570.0 + 4.50	100	70	90	86.67 ± 12.5
T6	665.0 + 5.25	100	90	100	96.60 ± 4.71
T7	Control	0	0	0	0 ± 0

Table 17. Results of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of 2,4-D and methyl parathion.

Exposure period (hrs)	Toxicant	LC_{50} (mg l^{-1})	95% confidence limit (mg l^{-1})		Slope (b)	Intercept (a)
			LLC_{50}	ULC_{50}		
48	2,4-D	389.884	342.547	438.521	5.978	-15.49
	Regression equation : Probit Y= -15.49 + 5.978 log X					
	Methyl parathion	3.078	2.704	3.462	5.978	-2.919
	Regression equation : Probit Y= -2.119 + 5.978 log X					

Table 18. Range of biological activity (S) and the range of additive index for 2,4-D- methyl parathion combination in rohu (*L. rohita*) juveniles.

Toxicant	48 h LC_{50} 95% confidence limits (mg l^{-1})		'S' value (range)	Additive index (range)
	Individually	In combination		
2,4-D	962.434 (954.016 to 970.806)	389.884 (342.547 to 438.521)	0.824 (0.717 to 1.024)	0.218 (-0.024 to 0.395)
Methyl parathion	7.34 (7.246 to 7.433)	3.078 (2.704 to 3.462)		

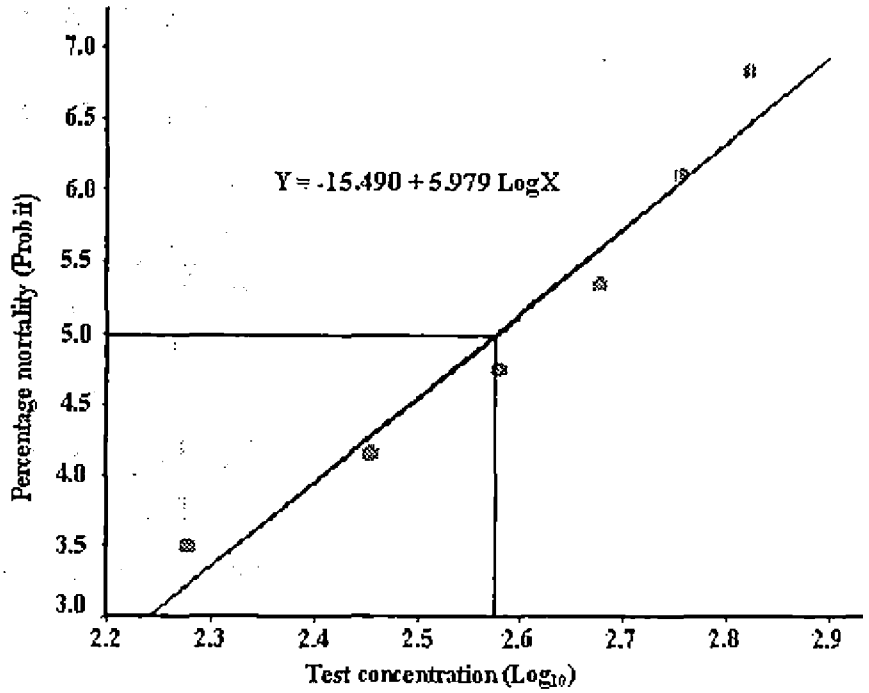

Fig. 10a. 48-h LC_{50} value of 2,4-D to juveniles of rohu (*L. rohita*) in the 2,4-D-methyl parathion combination.

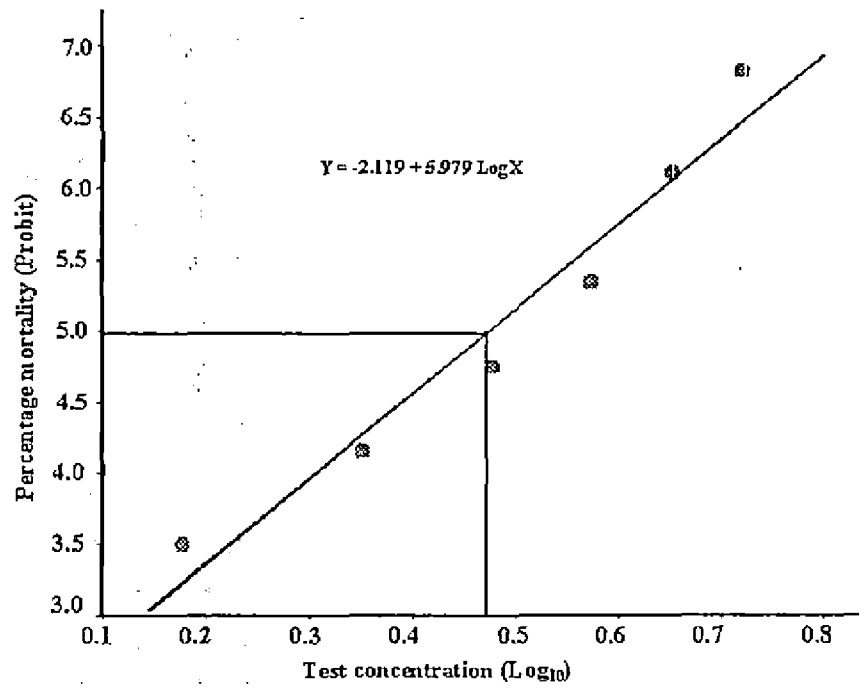

Fig. 10b. 48-h LC_{50} value of methyl parathion to juveniles of rohu (*L. rohita*) in the 2,4-D- combination.

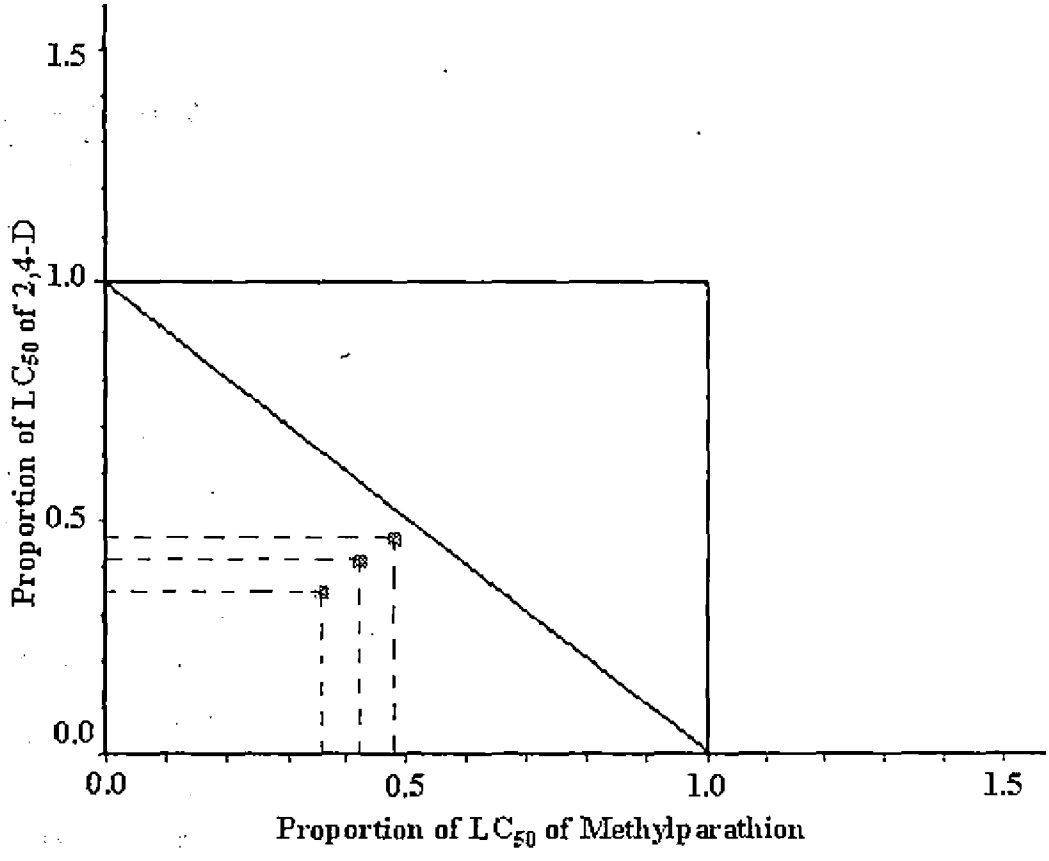

Fig. 11. The sum of biological activity and its range for the 2,4-D – methyl parathion combination.

4.3.4 Endosulfan-malathion combination

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of endosulfan-malathion combination is compiled in Table 19 and the results of probit analysis in Table 20. The concentration response curves based on probit analysis for the individual toxicants in mixture are represented graphically in Fig. 12a, Fig. 12b.

The calculated 48-h LC_{50} value of endosulfan in the mixture was 0.00078 $mg.l^{-1}$ (0.00063 to 0.00093 $mg.l^{-1}$). The calculated 48-h LC_{50} value of malathion in the mixture was 1.245 $mg.l^{-1}$ (1.013 to 1.483 $mg.l^{-1}$).

The results of 48-h LC_{50} values and their 95% confidence limits (individually and in combination), range of the sum of biological activity and the range of additive index values are given in Table 21.The sum of biological activity and its range are represented in Fig. 13.

The calculated value of sum of biological index was 0.378 and the range was 0.251 to 0.574. The calculated value of additive index was 1.648 and the range was 0.741 to 2.981.

Table 19. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of endosulfan and malathion.

Treatment	Test conc. (endosulfan and malathion) ($mg\ l^{-1}$)	R_1	R_2	R_3	Mean ± SD
T1	0.00025 + 0.4	0	10	0	03.33 ± 4.71
T2	0.0005 + 0.8	20	30	10	20.00 ± 8.61
T3	0.00075 + 1.2	40	50	30	40.00 ± 8.61
T4	0.001 + 1.6	60	80	40	60.00 ± 16.3
T5	0.00125 + 2.0	90	100	70	86.60 ± 12.5
T6	0.0015 + 2.4	100	100	90	96.60 ± 4.71
T7	Control	0	0	0	0±0

Table 20. Results of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of endosulfan and malathion.

Exposure period (hrs)	Toxicant	LC_{50} (mg l^{-1})	95% confidence limit (mg l^{-1})		Slope (b)	Intercept (a)
			LLC_{50}	ULC_{50}		
48	Endosulfan	0.0078	0.00063	0.00093	4.614	14.343
	Regression equation : Probit Y= 14.343 + 4.614 log X					
	Malathion	1.245	1.013	1.484	4.614	-0.439
	Regression equation : Probit Y= -0.439 + 4.614 log X					

Table 21. Range of biological activity (S) and the range of additive index for endosulfan-malathion combination in rohu (*L. rohita*) juveniles.

Toxicant	48 h LC_{50} 95% confidence limits (mg l^{-1})		'S' value (range)	Additive index (range)
	Individually	In combination		
Endosulfan	0.00355 (0.00251 to 0.00472)	0.00078 (0.00063 to 0.00093)	0.378 (0.251 to 0.574)	1.648 (0.741 to 2.981)
Malathion	7.885 (7.729 to 8.607)	1.245 (1.013 to 1.483)		

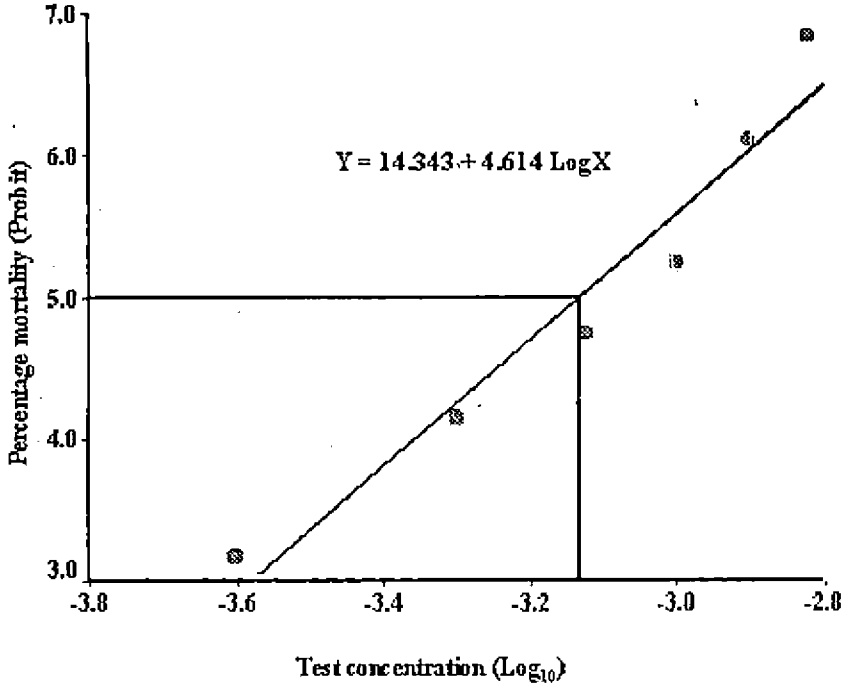

Fig. 12a. 48-h LC_{50} value of endosulfan to juveniles of rohu (*L. rohita*) in the endosulfan- malathion combination.

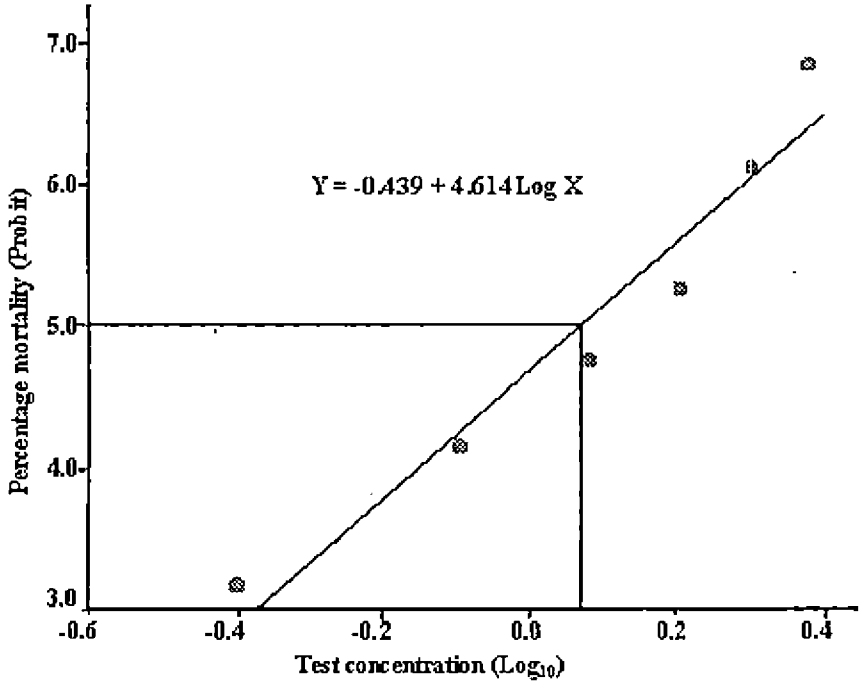

Fig. 12b. 48-h LC_{50} value of malathion to juveniles of rohu (*L. rohita*) in the endosulfan-malathion combination.

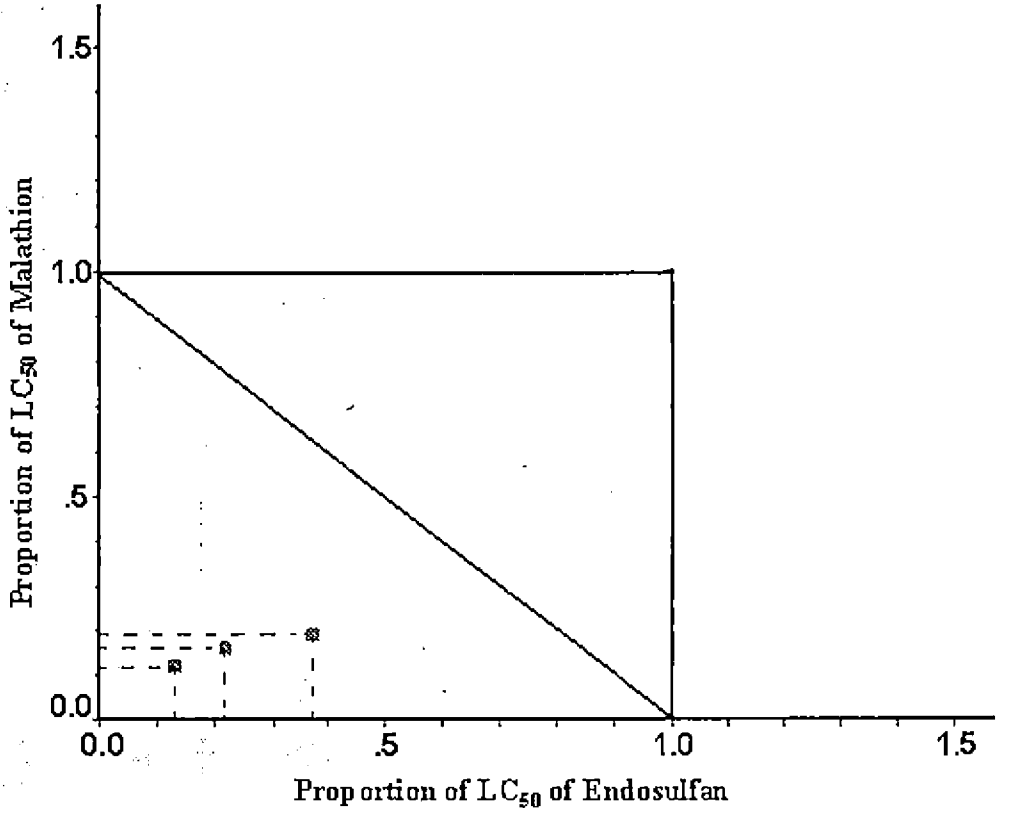

Fig. 13.The sum of biological activity and its range for the endosulfan-malathion combination.

4.3.5 Malathion-methyl parathion combination

The mean percentage mortality with SD values, of rohu juveniles during 48-h exposure to various concentrations of malathion-methyl parathion combination is compiled in Table 22 and the results of probit analysis in Table 23. The concentration response curves based on probit analysis for the individual toxicants in mixture are represented graphically in Fig. 14a, Fig. 14b.

The calculated 48-h LC_{50} value of malathion in the mixture was 3.18 $mg.l^{-1}$ (2.703 to 3.668 $mg.l^{-1}$). The calculated 48-h LC_{50} value of methyl parathion in the mixture was 2.981 $mg.l^{-1}$ (2.534 to 3.439 $mg.l^{-1}$).

The results of 48-h LC_{50} values and their 95% confidence limits (individually and in combination), range of the sum of biological activity and the range of additive index values are given in Table 23. The sum of biological activity and its range are represented in Fig. 15.

The calculated value of sum of biological index was 0.809 and the range was 0.655 to 0.979. The calculated value of additive index was 0.235 and the range was 0.022 to 0.527.

Table 22. Mean percentage mortality based on three replicates, with SD values, of rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of malathion and methyl parathion.

Treatment	Test conc. (malathion and methyl parathion)(mg l^{-1})	R_1	R_2	R_3	Mean ± SD
T1	1.6 + 1.50	10	20	0	10.00 ± 8.16
T2	2.4 + 2.25	20	40	20	26.66 ± 9.43
T3	3.2 + 3.00	40	70	40	50.00 ± 14.1
T4	4.0 + 3.75	70	70	60	66.66 ± 4.17
T5	4.8 + 4.50	80	80	70	71.66 ± 6.87
T6	5.6 + 5.25	100	100	90	96.60 ± 4.71
T7	Control	0	0	0	0 ± 0

Table 23. Results of probit analysis for rohu (*L. rohita*) juveniles during 48-h exposure to various concentrations of malathion and methyl parathion.

Exposure period (hrs)	Toxicant	LC_{50} (mgl^{-1})	95% confidence limit ($mg\ l^{-1}$)		Slope (b)	Intercept (a)
			Lower	ULC_{50}		
48	Malathion	3.18	2.703	3.668	4.765	-2.394
	Regression equation : Probit Y= -2.394 + 4.765 log X					
	Methyl parathion	2.981	2.534	3.349	4.765	-2.26
	Regression equation : Probit Y= -2.26 + 4.765 log X					

Table 24. Range of biological activity (S) and the range of additive index for malathion-methyl parathion combination in rohu (*L. rohita*) juveniles.

Toxicant	48 h LC_{50} 95% confidence limit($mg\ l^{-1}$)		'S' value (range)	Additive index (range)
	Individually	In combination		
Malathion	7.885 (7.729 to 8.607)	3.18 (2.703 to 3.668)	0.809 (0.655 to 0.979)	0.235 (0.022 to 0.527)
Methyl parathion	7.34 (7.246 to 7.433)	2.981 (2.534 to 3.439)		

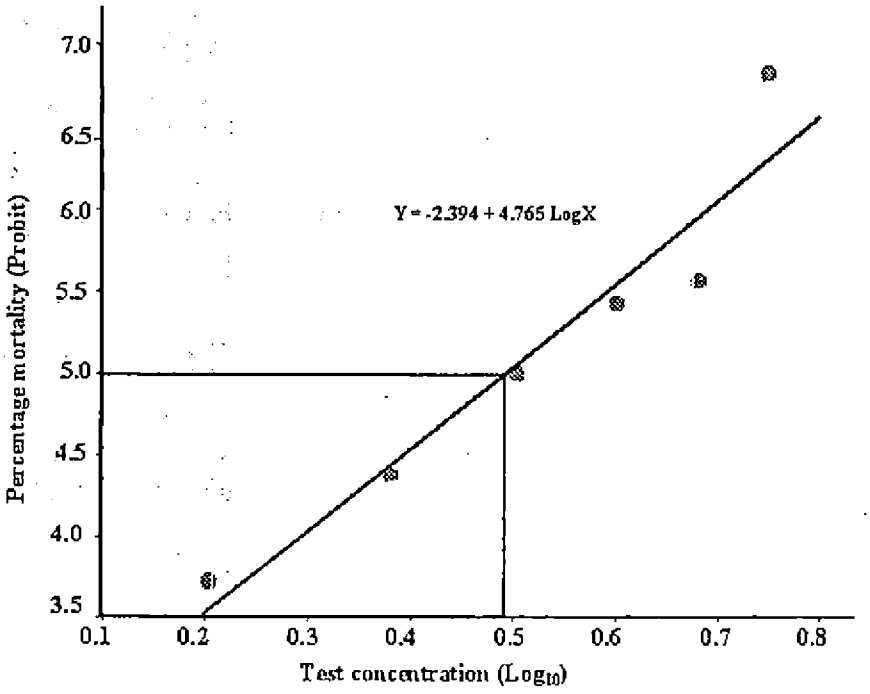

Fig. 14a. 48-h LC_{50} value of malathion to juveniles of rohu (*L. rohita*) in the malathion-methyl parathion combination.

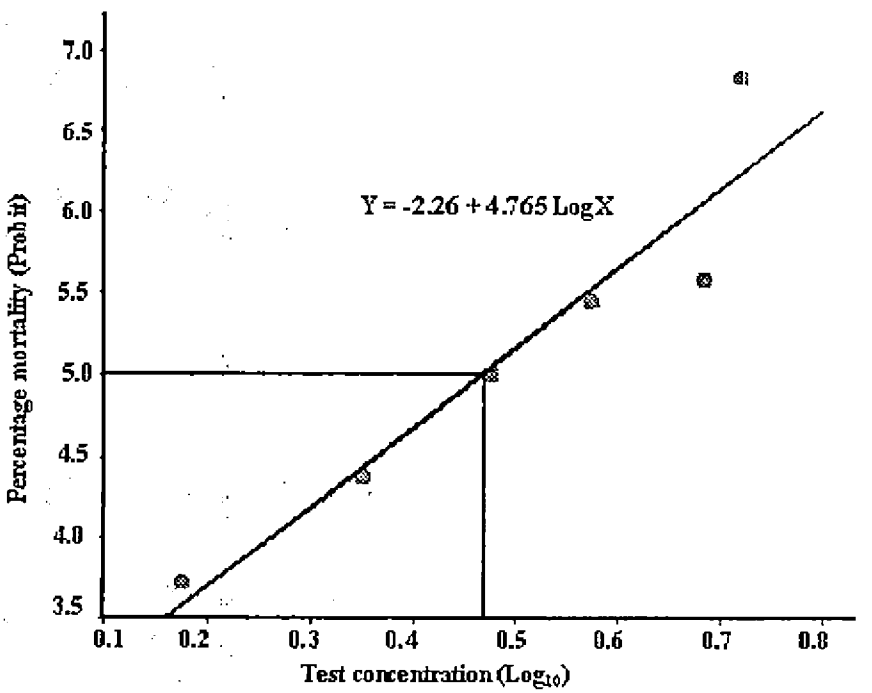

Fig.14b. 48-h LC_{50} value of methyl parathion to juveniles of rohu (*L.rohita*) in the malathion-methyl parathion combination.

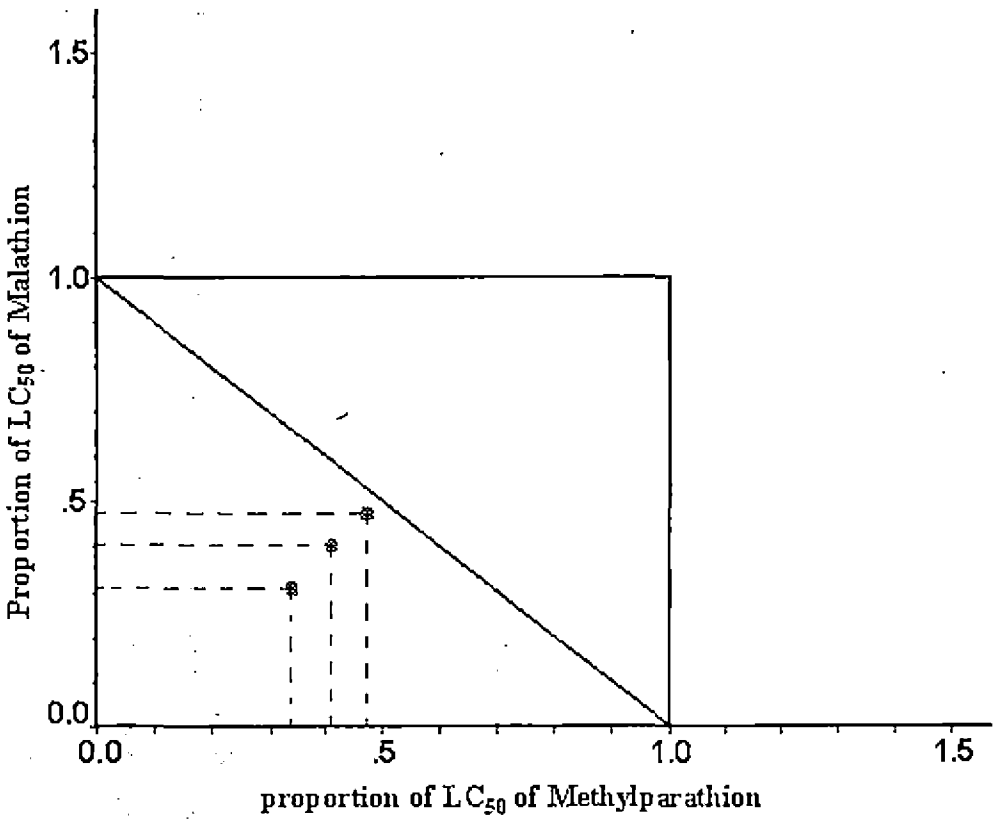

Fig. 15. The sum of biological activity and its range for the malathion – methyl parathion combination.

4.4 SUMMARISED RESULTS

The 48-h LC_{50} values of four pesticides (individual) on rohu juveniles to show the grade of toxicity are given in Table 25. The 48-h LC_{50} values of five pesticide pairs to show the additive toxicity are given in Table 26.

The additive index ranges for the five combinations are represented in Fig. 16.

Table 25. 48-h LC_{50} based on static renewal test of selected pesticides (individual) to rohu juveniles to show the grade of toxicity.

Sl. No.	Toxicant (Pesticides)	Compound	48-h LC_{50} (Range)	Grade of toxicity (Sprague, 1973)
1.	Endosulfan (Insecticide)	Organochlorine	0.0036 $mg.l^{-1}$ (0.0025 to 0.0047)	"Very toxic" (Below 1 $mg.l^{-1}$)
2.	Methyl parathion (Insecticide)	Organophosphate	7.34 $mg.l^{-1}$ (7.25 to 7.43)	"Toxic" (1 to100 $mg.l^{-1}$)
3.	Malathion (Insecticide)	Organophosphate	7.89 $mg.l^{-1}$ (7.38 to 8.61)	"Toxic" (1 to100 $mg.l^{-1}$)
4.	2,4-D (Herbicide)	Chlorophenoxy compound	962.43 $mg.l^{-1}$ (954.02 to 970.81)	"Moderately toxic" (100 to1000 $mg.l^{-1}$)

Table 26. 48-h LC_{50} based on static renewal test of selected pesticide pairs on the juveniles of rohu to show the additive toxicity.

SL. No.	Pesticide combination	'S' value (Range)	Additive index (Range)	Additivity (Median Magnification factor)
1.	2,4-D and malathion	1.018 (0.95 to 1.09)	-0.018 (-0.09 to 0.05)	Strictly additive (x0.98)
2.	2,4-D and methyl parathion	0.824 (0.72 to 1.02)	0.218 (-0.02 to 0.40)	Strictly additive (x1.22)
3.	2,4-D and endosulfan	1.073 (0.86 to 1.42)	-0.073 (-0.04 to 0.16)	Strictly additive (x0.93)
4.	Malathion and methyl parathion	0.809 (0.66 to 0.98)	0.235 (0.021 to 0.53)	More than additive (x1.24)
5.	Malathion and endosulfan	0.378 (0.25 to 0.57)	1.648 (0.74 to 2.98)	More than additive (x2.65)

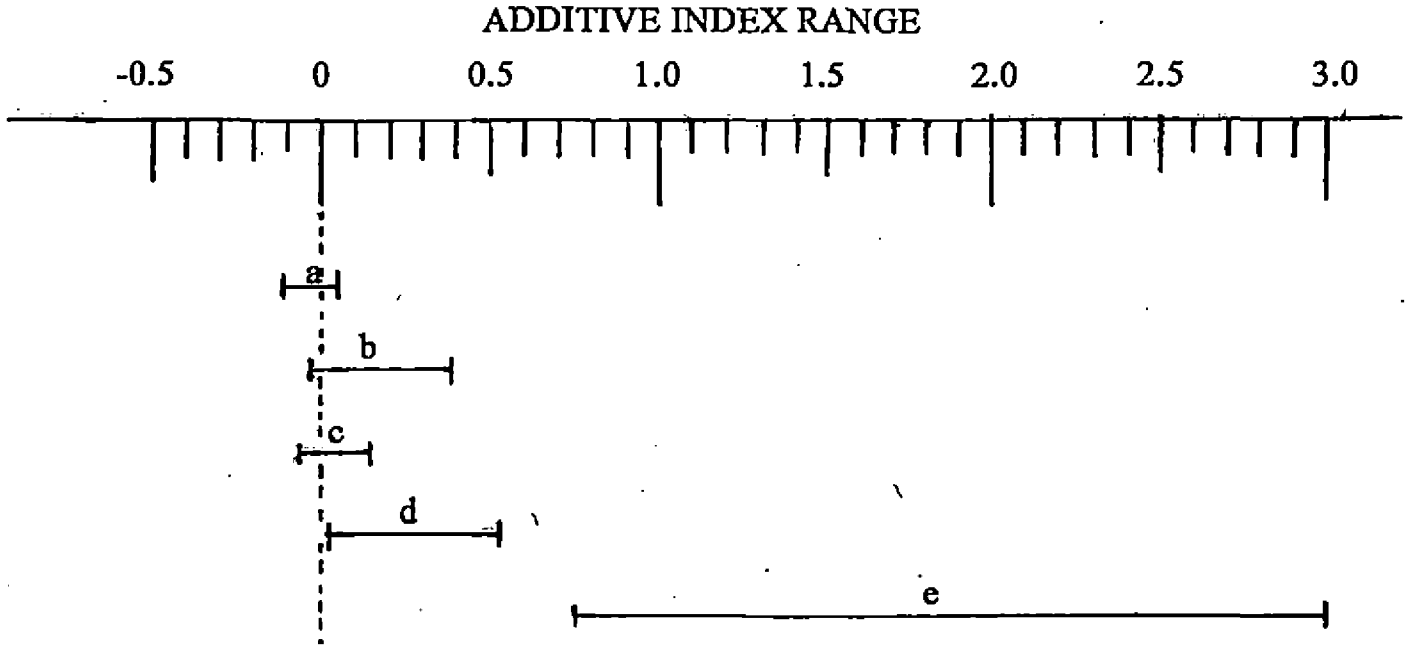

Fig. 16. The additive index range for the five pesticide combinations

Discussion

5. DISCUSSION

5.1 INDIVIDUAL TOXICITY

Acute lethality tests are considered to provide rapid and reproducible concentration response curves for identifying and estimating the effects of chemicals on aquatic organisms. Static acute lethality tests provide the most practical means for (a) deriving estimates of the upper limit of the range of concentration producing toxic effects, (b) evaluating the relative toxicity of a large number of chemicals and relative response of test animals and (c) for evaluating the combined toxicity of chemicals (Macek *et al.*, 1978).

5.1.1 Endosulfan

The calculated 48-h LC_{50} of endosulfan to juveniles of rohu is 0.0036 $mg.l^{-1}$ (0.0025 – 0.0047 $mg.l^{-1}$). Das and Mukherjee (2002) reported the 48-h LC_{50} to larger juveniles of rohu as 0.015 $mg.l^{-1}$. The 48-h LC_{50} to rainbow trout, a sensitive species was reported to be 0.001 $mg.l^{-1}$ (Pimentel, 1971). The toxicity of endosulfan to several species of freshwater fish (*Channa punctata*, *Cirrhinus mrigala* and three species of catfishes) was in the range of 0.002 to 0.0081 $mg.l^{-1}$ (Devi *et al.*, 1981; Swarup *et al.*, 1981 and Rao and Murty, 1982). The United States Environmental Protection Agency (EPA, 1980) in its report on ambient water quality criteria for endosulfan reported that the 96-h LC_{50} for salt-water fishes ranged from 0.0003 to 0.0029 $mg.l^{-1}$. Naqvi and Hawkins (1988) exposed mosquitofish (*Gambusia affinis*) to endosulfan and the 96-h LC_{50} value was found to be 0.0013 $mg.l^{-1}$. Jagan *et al.* (1989) reported the 48-h LC_{50} of endosulfan to common carp (*Cyprinus carpio*) juveniles was 0.0336 $mg.l^{-1}$. Schimmel *et al.* (1976) reported the 96-h

LC_{50} of heptachlor, another cyclodiene (high toxic insecticide) to be 0.001 to 0.004 $mg.l^{-1}$, for several species of estuarine fish.

The 96-h LC_{50} of endrin to various freshwater fishes ranged from 0.0007 to 0.0021 $mg.l^{-1}$ (Henderson *et al.*, 1959). The 96-h LC_{50} of chlordane to pinfish and sheepshead minnow was 0.0064 and 0.025 $mg.l^{-1}$ respectively (Parrish *et al.*, 1976)

The result of the present study compares well with those available for endosulfan and other cyclodiene organochlorines. Endosulfan can be classified as 'very toxic' according to the classification of Sprague (1973) where the acute lethal threshold is below 1.0 $mg.l^{-1}$.

5.1.2 Malathion and methyl parathion

In the present study the organophosphate insecticides malathion and methyl parathion showed almost the same 48-h LC_{50} values to juveniles of rohu. The 48-h LC_{50} of malathion is 7.885 $mg.l^{-1}$ (7.279 to 8.607 $mg.l^{-1}$) and that of methyl parathion is 7.34 $mg.l^{-1}$ (7.246 to 7.443 $mg.l^{-1}$) being marginally more toxic.

The 96-h LC_{50} of methyl parathion and malathion to gold fish is reported as 9.0 $mg.l^{-1}$and 10.7 $mg.l^{-1}$; to fathead minnows as 8.9 $mg.l^{-1}$and 8.6 $mg.l^{-1}$; to common carp as 7.1 $mg.l^{-1}$ and 6.6 $mg.l^{-1}$; to channel cat fish as 5.7 $mg.l^{-1}$ and 9 $mg.l^{-1}$ and bullhead as 6.6 $mg.l^{-1}$and 12.9 $mg.l^{-1}$ respectively (Macek and McAllister, 1970). Pimental (1971) reported the 48-h LC_{50} of methyl parathion and malathion to rainbow trout to be 2.75 $mg.l^{-1}$and 0.196 $mg.l^{-1}$ respectively. Arora *et al.* (1971) found rohu to be the most resistant of the four carps tried, the 24 and 96-h LC_{50} value for malathion being 7.15 $mg.l^{-1}$ and 5.05 $mg.l^{-1}$ respectively. Das and Mukherjee (2002) reported the 48-h LC_{50} of malathion to larger juveniles of rohu as 20.13 $mg.l^{-1}$. Jagan *et al.* (1989) reported the 48-h LC_{50} of malathion to common carp juveniles as 0.138 $mg.l^{-1}$.

Thus the perusal of literature shows discrepancies regarding the relative toxicity of the two organophosphate insecticides. In the present study they are found to exhibit almost same toxicity to juveniles of rohu. Malathion and methyl parathion can be classified as 'toxic' according to Sprague (1973) classification where the acute lethal threshold is between 1.0 to100.0 $mg.l^{-1}$.

Organophosphates in general show moderate to high acute toxicity, but have negligible chronic toxicity (Murty, 1986 b). In mammals they are known to act primarily as cholinesterase inhibitors (Costa *et al.*, 1990). Moreover several liver enzymes activities are also modified (Costa and Murphy, 1983).

5.1.3 2,4-D

Few of the herbicides have chronic toxicity, and the acute toxicity is also low (Murty, 1986 b). But the problem with herbicides is the very high quantities that have to be initially applied for effective weed control. Also the toxicity of many herbicides to plants is less by several orders of magnitude when compared to animals (Frank, 1972). 2,4-D (dichlorophenoxyacetic acid) is a selective translocated phenoxy herbicide used in wheat, sorghum, com, oats, apples, rice, sugarcane, etc., for weed control. It is also used to control aquatic plants, for bush control and on turf (Thompson, 1982).

The 48-h LC_{50} of 2,4-D on the juveniles of rohu is 962.4 $mg.l^{-1}$ (954.02 to 970.81 $mg.l^{-1}$). Elezovic *et al.* (1994) reported the 48-h LC_{50} of 2,4-D on juveniles of common carp as 295.0 $mg.l^{-1}$ (262.0 to 312.5 $mg.l^{-1}$) at 20 ± 1°C. Nair *et al.* (2000) working with juveniles of pearl spot (*Etroplus suratensis*), a very sensitive species, found the 48-h LC_{50} to be 267.0 $mg.l^{-1}$ (228.9 to 305.9 $mg.l^{-1}$) at 27 ± 1°C. The present study clearly indicates the high tolerance of rohu juveniles to the herbicide. The 48-h LC_{50} of the weedicide glysophate to common carp juveniles is

given as 645.2 $mg.l^{-1}$ (632.5 to 655.0 $mg.l^{-1}$) by Elezovic *et al.* (1994). 2,4-D can be classified as 'moderately toxic' as per the classification of Sprague (1973), where the acute lethal threshold is 100.0 to 1000.0 $mg.l^{-1}$.

In the present study the relative toxicity of the pesticides tried is that endosulfan is 'very toxic', malathion and methyl parathion are 'toxic' and 2,4-D is 'moderately toxic' to rohu juveniles. A comparison with the available literature shows that rohu juveniles are more tolerant to these toxicants. Henderson *et al.* (1959), Pickering *et al.* (1962), Mecek and McAllister (1970) and Eisler (1970) worked with large number of pesticides and fish species. The general conclusion that can be drawn from these studies are (i) the organochlorines are more toxic to fish than organophosphates and carbamates and (ii) the cyprinid species are the least sensitive (more tolerant) of the test species.

5.1.4 Behaviour

The fishes in the toxicants exhibited darting movements, excitations with frequent attempts to leap out of water and later on leading to muscular spasm causing short jerky movements and convulsion. This leads to loss of balance and rolling on to the belly and coming to rest at the bottom and subsequent death. The degree of reaction varied with the four pesticides tried. Holden (1965) states that acute toxicity primarily damages the central nervous system resulting in instability, respiratory difficulties and sluggishness. It is compounded by the fact that the principal route of entry of toxicants for non-feeding fish is via the gills.

5.2 COMBINED TOXICI

It is uncommon to fi. . river or lake or coast, polluted by a single toxicant and usually several harmful substances are present together in

significant quantities. Concentration addition model appears to be adequate to describe the joint-effects of commonly occurring toxicants (Alabaster and Lloyd, 1982). The additive index ranges for the five combinations tested are represented in Fig 16.

5.2.1 Insecticide –weedicide combinations

In the present study the three combinations of the insecticides and the weedicide (2,4-D-endosulfan; 2,4-D-malathion; 2,4-D-methyl parathion) show simple or strictly additive toxicity at 48-h LC_{50} with rohu juveniles. When the additive index value ranges overlap zero, simple or strictly additive toxicity is indicated (Marking, 1977). Nair *et al.* (2000) working with the juveniles of *Etroplus suratensis* (Pearl spot) and the combination of monocrotophos (an organophosphate) and 2,4-D reported simple additive toxicity.

Lichenstein *et al.* (1973) found that interaction between parathion and 2,4-D was more than additive (x3.2) when tested with third instar of mosquito larvae. Similarly Fabacher *et al.* (1976) showed that mortality of mosquitofish (*Gambusia affinis*), in a mixture of methyl parathion and a defoliant, tributyl phosphorotrithioate was several fold more than additive. In the present study among the 2,4-D-insecticide pair, 2,4-D-methyl parathion combination showed the maximum magnification factor (x1.22).

5.2.2 Organophosphate-organophosphate combination

The malathion-methyl parathion combination showed more than additive (x1.24) toxicity with rohu juveniles at 48-h LC_{50}. Marking and Dawson (1975) measured the 96-h LC_{50} of malathion and delvan to *Lepomis macrochirus* and found that joint action was markedly more than additive (x8.2). Bender (1969) found enhanced joint toxicity of malathion and its alkaline hydrolysis product, dimethyl fumarate on

fathead minnow. Combination of quinalphos and phenthoate showed synergistic toxicity to tilapia at 96-h LC_{50} (Durairaj and Selvarajan, 1995). Denton *et al.* (2003) conducted 96-h static renewal test on larvae of fathead minnows with diazinon and esfanvalerate (pyrethroid) to study acetylcholinesterase activity, histopathology and biochemical changes. The combined acute toxicity appeared to be greater than additive (synergistic) in all three tests.

5.2.3 Organochlorine-organophosphate combination

In the case of the endosulfan-malathion pair the combined toxicity to rohu juveniles at 48-h LC_{50} is markedly more than additive (x2.65). When rainbow trout was simultaneously exposed to endosulfan and disulfoton, the acute toxicity concentrations (96-h LC_{50}) were considerably reduced with a combination of the two pesticides, when compared to the 96-h LC_{50} for the single compounds suggesting more than additive toxicity (Arnold and Braunbeck, 1994). They also suspect that synergistic effects may be the cause for large-scale fish kill when disulfoton was spilled into the river Rhine in November 1986. Mirex clearly increased DDT toxicity to larvae of the salt marsh fish *Adinia xenica* (Koenig, 1977). Gill *et al.* (1991) carried out 48-h LC_{50} test to evaluate combined toxicity of endosulfan, phosphamidon and aldicarb to *Puntius conchonius*. Enhanced toxicity was shown when pesticides were in combination rather than as individual compounds.

Ludke (1972) found, with several species of fish, a less than additive toxic effect with mixtures of parathion and aldrin and similar results were obtained by Ferguson and Bringham (1966) with mosquitofish exposed to all possible paired combinations of endrin, DDT, toxaphene and methyl parathion.

5.3 GENERAL

Macek (1975) exposed bluegill (*Lepomis macrochirus*) to 29 different mixtures of pairs of pesticides. He found the average value of the results was slightly more than additive. Marking and Mauck (1975) working with rainbow trout and seven insecticides and 20 combinations found that in nine pairs the joint toxicity was 0.5 to 0.7 times less than additive, in nine others, the response was not significantly different from additive and for the remaining two it was 1.4 to 1.7 times more than additive.

Interestingly Alabaster and Lloyd (1982) concludes "while many data on the acute lethal toxicity of mixtures of pesticide and other substances to fish show that action is close to additive, a relatively high proportion, compared with toxicants commonly found in sewage and industrial wastes, show that it is several fold more than additive".

5.4 CONCLUSION

Individually it is found that 2,4-D is 'moderately toxic', malathion and methyl parathion are 'toxic', and endosulfan is 'very toxic' to juveniles of rohu under static renewal 48-h LC_{50} test. But the 'strictly additive' nature of the insecticide-weedicide combinations and the 'more than additive' (x1.24 and x2.65) nature of the insecticide pairs coupled with the sequential or even simultaneous application of these chemicals in the paddy fields and plantations increase the potential for pollution of these pesticides in the freshwater and coastal ecosystems of the state. Studies on the combined chronic sublethal toxicity of pesticide pairs would throw more light on this subject.

Summary

6. SUMMARY

The present study was made to understand the individual and combined toxicity of malathion, methyl parathion, endosulfan and 2,4-D on the juveniles of rohu *Labeo rohita* (Ham.). The methodology, results and the conclusions of the study are as follows:

1. The 48-h static with renewal (12 hrs) toxicity bioassays were conducted for both the individual and combined toxicity studies.
2. Rohu juveniles, having an average size of 48.39 ± 3.9 mm and 956.47 ± 268.24 mg were used for the experiments. The average animal load factor was around 1.366 $g.l^{-1}$.
3. Fishes were acclimated for 10-14 days under laboratory conditions. They were starved for a period of 24 hrs prior to the experiment and during the experimental 48 hrs.
4. Ten fishes were used in seven litres of water. With each set of treatments a control was also kept. All the treatments were carried out in triplicate.
5. The pesticides used are 2,4-D (chlorophenoxy herbicide), malathion and methyl parathion (organophosphate insecticides) and endosulfan (organochlorine insecticide).
6. The 48-h LC_{50} values were calculated based on the probit analysis method of Finney (1971)
7. The 48-h LC_{50} value of 2,4-D is 962.43 $mg.l^{-1}$ (954.02 to 970.81); malathion is 7.89 $mg.l^{-1}$ (7.28 to 8.61); methyl parathion is 7.34 $mg.l^{-1}$ (7.25 to 7.43) and endosulfan is 0.0036 $mg.l^{-1}$ (0.0025 to 0.0047).
8. Based on the grade of toxicity (as per Sprague, 1973), 2,4-D is 'moderately toxic', malathion and methyl parathion are 'toxic'

and endosulfan is 'very toxic' to juveniles of rohu under static with renewal 48-h LC_{50} test.

9. The combined lethal toxicity tests (48-h LC_{50}) are based on the simple addition (simple similar action) model of Sprague and Ramsey (1965).
10. The additive index values are calculated based on the sum of biological activity as per Marking (1977) to arrive at the mode of additivity.
11. The herbicide-insecticide combinations are found to be 'simply additive' or 'strictly additive' in the combined toxicity.
12. The additive index value of 2,4-D-malathion is -0.018 (-0.09 to 0.50) and the magnification factor is x0.98 (0.91 to 1.05).
13. The additive index value of 2,4-D-methyl parathion is 0.218 (-0.02 to 0.40) and the magnification factor is x1.22 (0.98 to 1.40).
14. The additive index value of 2,4-D-endosulfan is -0.073 (-0.40 to 0.16) and the magnification factor is x0.93 (0.60 to 1.16).
15. The insecticide-insecticide combinations are 'more than additive' in combined toxicity.
16. The additive index value of malathion-methyl parathion (organophosphate-organophosphate) is 0.235 (0.021 to 0.53) and the magnification factor is x1.24 (1.021 to 1.53).
17. The additive index value of malathion-endosulfan (organophosphate-organochlorine) is 1.648 (0.74 to 2.98) and the magnification factor is x2.65 (1.74 to 3.98).
18. Thus the combined toxicity (due to simultaneous or persistent use) of these pesticides have greater potential for pollution in the natural water bodies of the state.
19. Chronic combined sublethal toxicity studies are needed to know more about the intricacies of toxicity of pesticide pairs and mixtures, under tropical conditions.

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INDIVIDUAL AND COMBINED LETHAL TOXICITY OF PESTICIDE COMBINATIONS ON THE JUVENILES OF ROHU *LABEO ROHITA* (HAM.)

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ABSTRACT OF THE THESIS

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ABSTRACT

In the natural aquatic ecosystems, fishes are exposed to more than one biocide or contaminant at a given time. In the present study an attempt is made to understand the individual and combined toxicity of the common biocides- malathion, methyl parathion, endosulfan and 2,4-D on the juveniles of rohu (*Labeo rohita*) under laboratory conditions. The 48-h LC_{50} values were computed based on the probit analysis method of Finney (1971). The 48-h LC_{50} value of malathion was 7.89 $mg.l^{-1}$ (7.28 to 8.61); methyl parathion was 7.34 $mg.l^{-1}$ (7.25 to 7.43); endosulfan was 0.0036 $mg.l^{-1}$ (0.0025 to 0.0047) and 2,4-D was 962.43 $mg.l^{-1}$ (954.02 to 970.81). The 'additive index' values and 'magnification factors' for the combined toxicity were calculated for the different pesticide pairs based on the method of Marking (1977). For 2,4-D-malathion it was –0.018 (-0.09 to 0.50) and x0.98 (0.91 to 1.05) respectively; for 2,4-D-methyl parathion it was 0.218 (-0.02 to 0.40) and x1.22 (0.98 to 1.40) respectively; for 2,4-D-endosulfan it was –0.073 (-0.40 to 0.16) and x0.93 (0.60 to 1.16) respectively; for malathion-methyl parathion it was 0.24 (0.021 to 0.53) and x1.24 (1.0214 to 1.53) respectively and for malathion-endosulfan it was 1.648 (0.74 to 2.93) and x2.65 (1.74 to 3.98) respectively. Individually it is found that 2,4-D (chlorophenoxy herbicide) is 'moderately toxic', malathion and methyl parathion (organophosphate insecticides) are 'toxic' and endosulfan (organochlorine insecticide) is 'very toxic' to juveniles of rohu under static with renewal 48-h LC_{50} test. But the 'strictly additive' nature of the insecticide-weedicide combinations and the 'more than additive' nature of the insecticide pairs coupled with the sequential or even simultaneous application of these chemicals in the paddy fields and plantations increase the potential for pollution of these pesticides in the freshwater and coastal ecosystem of the State. Chronic combined sublethal toxicity

studies under tropical condition would throw more light on these aspects.