ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Acute toxicity and synergetic action of some pesticides on *Cyprinus carpio*

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Manuscript Info

Manuscript History:

Received: 10 December 2013
Final Accepted: 28 December 2013
Published Online: January 2014

Key words:
Acute toxicity, insecticide,Cyprinus carpio

Abstract

Acute toxicity studies are the test to determine the strength of toxicity of the material by the reaction of the living organism to it**.** In the present study conducted on Cyprinus carpio, Fenthion was found to be highly toxic as compared to Malathion, Metasystox and Nuvan. Acute toxicity studies of insecticide mixture pointed out that the action of additional insecticide increases the toxicity level**.** Among the synergetic action of pesticides selected, Thiodan + Fenthion was more toxic than Fenthion + DDT.

Introduction

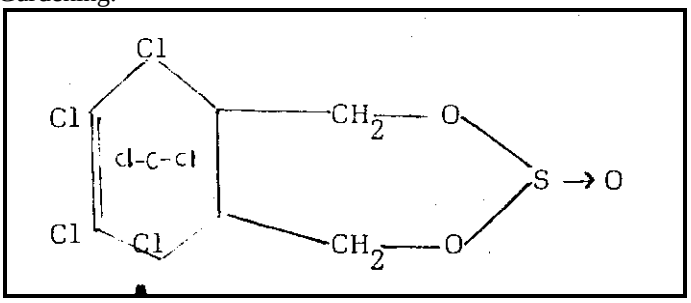
Acute toxicity study provides the basis to measure the relative toxicities of different materials and the relative sensitiveness of different species under the conditions that rapidly lead to the death of the animals. When this material proves harmful to the test organism, it is termed as toxic substance. Depending upon the exposure period of toxic substance bioassay study may be divided into acute toxicity and chronic toxicity studies. The former determines immediate response to metabolic activities for short term exposure, the latter records slower disruption of metabolic activities to long term exposure. Acute toxicity tests results are generally reported in terms of median lethal dose Ld_{50} or median tolerance limit TLm or median lethal concentration Lc_{50}. TLm or Lc_{50} or Ld_{50} is a concentration at which 50% of the test animals survive or it is an interpolated value based on the percentage of organisms surviving at two or more concentrations at which less than or more than 50% of the test organisms survive. Exposure period of an animal to applied stress is normally 24, 48, 72 and 96 hrs and the results are expressed in relation with time factor as 24, 48, 72 and 96 hrs Lc_{50}.

Studies on acute toxicity tests using various pollutants have been done so far on many aquatic animals. In India most of the farmers use a mixture of pesticides to clear away pests more effectively than single toxicant. Very few studies have been carried out on toxic effects of pesticide mixtures on aquatic organisms. Verma et. al. (1982) studied on the effect of three pesticides Thiotox, Dichlorovos and Carbofuran and their synergetic actions on Mystus vittatus exposed for 30 days. Markings & Bliss (1985) reported that extremely small quantities proved lethal to 3 species of fish, rainbow trout, white sucker and fat head minnow, when exposed to a mixture of TFM, Delnav and Malathion. Soman (1987) noted synergetic action of Thiodan and Fenthion mixture to be more, toxic than that of DDT and Fenthion to `the fresh water fish C. fasciata. It can be seen from the above review that quantitative information regarding acute toxicities of pesticides on fresh water fishes is rather insufficient. The present study was an effort to fill some gap in the information in terms of acute toxicities.

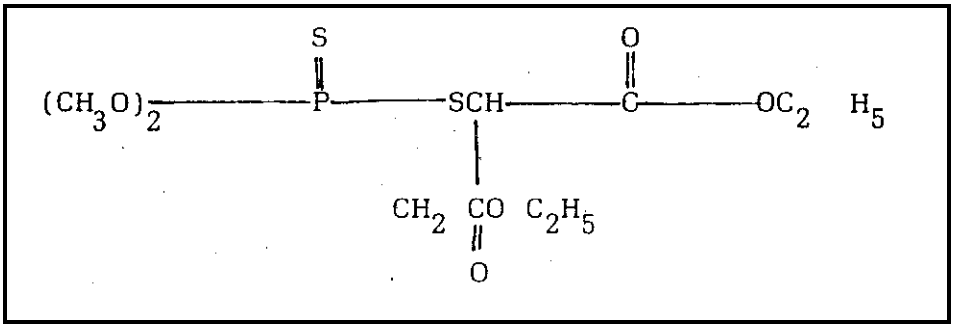
Materials and Methods:

Endosulfan or Thiodan:– A cyclodiene organochlorine insecticide is the addict of hexachloro cycle pentadiene and 1, 4, dihydroxy-x-butane subsequently chlorinated with' SOZ C12 to produce 6, 7, B, 9, 10-hexachloro-1, 5, 5a, 6, 9, 9a hexa hydro-6-9-methano 2, 4, 3-benzodioxathiepin-3-oxide. The technical grade material is a brownish solid having m.p. 70-100°C. Thiodan contains two stereo isomers, the alpha of melting point 108-110°C and beta of melting point 108 to 210°C. It is Stable in sunlight though subject to a slow hydrolysis to alcohol and sulphuric

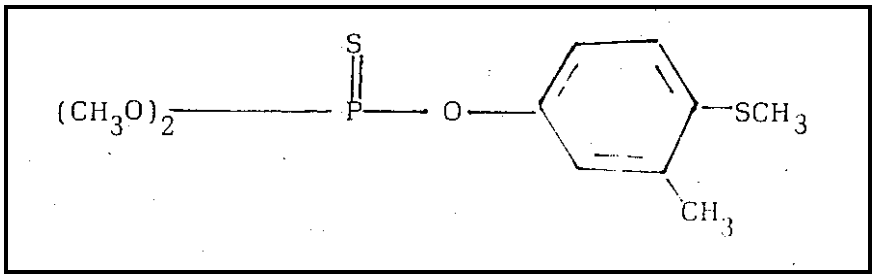
acid. Thiodan is readily absorbed by the insects or pests cuticle and affects the ganglia of CNS leading to hyper activity and hypersensitivity with convulsive movements.This broad spectrum insecticide has wide applications in Agriculture, Forestry and Gardening.



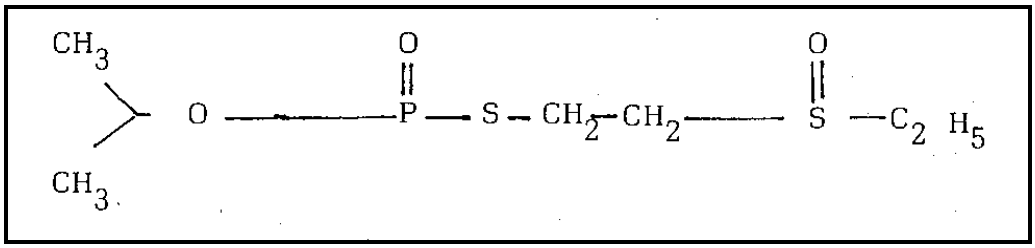
Malathion: .:Malathion is 0,0, dimethyl s-(1,2 dicarboxy ethyl) phosphorodithioate a brownish liquid B.P. 156 °C at 0.7 mm. The technical material is 95 to 98% pure with an unpleasant odour. Malathion is easily hydrolyzed above pH 7.0 and below pH 5.0 and is compatible with alkaline materials.



Fenthion: Fenthion is O,-o-dimethyl-04 methyl thio-m-tolyl phosphorothioate. It was developed by G. Schrader and E-Schegk and was introduced in 1957 by Bayer A.G. under Code No. Bayer 29493, S 1752, trade mark Baycid, Lebaycid, Baytex, Mercaptopos, Entex, Tiguvon. Brownish liquid B.P. 105°c at 0.01 mm d^{25} . 1.245 P. 2.15×10^{-6} mm at 20°C. Fenthion is a Contact and stomach insecticide with a useful penetration and persistent action. It is effective against various pests of stored product, public hygiene, crop protection. lt is also effective against fruit flies, aphids, plant bugs, saw flies and also weaver birds. Fenthion solubility at room temperature is 54-56 mg/l H20 Q readily soluble in most of the organic solvents and in glyceride oils. The technical product is pure with weak garlic odour. It is stable at 210°C to light and to alkali. Formulation of Fenthion includes Baytex for house hold use, a Lebaycid w.p. (250 and 400g/kg) E.C. (500 and 1000 g/l) and ULV (1000 g/l) and dust 30 g/kg for use against crop pests, Qualatose for use against weaver birds, Tiguvon against animal parasites.

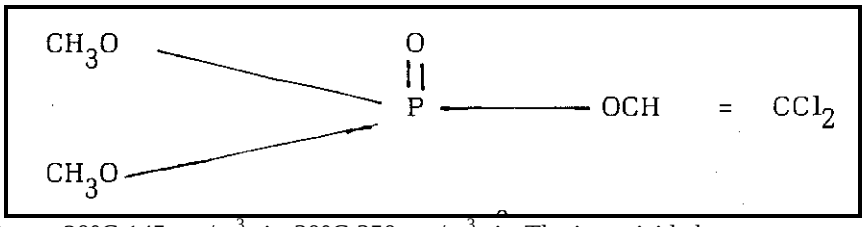


Metasystox: Metasystox is a mixture of O-O-dimethyl O-2 ethyl thioethyl phosphorothioate and O-O dimethyl S-[Zethyl thio) ethyl phosphorothionate. Both the isomers are liquid impure form but are soluble in solvent and slightly soluble in water. In aqueous solution the active ingredient is slowly hydrolyzed. It is not stable in alkaline media.



Yellowish liquid practically odorless. Molecular weight 246.3 B.P. 106°C at 0.01 mm Hg volatility 0.3 mg/m3 at 30°C and specific gravity 1.289 at 20°C.The toxic action of Metasystox is mainly due to sulphoxide and sulfone of thioate metabolically produced in animals and plants. However, both the isomers do undergo reaction and liberate sulphonium compounds in small amount which is highly toxic. Metasystox is recommended against sucking insects, aphids, mites and sawflies.

Nuvan 100 EC: Nuvan is a .water emulsifiable concentrate containing 760gms Dichlorovos (DDVP) as its active ingredient (w/w) per Kg product with adequate emulsifier. DDVP is a volatile phosphoric ester. The chemical formula for which is O,O-dimethyl 2 : 2 dichlorovinyl phosphate. Nuvan is a highly volatile liquid



with great saturation at 20°C 145 mg/m^3 air, 30°C 350 mg/m^3 air. The insecticide has a contact and fumigant action and is supplied in 100% emulsifiable concentration. It is recommended against large number of insects and pests.

The stock solution of two mixtures of insecticides used for the present tests are Organochlorine + organophosphorous: Thiodan + Fenthion and Organochlorine + organophosphorous : DDT + Fenthion. Both in 1:1 proportion .

Test animal:

The common carp Cyprinus carpio (Linn) is the most extensively transplanted species of fish in the world. thus supplying essential sources of protein for human food consumption. The fish was very much preferred for cultivation in ponds in Asia, near and far east, alone or in combination with other fishes, because of its excellent growth rate, omnivorous habit, breeding in confined water, hardy nature and easy adaptability to artificial feeds. The possibility of rearing carp in flooded rice fields has been contemplated for many years, superior rice fields were obtained from field in which carp was reared. Today, intensive modern farming includes use of more fertilizers, herbicides, fungicides, insecticides and other agricultural chemicals. Since the inland fishes like Cyprinus carpio (Linn) and other carps are important sources of proteins in the nation's diet, a thorough understanding of pesticides effects on fish would be more rewarding for fish conservation and fisheries. Thus, with a view to finding out the toxic effects of the pesticides, the test animal selected for the study was Cyprinus carpio (Linn). Only the Juveniles of C. carpio, more or less of uniform size 13-14 cm. and 15-20 gm. wt. irrespective of sex, were selected for this study.

Test fish selected for the experiment purpose were brought from pollutant free water. In order to protect them from infection 0.001% $KMnO_4$ bath was given before acclimatization. The fishes were acclimatized to laboratory conditions for two weeks prior to bioassay test. During this period they were fed on tubifex and dry pellets of oil cake, once a day. The fishes were maintained in large glass aquaria containing aged tap water. The water was changed every alternate day. The tanks were kept away from serious visual and mechanical disturbances. Only those stock animals showing no mortality signs were selected for bioassay studies. At the end of successful acclimatization healthy looking fishes of approximately the same size (13 cm.) and 18-20 gm weight were selected as test animals [APHA (1975)].

Glass aquaria measuring 46 x 24 x 24 cms were used for conducting bioassay studies. They were properly cleaned before use by washing with detergent followed by a wash with clean water. Tanks were finally rinsed with chlorine free aged tap water. Each test aquarium contained twenty litres of tap water and five fishes. The required

dose of toxicant was added to each tank. Replicate test for each concentration was conducted along with a control group for three times.

Test water:

For bioassay tests aged chlorine free tap water was used. Physiochemical properties records of the test water such as temperature, dissolved oxygen, free chlorine, acidity, alkalinity and hardness were noted regularly during the test period as per Barnes (1959) in APHA (1975). The water samples from all the test aquaria were routinely analyzed. The oxygen content of test water was never allowed to fall below 6 mg/l. Care was taken to see that water temperature was never allowed to differ or vary beyond a range of 11°C during the complete bioassay period.

Test solution:

Stock solutions of appropriate concentration of various toxicants were prepared in analar grade acetone. The necessary concentrations in aquaria were obtained by adding the stabilized aliquots of stock solutions to test water. Pilot runs were performed using two or more concentrations of the toxicants. This gave an idea about the concentration to be selected for the full scale test. The range of concentrations selected was such that they result in zero to 100% mortality. The effect of these concentrations on the mortality rate of test species helps in selecting a second series of concentration of pesticide which was taken into consideration while calculating Lc_{50} values.

Test procedure:

The required test concentration was selected from the pilot tests for full scale experiments. The test water was changed after every 24 hrs during 96 hrs test period in' order to maintain the desired concentration. During the addition of toxicant, test animals were not fed two days prior to and during the exposure period to avoid contamination and increase in metabolic activity of the animal. A tank with free aged water was concurrently maintained in exactly the same manner under the same conditions as mentioned for experimental except that no toxicant was added to this water But acetone was added to this control water since it was used as a solvent, in an amount equal to the one used for the highest concentration dilution. These fish were treated as controls. All necessary precautions were taken to keep the test fish away from any serious mechanical or visual disturbances during test period.

Observations on mortality:

Fishes were examined after every 24 hrs for a total test period of 96 hrs. Mortality was recorded after every 24 hrs for four days and dead fish were removed from experimental tanks immediately. Criteria for death were failure to respond to any kind of mechanical stimulus over a period of two to three minutes. During the test period observations on general behavior and symptoms of intoxication were made The tests were repeated at least three times to confirm the results.

Measurements of toxicity and calculations:

Lc_{50} values were calculated from the mortality rate recorded for 24, 48, 72 and 96 hrs by straight line graphical interpolation method. The 95% confidence limit (C.L.) of Lc_{50} values and slope function (slope F) of the line has been calculated according to the method suggested by Litchfield and Wilcoxon (1949).

Results and Discussion:

Mortality:

No mortality was observed in any of the control tanks till the end of this experiment. Table 1.1 gives comparative Lc_{50} values at 24, 48 72 and 96 hrs for, different pesticides tested. From this table it is evident that Thiodan, the organochlorine compound is the most toxic and Nuvan the organophosphorous compound the least toxic. The Organophosphorous insecticides Fenthion, Malathion and Metasystox fall in intermediate ranges between the two extremes Table 1.2 shows comparative Lc_{50} values of mixtures of insecticides used. Thiodan + Fenthion mixture proved to be more toxic than DDT and Fenthion mixture.

The degree of toxicity of Thiodan, Malathion, Metasystox, Fenthion and Nuvan is reported by a few workers using various aquatic organisms as experimental animals. Bender (1969) demonstrated that residual half-life was just 12 hrs for Malathion treated fish Cyprinus Carpio. Arora, et al. (1971a,b) studied the toxic effect of four commercial insecticides-Malathion (50% EC), Monocide (40% EC), Thiodon (35% EC) and Ekatin (25% EC) to exotic carp and reported Thiodan to be the most toxic and Malathion the least toxic. The lesser toxicity of Malathion compared to

Fenthion in the present study was due to rapid hydrolysis to non toxic product. Jouch (1979) demonstrated that at a concentration of 8.5 mg/l of Fenthion, 5% of Tilapia mossambica and at 2.8 mg/l 19% of the fish Hetero tilapia died at 96hrs. Tilapia mossambica turned out to be much less sensitive to Fenthion than Hetero tilapia. .

Organochlorine compounds are highly volatile and persistent. They act as central nervous system stimulants. According to S. Gaikwad (1981) high toxicity of Thiodan is related to its direct effect on ganglia of CNS leading to sudden behavioural changes in Tilapia Mossambica. As suggested by Wooley and Talens (1971) toxic effect Thiodan is due to its accumulation in adipose tissue. Thus, it can be co-related that in the present study, high toxicity of Thiodan may be due to its direct effect on CNS and its accumulation in tissues. According to Sheela J et. al. (1982) high toxicity of Fenthion is due to its interference with normal mechanism of nerve impulse and enzyme cholinesterase and suggested that organophosphorous insecticide rapidly hydrolyses acetylcholine in CNS that cause death.

In the present study, Fenthion was highly toxic as compared to Malathion, Metasystox and Nuvan. The variation in percentage inhibition of AChE with respect to exposure period could be one of the reasons for different mortality rates for different organophosphorous pesticides and thus the degree of toxicity. Second reason could may be due to its change in ability to hydrolyse the particular insecticide.

Macek & McAllister (1970) worked on acute toxicity studies of insecticide mixture and they pointed out that the action of additional insecticide increases toxicity level. Marking & Bliss [1985] studied synergetic action of JFM, Delnav and Malathion and proved that an extremely small quantity of their mixture became lethal to 3 different species of fish, rainbow trout Salmo giardneri, white sucker Catostomus commersoni and fat head minnow Pimephales pronidas. Soman (1987) reported that Fenthion + Thiodan was more toxic than DDT + Fenthion to the fresh water fish C. fasciata. In the present study, mixture of Fenthion + Thiodan was more toxic than Fenthion + DDT which can be explained on the basis that organochlorine compound Thiodan was more toxic than DDT and both being central nervous system stimulants, might have increased their toxicity to fish when used in combination with organophosphate insecticide, Fenthion.

Table 1.1 COMPARATIVE LC5O VALUES FOR THE DIFFERENT TOXICANT5 TESTED FOR C. CARPIO

Toxicant	Exposure period (hrs.)	LC_{50} ppm (mg/l)	95% C.L.	Slope
Thiodan	24	0.0032	0.004 -0.0025	1.3229
	48	0.0025	0.0028 -0.0022	1.1481
	72	0.001	0.0023 -0.0015	1.2649
	96	0.0014	0.0017 -0.001	1.325
Fenthion	24	2.5	2.875 - 2.173	1.1517
	48	2.2	2.424 - 1.996	1.118
	72	1.85	1.9166 - 1.785	1.042
	96	1.55	1.638 - 1.466	1.066
Malathion	24	6.4	7.14 - 5.73	1.133
	48	4.8	5.16 - 4.46	1.087
	72	4.4	4.96 - 3.9	1.147
	96	3.3	4.113 - 2.64	1.286
Metasystox	24	8.1	8.52 - 7.59	1.076
	48	6.3	6.74 - 5.88	1.080
	72	5.8	6.29 - 5.34	1.098
	96	4.8	5.4 - 4.26	1.144
Nuvan	24	8.4	9.13 - 7.72	1.1002
	48	6.8	7.88 - 6.94	1.075
	72	5.8	6.43 - 5.22	1.126
	96	4.3	5.50 - 4.53	1.325
Control (Aged tap water + acetone)	24	0.0	-	-
	48	0.0	-	-
	72	0.0	-	-
	96	0.0	-	-

Fresh water characteristics (Average values)
Dissolved oxygen : 6.9 ±0.2, pH = 7.8 ±0.5
Carbondioxide = 0.3 ±0.2, Temperature = 29 ± 1°
Acidity in ppm = 5.6,A1ka1inity in ppm = 45.9
Total hardness (CaCO3) in ppm = 31.0

Table 1 . 2 COMPARATIVE LC50 VALUES FOR THE DIFFERENT TOXICANTS TESTED FOR C. CARPIO

Toxicant	Exposure period (hrs)	LC_{50} mg/l (ppm)	95% C.L.	Slope
Fenthion + Thiodan	24	$2.5x10^{-7}$	$3.0x10^{-7}$-$2.078x10^{-7}$	1.235
	48	$2.0x10^{-7}$	$2.46x10^{-7}$-$1.62x10^{-7}$	1.270
	72	$1.8x10^{-7}$	$2.52x10^{-7}$-$2.28x10^{-7}$	1.472
	96	$1.1x10^{-7}$	$1.788x10^{-7}$-$0.676x10^{-7}$	1.742
Fention + DDT	24	$3.1x10^{-5}$	$3.5x10^{-5}$-$2.73x10^{-5}$	1.1342
	48	$2.85x10^{-5}$	$3.31x10^{-5}$-$2.44x10^{-5}$	1.190
	72	$2.45x10^{-5}$	$3.0x10^{-5}$-$2.0x10^{-5}$	1.2608
	96	$2.2x10^{-5}$	$2.62x10^{-5}$-$1.84x10^{-5}$	1.2247

Fresh water' characteristics (Average values) :
Dissolved oxygen : 6.9 ± 0.2 pH = 7.9 ±0.5
Carbondioxide = 0.3 ±0.2, Temperature = 29 ±1°
Aoidity in ppm = 5.6 Alkalinity in ppm = 45..9
Total hardness (CaCO3) in ppm = 31.0

Behaviour:

Behavioral results provide a valuable tool to assess the toxicity impact because of the cause by which one can record the behavioral response without causing any mechanical injury to the exposed animal. There was positive correlation between the exposed concentration of insecticides selected in the present study and the degree of abnormal behavior of the test fish.

When the fish understudy was exposed to Thiodan (0.0032 ppm), showed erratic swimming leading to jerky movements and the fish tried to leap out, to avoid toxic medium. Further, one side rolling of the body and convulsions occurred prior to death. While C. carpio exposed to Thiodan for 96 hrs (0.0014 ppm) showed all the above mentioned effects except convulsions but to a lesser extent. Similar observations were marked by Mount (1962) in blunt nose minnow exposed to Endrin; and in fishes Gambusia affinis and Tilapia mossambica exposed to Thiodan by Joshi, (1978) and Gaikwad (1981). According to Gaikwad (1981) changes in behavior noted in fish T.mossambica exposed to Thiodan could be due to its accumulation in tissues and may be due to its direct effect on CNS thus causing similar respiratory failure. The similar changes in behaviour observed in C. carpio exposed to Thiodan ,Fenthion, Malathion, Metasystox and Nuvan in present study may also be attributed to possibly to its direct effect on CNS and possibly to its accumulation in tissues causing respiratory failure.

Fish exposed to Fenthion showed irritability with increase in opercular movements and the tremors were observed more severely as compared to other fishes exposed to other pesticides. All the exposed fish sank to the bottom before death. C. carpio exposed to Metasystox showed rotation of its own body with jerky movements before loss of equilibrium. The frequent jumping of the fish as observed could be an effort made by the fish to avoid the toxic solution. According to Arunachalam et.al., (1980) inhibition of cholinesterase could be the reason for changes in the normal behavior like erratic movements and imbalance in fish when exposed to toxicant. Noted increase in frequently of opercular movements and surfacing in the treated fish C. carpio in the present study may be due to the

effect of toxicant on respiratory process resulting in failure of energy production or it could be due to release of stored metabolic energy causing severe stress to the fish and thus leading to its death.

Conclusions

.Cyprinus carpio is comparatively sensitive to different pesticides tested. Thiodan proves to be the highly toxic and Nuvan least toxic. Fenthion, Malathion and Metasystox fall in the intermediate range between Thiodan and Nuvan. Among the mixtures of pesticides selected, Thiodan + Fenthion is more toxic than Fenthion + DDT. 2. Fish exposed to various pesticides showed similar symptoms of poisoning with reference to respiratory distress.

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