Introduction
Ever since the dawn of civilization, man has continually endeavoured to improve his living conditions; in his efforts to produce adequate supplies of food, man has been hampered by the ravages of the pests and crop diseases. There are many examples of mass destruction of different crops by plant pests all over the world, leading to widespread shortage of food grains. This problem became much more acute in view of the staggering increase of human population. They damage economic goods such as wood, paper, cotton, rubber etc., and are vectors for the diseases such as encephalitis, typhus, sleeping sickness, filaria, malaria etc. Hence, it became necessary to invent or devise measures to combat the problems. One of the most prominent of these measures is the invention and application of chemical substances to control the ravages of different pests.

The chemicals used to destroy selectively any species of pest in a biological community are known as pesticides. Pesticides can remove weeds and can kill bacteria, protozoa, viruses, helminth parasites, rodents and birds. They not only kill the pests but also affect the organisms whose control is not intended. Hence a general term "Biocide" has been preferred. The use of pesticides sharply decreased the losses to the natural economy from various pests and the high economic efficiency achieved with the use of pesticides in agriculture and other branches of economy has favoured the rapid development of these pesticides both quantitatively and qualitatively. No doubt much success has been achieved through these pesticides. However, excessive and indiscriminate use of these chemicals has led to several deleterious effects in the environment, especially aquatic system. The translocation of these pesticide chemicals into the aquatic system is mostly through their application in agricultural and public health operations, direct
and deliberate discharge into water resources to eradicate the vectors of diseases, and accidental spillage and carelessness during operations. So they posed a great threat to man and his environment by eliminating beneficial insects causing ecological imbalance, becoming phytotoxic by destroying useful plants and entering into the food chain and causing toxicity to both target and non-target animals including human beings. Besides these pesticides are being carried out by natural forces such as wind, rain, and flow of rivers and ocean currents. Residues of pesticides began to appear everywhere on the globe, from tropical forests to antarctic snows. Still worse, the slowly decomposing chemicals were taking their toll among many non-target fish, crabs, prawns and wild species.

It became clear that the appearance of residues in food stuffs (Egan and Hubbard, 1975; Mukherjee et al., 1980; Passino, 1981), human milk (Kalra and Chawla, 1981; Dillon et al., 1981), human fat (Mukherjee et al., 1980; Rosival et al., 1980) and human placenta and accompanying fluid (Saxena et al., 1980). High mortality rate and reduced reporduction potentiality of organisms such as birds and fish and development of pesticide resistance in target and non-target species have proved the residue problem (Braun and Frank, 1980; Hama-Hiroshi, 1980; Hall et al., 1980; Motoyama et al., 1980; Yockim et al., 1980; Conway and Comins, 1981; Liberman and Alexander, 1981). This problem is due to lack of appropriate knowledge on pesticide usage. Exposure of human beings to pesticides may occur in laboratories where these substances are synthesized and examined for their chemical, physical and biological properties, and several instances of acute poisoning of farm workers have resulted from dermal exposure to pesticides during spraying operation in the fields. Hazards of the pesticides are variable
depending on a large number of factors such as (i) Chemical characteristics, physical properties of the formulation to be used and mode of application, (ii) environmental conditions i.e., air temperature, wind velocity, humidity etc. determining the dispersion and fate of chemicals in the environment. Soil, water reservoir and the micro flora determine the persistence of these chemicals to some extent and (iii) health status, genetic disposition of exposed population of individuals. Therefore, hazards from pesticides will vary from one place to another depending upon environmental variables favouring or disfavouring their absorption by the body in the exposed population.

Pesticides are classified as insecticides, fungicides, rodenticides etc., depending on the nature of the pest intended to be controlled. A detailed nomenclature and classification of pesticides with emphasis on practice in Yugoslavia was made (Mojasevic Milica, 1980). Most of these pesticides are not specific in their action. Based on the chemical nature of these pesticides, they are classified into three general groups, (a) inorganic compounds including arsenicals, mercurials, borates and fluorides, (b) natural organic compounds derived from plants like nicotine, pyrethrum, rotenone and derris etc., (c) synthetic organic compounds like organochlorides, organophosphates and carbamates.

The development of synthetic organic pesticides passed through an accelerated phase during the decades following the world war II. Organochlorides are the first in the series of pesticides that are widely used for a variety of purposes. Human health has been improved by reducing the incidence of malaria and other diseases carried out by insect vectors. The World Health Organisation proposed a campaign for control of malaria in
1955 by using DDT and the disease has been eradicated in 36 countries (total population 710 millions). However, during 1950's and 1960's, reports of large residues of these long-persistent pesticides were made. Increased levels of DDT and other organochlorine insecticide residues in man and the environment and increasing appearance of resistance among insects were among several factors that contributed to replace the organochlorides by the most versatile groups of insecticides namely organophosphates and carbamates. In 1972, 10 million pounds of parathion and 40 million pounds of methyl parathion were used for insect control. They were proved to be valuable substitutes for organochlorine insecticides being less persistent either in environment or within the organism. Hence, they are being used as high-use insecticides.

In addition to organophosphates, organochlorides and carbamates, another group has come into the list of insecticides, viz., pyrethroids. They have quick knock-down effect and are normally not harmful to warm-blooded animals. They are quickly decomposed in air and do not leave undesirable residues and posed least problems of pest resistance.

It is inevitable to man, now a days, to use insecticides though it was clear that using of pesticides has deleterious effects on the environment and human beings from the following incidents: (A) In 1958 more than 100 people died in Cochin (India) after consuming the food contaminated by parathion, (B) In 1970, a large number of labourers in the Maluad region of the Karnataka state in India were affected with a mysterious crippling disease which resulted in cases of severe bone degeneration leaving the victims totally helpless. In the analysis that
followed, it was proved that consumption of crabs from paddy fields in the area which were sprayed indiscriminately with pesticides was responsible for this disaster, (C) In 1972, the WHO's Expert Committee on insecticides estimated that there were about 5,00,000 cases of accidental pesticide poisoning in the world, (D) According to conservative estimate, the total number of deaths every year would be around 9,200. This kind of deleterious effects of pesticides could be minimized only by using safe insecticides. To achieve desired effects without affecting the non-target organisms, we have to depend more on biodegradable pesticides and lessen our sole dependence on persistant pesticides.

At this juncture there is an urgent need to develop new class of pesticides with target site action similar to the earlier conventional pesticides to minimise the environmental pollution. These compounds must incorporate the principles of biodegradability without compromising the cost and efficacy. Already some work in this direction has been undertaken. Pyrethroids have made their appearance and have come into the light of publicity because of their unique physico-chemical characters and being less toxic to the environment. These are more powerful than the classical insecticides viz., organochlorides, organophosphates and carbamates. There is a great scope for increasing pyrethroid production in this country, some of which are photostable also have a great role to play in future.

History of Pyrethroids

A large number of different plant species contain insecticidal materials. Some of these have been used by man as insecticides since very early times. The most important insecticide in this category is pyrethrum.
In early 1800, Jumtikoff of America discovered that certain caucasian tribes used ground flowers of a plant as a insecticide. Since then, the material, called "Pyrethrum" came into wide use. Various species of the genus, Chrysanthemum, family Asteraceae, yield a useful product, but in 1840 the species Cinerariae folium was found to be the most effective and displaced other species such as Chrysanthemum coccineum and Chrysanthemum carneum. For years, Yugoslavia was the primary producer of the flowers but Japan moved into the ascendent by about 1915. The production of synthetic pyrethroids on a commercial basis started with allethrin and cyclethrin around 1950 (O'Brien, 1967). The last decade has seen the development of a number of new photostable and highly insecticidal pyrethroids (Elliott, 1977), the use of which as "nerve poisons" is rapidly becoming widespread.

Development of synthetic pyrethroids in India

In India synthetic pyrethroids have been evaluated since 1976 and in the beginning they were tested on cotton and rice fields etc. The use of these products has been increasing at an extremely fast rate in many countries of the world including India. At present pyrethrin, cypermethrin, decamethrin and fenvalerate are the main synthetic pyrethroids being developed commercially.
Synthetic pyrethroids available in India

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<thead>
<tr>
<th>Name of Insecticide</th>
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<tr>
<td>1. Permethrin</td>
<td>Permaset 25.0 EC</td>
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<td>Decis 2.8 EC</td>
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<td>3. Fenvalerate</td>
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<td>4. Cypermethrin</td>
<td>Ripcord 10.0 EC</td>
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General features of the pyrethroids

Classical pyrethroids are esters of cyclopropane carboxylic acids with alkenylmethyl cyclopentanolone alcohols, e.g., chrysanthemic acid and pyrethrolone in pyrethrine I. Fig 1 shows the structural formula of pyrethrin I, a compound which contains all the basic features of good insecticidal activity. Both dihalovinyl substitution in the acid moiety and incorporation of 3-phenoxy alcohol (Fig. 1) have improved the stability of molecule against breakdown and metabolism. Activity also depends on the intact ester, and derivatives of either the alcoholic or acidic component (Elliott, 1976). For example, changing the esterlink between acid and alcohol to form a ketone or carboxamide, markedly reduces the compound's toxicity to insects, although the type of biological activity may remain the same (Berteau et al., 1968). The improvement on insecticidal activity was achieved by replacing the vinyl methyl moiety by halogens (permethrin) (Fig. 1) and also by addition of cyano group (cypermethrin) (Fig. 1) at the \( \alpha \)-position of the alcohol moiety.
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A) PYRETHRIN-1

B) PERMETHRIN

C) FENVALERATE

D) CYPERMETHRIN

E) DECAMETHRIN
It is not clear whether the insecticidal activity of pyrethroids depends on the structural configuration and the activity of a particular optically pure isomere is often far greater than that of its enantiomer. For this reason it is extremely important to know about the chemical structure of pyrethroids. The structure of pyrethrin-I reveals that it has three optical centres at carbons 1 and 3 of cyclopropane ring and another at the cyclopentane moiety in the alcohol. By systematic experimentation it was found that cyclopentenolone increases the toxicity to insects, without altering its low mammalian toxicity (Elliott, 1971). Further advance was made when the cyclopentenolone alcohol was replaced by 3-phenoxybenzyl alcohol. This in combination with a dichlorovinyl side chain at C-3 of the cyclopropane ring has resulted in the first photostable pyrethrin, i.e., permethrin (Elliott et al., 1973). Introducing an \(\alpha\)-cyano group in an S-configuration on to 3-phenoxy benzyl alcohol resulted in exceptionally high activity and has led to the discovery of decamethrin (NRDC 161, Fig. 1), the most active pyrethroid presently known with an \(LD_{50}\) for insects in the order of 0.03 mg/kg (Elliott et al., 1974). Another important development is the discovery that esters of (S)-\(\alpha\)-substituted isovaleric acid with known pyrethroid alcohols are highly active insecticides, which led to the synthesis of fenvalerate and related compounds (Ohno et al., 1976). Several recently synthesized alcohols have yielded highly active pyrethroids (Naumann, 1981) and the search for even more active and selective pyrethroids will undoubtedly continue in the coming years.

**Symptomology**

The first step in assessing the toxic effect of a poison is to observe physical and behavioural responses. They are called the symptoms of
poisoned animals and are helpful to understand the mode and site of action of insecticides. Explaining the symptomology of poisoning in neurophysiological terms can be approached from many levels. In most cases interpretation is most difficult due to the complexity of the nervous system and the widespread actions of the pyrethroids in sensory, motor and central nervous elements. Furthermore, many of the overt poisoning symptoms were also probably side effects not directly involved in toxicity; for example, hyperexcitability increases at higher temperatures, where mortality is reduced (Gammon, 1978; Adams and Miller, 1979).

Pyrethrum and many of the synthetic pyrethroids cause a progressive series of poisoning symptoms, including hyperactivity, ataxia, convulsions and eventual paralysis (Blum and Kearus, 1956; Burt and Goodchild, 1971a, b; Camougis and Davis, 1971; Narahashi, 1971a; Clements and May, 1977; Leake, 1977; Gammon, 1978). The rate at which symptoms develop depends on the chemical applied, the mode of application, and the temperature. With many of newer synthetic compounds, in particular the α-cyano-3-phenoxybenzyl esters (for example deltamethrin, cypermethrin and fenvalerate), symptoms of excitation are absent. Instead, ataxia and lethargic state develop, leading to the condition described as flaccid paralysis (Ford et al., 1977; Adams and Miller, 1980).

Gammon et al., (1981) noted differences in symptoms upon in vivo poisoning symptoms with two classes of pyrethroids. Type-I compounds cause restlessness, incoordination, and hyperactivity followed by prostration and paralysis, while Type-II compounds cause a characteristic pronounced convulsive phase; i.e., within minutes of dosing, cockroaches become ataxic
and incoordinated. There were periods of convulsions, hyperactivity, and sporadic, sustained contractions involving extension of the metathoracic legs. The actual speed with which the compounds paralyze the insects depends on several factors. The size of the insect is obviously important. The rapid action on houseflies or mosquitoes (Page et al., 1949) appears to be related to their large surface-to-volume ratio; large insects such as cockroaches and locusts exhibit a more drawn out, distinct series of behavioural stages preceding paralysis (Burt and Good Child, 1971a; Gammon, 1978). Leake (1977) reported an interesting correlation between symptoms and aberrant electrical activity in the central neurons of leech, Hirudo medicinalis during S-bioallethrin poisoning. General incoordination was correlated in time with repeated depolarizations in P cells and Retzius cells. Subsequent "rigor" was associated with a block of impulse conduction in sampled neurons. Other studies have shown that the flightmotor neurons of the housefly, Musca domestica remain highly active long after prostration and paralysis have set in (Adams and Miller, 1980).

Selective action

The pyrethrum extract appears to be toxic not only to insects but also selectively to some mammals. The pharmacological results of purified extract of pyrethrum were first published by Fujitau in 1990. Pyrethrins were once labeled as non-toxic to humans and pests and are generally considered to be the safest insecticides.

Synthetic pyrethroids have comparatively high potentiality than that of other insecticides. The potentiality depends on the integrity of molecules, as derivatives of either alcoholic or acidic component are inactive
(Elliott, 1977). The cis isomers are generally toxic than the corresponding trans isomers. The trans isomers of resmethrin, phenothrin and permethrin have very low mammalian toxicity despite their similar insecticidal potential to their cis-isomers. The non $\alpha$-cyano group (Type I action) caused repetitive firing in cockroach cercal sensory nerves, whereas $\alpha$-cyano group did not. Introduction of $\alpha$-cyano group increases both the insect and mammalian toxicity. The mode of metabolic detoxification is dependent on such structural features as cis or trans configuration of the acid moiety and the presence or absence of the cyano substituent in the alcohol moiety.

Obviously small structural changes sometimes have a large influence on the relative potency and species selectivity of pyrethroids. For example Lacewing larvae are highly tolerant to pyrethroids, especially deltamethrin, which for other insects is usually highly toxic (Ishaaya and Casida, 1981). Pyrethroids are very toxic to honey bees, but the structural modifications for optimal potency to flies substantially differs from those to honey bees (Elliott et al., 1978). Several insect species resistant to DDT are cross-resistant to pyrethroids (Elliott et al., 1978).

Mode of action of Pyrethroids

The primary target of the pyrethrin group of insecticides is considered to be the nervous system, as judged by their quick action. The symptoms of pyrethrin poisoning are: (1) Excitation, (2) Convulsions, (3) Paralysis and (4) Death. The effects of pyrethrins on the insect nervous system closely resemble those of DDT (Welsh and Gordon, 1947; Yamasaki and Ishii, 1952), but are apparently much less persistent. The primary target of pyrethrins seems to be the ganglion of the insect central nervous system
(Roy et al., 1943) although some pyrethrins injected into the abdomen or a spiracle produce progressive symptoms in the leg innervated by the ganglion nearest to the point of injection.

As far synthetic pyrethroids, it was first reported by Narahashi et al., (1977), that there appears to be two types of compounds, those that cause excitation in the abdominal nerve cord of the crayfish with a clear-cut relationship to their insecticidal properties (i.e., greater the neurotoxicity, greater the insecticidal potential) and those that show no overt excitatory action, still be insecticidal. Gammon (1980) compared the action of pyrethrin and cypermethrin on a pyrethroid resistant strain of Spodoptera littoralis and found that the former induced a clear-cut excitation in the nerve cord at $10^{-7}$ M, while the latter did not affect even at $10^{-6}$ or $10^{-7}$ M. The most significant finding was that the above excitation by permethrin was suppressed at high external calcium and at lower calcium concentration. In addition, pyrethrin exhibited a more rapid blocking action at lower temperatures, while cypermethrin did not. Gammon et al., (1981) made an extensive survey of the effects of various pyrethroid analogues by using central sensory nerves of the American cockroach, and concluded that there are two types of pyrethroids. Type-I compounds include repetitive firing in a cercal sensory nerve following a single electrical stimulus, while Type-II compounds do not. Gammon et al., (1981) noted differences in in vivo poisoning symptoms between the two classes of pyrethroids.

Nicholson et al., (1983) found that permethrin and deltamethrin substantially enhanced the release of $\gamma$-aminobutyric acid (GABA) from
synaptosomes isolated from the guinea pig cortex. Deltamethrin was more active than DDT or permethrin. In this case, Tetrodotoxin at 0.45 x 10^{-6}M could abolish most of the increased transmitter released by DDT and Deltamethrin, thus indicating that this aspect of DDT and pyrethroid action is likely due to their influence on sodium channels.

Staatz et al., (1982) studied the effects of permethrin and deltamethrin on the central nervous system of mice. They found that pyrethroid-induced signs of toxicity (eg., hyperactivity, whole body tremor etc.) appeared more quickly when the toxins were given into the central nervous system via intra cerebroventricular injection than when they were administered via the peripheral (intravenous) route. Pretreatment of mice with drugs known to affect central noradrenergic, cholinergic or serotoninergic transmission potentiated the manifestations of pyrethroid toxicity, while some of the symptoms could be partially alleviated by pretreatment (intraperitoneal) with diazepam, amino-oxyacetic acid or cyclohexamide. They therefore concluded that in mice the main site of action of these pyrethroids is the central nervous system. Cole and Casida (1983) have also shown that some of the signs of toxicity of deltamethrin in frog are effectively antagonised by treatment with diazepam, the action of which is known to be largely central.

**Effects of pyrethroids on the invertebrate nervous systems**

Since the new class of pyrethroids has become available, several interesting studies have been made on invertebrates. It is generally believed now that the pyrethroid compounds act directly on excitable membranes interfering with the changes in ionic permeabilities and accompanying
electrical phenomena (Narashahi, 1976a). Therefore, electrophysiological methods are most appropriate ones to study the action of pyrethroids.

Pyrethroids exert widespread potent actions on both peripheral and central elements in arthropods. These actions have been studied at various levels, from extracellular recordings of the arthropod ventral nerve cord activity (Burt and Good Child, 1971a, b; Gammon, 1978, 1979) to single cell intracellular recordings (Narashahi, 1971b; Leake, 1977) and ultimately membrane ionic channels (Narashahi, 1971b, 1976; Narashashi and Lund, 1980). Miller and Adams (1977) and Miller and Kennedy (1972) reported that tetramethrin and cismethrin produced repetitive activity in the crural nerve of isolated metathoracic leg of both the housefly, Musca domestica, and of the cockroach, Periplaneta americana. There was a good correlation between the appearance of trains of nerve impulses in peripheral sensory axons and knockdown action. Application of S-bioallethrin to Retzius medicinalis cells in the central nervous system of the leech, Hirudo medicinalis, caused an increase in spontaneous activity (Leake, 1977). The pyrethrins caused a very rapid paralytic action in insects (Yamamoto, 1970). They had disruptive effects on a variety of arthropod sensory preparations.

Effects of pyrethroids on the vertebrate nervous system

Pyrethroids that contain the α-cyano-3-phenoxy benzyl ester are reported to cause poisoning symptoms such as chewing, salivation, pawing followed by death. They are characterized as largely central in origin (Ray and Cremer, 1979) whereas other pyrethroids such as bioresmethrin or cisresmethrin produced tremors or an increase in body temperature in rats (Verschoyle and Barnes, 1972; Carlton, 1977) suggesting their action on the
peripheral nervous system. White et al., (1976) found that lower brain levels of cismethrin were correlated with tremors or death in rats when compared with bioresmethrin. They concluded that cismethrin had a higher intrinsic toxicity than bioresmethrin, whereas the latter was metabolized somewhat faster by liver microsomal esterases.

Pyrethroids appear to be active on vertebrate sensory nerve and motor nerve-muscle preparations at highly specific sites. They do not appear to cause repetitive discharges in nerve terminal regions. These effects are similar to those of pyrethroid on invertebrate nervous system. Gray et al., (1980) suggested that higher toxicity of cismethrin compound to that of bioresmethrin was due to higher intrinsic activity at the site of action in the nervous system of rat. Both the compounds entered the nervous system rapidly, reaching peak concentrations within minutes after intravenous injection.

Biochemical and physiological changes

Generally, pyrethroids affect the metabolism of organisms, resulting in the physiological and biochemical changes of the tissues.

Several studies have shown that pyrethroids such as cypermethrin and deltamethrin inhibit the activity of ATPase in animals (Desaih et al., 1975; Casida et al., 1983). In the Squid, Loligo the natural type pyrethroids (Permethrin, Allethrin) primarily inhibit Ca\(^{++}\)-ATPase activity, whereas the highly modified pyrethroids (Cypermethrin, Decamethrin) mainly inhibit the Ca\(^{++}\), Mg\(^{++}\)-ATPase activity (Clark and Matsumura, 1982). Ahmed et al (1987) reported inhibition of AChE in different areas of rat
brain on different days after injection of decamethrin intraperitoneally. ATPase and AChE are very sensitive to fenvalerate stress in the field crab, *Oziotolphusa senex senex* (Neeraja Kumari, 1986). Pyrethroids have certain features in common with acetylcholine and could possibly interact with the cholinoreceptors (Korolkuos, 1970). In vivo and in vitro studies on cockroach, *Periplaneta americana* showed the inhibition of AChE and ATPases activity under fenvalerate stress (Lakshmirajjyam, 1986). Besides their effects on ATPases and AChE, inhibition of hormone release from isolated rat neurohypophysis (Dyball, 1982) has also been shown under pyrethroid stress. Total lipid content was increased in rats administered with pyrethroid compounds (Nagy et al., 1983). Significant increase in P-glucuronidase and β-galactosidase was found in the distal portion of sciatic posterior tibial nerves in pyrethroid treated rats (Rose and Dewar, 1983). Anastasi and Banister (1980) reported the changes of fish muscle enzymes like pyruvate kinase dehydrogenases (LDH, SDH and MDH) under pyrethrin exposure. There was a gradual depletion in the activity levels of SDH, MDH, cytochrome-C-oxidase and Mg²⁺-ATPase under fenvalerate intoxication in liver, brain and muscle tissues of fishes (Ghosh, 1990). Cremer et al., (1980) reported localized changes in brain glucose utilization in rats administered with deltamethrin.

Kumarguru and Beanish (1983) reported that the basal metabolic rate of oxygen-consumption of trout exposed to permethrin increased at initial exposure and declined to the control level after 13 and 32 days exposure. Decreased oxygen uptake was observed in rainbow trout exposed to fenvalerate (Bradburg et al., 1986). Some pyrethroids such as bioresmethrin or cismethrin produced fine tremors and an increase in body temperature in rats
(Verschoyle and Barnes, 1972; Carlton, 1977). Repetitive discharges were recorded postsynaptically from the sartorius muscle when the frog motor endplate preparation was perfused with allethrin (0.1 - 1 uM) (Wouters et al., 1977). Hefez et al., (1981) reported that fenvalerate-treated females of oribatid mite, oppiasticta started to lay eggs after a significant longer period than untreated females, and oviposition and post-oviposition periods were significantly shorter in treated females. Berlin et al., (1984) studied the nature of Deltamethrin-induced increase in the tension of the heart muscle by using guinea pig heart muscle system.

Influence of temperature

Most pyrethroids have negative temperature coefficients of toxicity; that is, they are more toxic at colder temperatures. The effective dose of given compound is usually temperature-dependent, whether one considers behavioural effects or toxicity. Measures of both KD50 (knockdown) and LD50 (toxicity) can be quite different, for example, between 15° and 32°C (Blum and Kearns, 1956; Gammon, 1978; Miller et al., 1979). Approximately dosed insects paralyzed at 15°C return to normal if the temperature is raised above a certain level. Poisoning symptoms return as soon as temperature is again lowered. This ability to control the development of or reverse symptoms quickly by merely manipulating the ambient temperature is suggestive of a membrane site of action for pyrethroids.

Many pyrethroids (Deltamethrin, Kodethrin, Fenvalerate) caused no repetitive firing during poisoning. In all cases examined, repetitive firing reverted to single potentials reversibly when flies were cooled below a characteristic transition temperature. Pyrethroid-induced multiple discharges
reverted to single potentials below 19°C, where as DDT type discharges persisted down to 10°C - 13°C. A temperature sensitive physical process thus seems to be important in the action of pyrethroids. Besides, increased rates of transmitter release and block appeared to have a negative temperature coefficient and were correlated with toxicity.

There are two types of actions of synthetic pyrethroids, type I action is mainly associated with compounds that cause nerve excitation symptoms characterised by the appearance of repetitive discharges and negatively correlated temperature reversible knockdown property. Type II action is related to the positively temperature correlated with killing action and nerve blocking action. It has been firmly established that the major cause of neuro excitation and eventual death by type I pyrethroids is negatively temperature correlated, as in the case of DDT. Thus, the effects positively temperature correlated or temperature dependent may be excluded from the list of suspected primary actions.

**Synergism**

Synergism is a striking phenomenon for pyrethroids in increasing the potency or decreasing the necessary dosage of costly pyrethroids. Many theories on synergism have been proposed, and now it is established that synergists inhibit the in vivo detoxification of pyrethroids and consequently the insecticides increase in their persistence and toxicity. Most of the synergists are methylenedioxy phenyl compounds, but other types of compounds are also active. A methylenedioxy group is essential for synergistic activity and modification of methylenedioxy group usually results in loss of activity. Some C^14-methylenedioxy phenyl synergists are
metabolised through the same hydroxylation responsible for the metabolism of pyrethroids. It has been suggested that methylene dioxyphenyl compounds serve as substitutes for pyrethroids in the enzyme system and inhibit the pyrethroid metabolism.

Pyrethrins are highly toxic as contact insecticides, but generally less toxic when fed to insects. Pretreatment of house flies with a synergist before the application of pyrethrins gives a result similar to that of simultaneous administration of toxicant and synergist.

Insect resistance to pyrethrins is due partly to reduce absorption of the insecticides, but increased metabolism is the major cause of resistance. Piperonyl butoxide increases the effect of pyrethrins on *Calandra granaria* by 4.6 fold for the susceptible strain and by 37 fold for the resistant strain. This result indicates a strong supression of metabolism by the synergist in the resistant insect. Pyrethrins are more active against flies at low temperature, but in the presence of piperonyl butoxide such negative temperature coefficient disappears, because the synergist inhibits metabolism which increases at higher temperatures.

**Degradation of pyrethroids**

Pyrethroids are comparatively new to the field of toxicology and so the literature on pyrethroid metabolism is not so vast as on organophosphates, organochlorides and carbamates.

Pyrethroids are highly degradable insecticides according to present knowledge. They are much less persistent and do not accumulate in
the environment. All pyrethroids are metabolized by ester hydrolysis and oxidation of methyl, methylene, alkenyl or aryl substituents. The metabolites are generally excreted by insects, birds and mammals as alcohols, phenols or carboxylic acids and their glycine, sulfates, glycoronide, or glucoside conjugates. Previously it was assumed that hydrolysis at 3-phenoxy-benzyl alcohol group as main degradation mechanism (Martin and Crosby, 1971), but the studies on the labelled C\textsuperscript{14} pyrethroid metabolism proved that the pyrethroid degradation is through oxidation.

Metabolism of pyrethroids results in detoxification; esterases and oxidases are involved in the detoxification of pyrethroids. Esterases and oxidase inhibitors increase the potency of pyrethroids when metabolism is the limiting factor in toxicity. Atleast 80 metabolites were identified from cis- and trans- permethrin alone in various species and systems. Some important metabolites of permethrin are: 4-hydroxy permethrin in housefly adults, rainbow trout, chickens, rats, goats and cows (Gaughan et al., 1977, 1978; Ivie and Hunt 1980; Glickman et al., 1981), 2-hydroxy permethrin in rats (Gaughan et al., 1978) and 6-hydroxy permethrin in houseflies (Shono et al., 1978).

Carp liver microsomal esterases hydrolyze transpermethrin much more extensively than cis-permethrin and the same relationship holds good for rainbow trout liver microsomes, although they appear to be less active (Glickman, 1979).
Proposed degradation pathways for Cypermethrin in soils

[] - Degradation products of Cypermethrin detected only in the teaching study
The detoxification of all pyrethroids in mammals and at least some pyrethroids in insects and fish is due to the involvement of the microsomal mixed function oxidase (MFO) system. The sites of oxidation and the rates vary with the pyrethroid structure and with different species (Soderlund and Casida, 1977b; Shono and Casida, 1978). The cyano-phenoxymethyl pyrethroids are known to oxidize more slowly than the other compounds. The low sensitivity of mammals to orally administered pyrethroids appears to result from a rapid metabolism together with poor absorption from the gastrointestinal tract (Miyamoto, 1976; Soderlund and Casida, 1977a, b).

Shinoi Sakata et al. (1986) described the degradation pathways of cypermethrin on soil surface. He reported that cypermethrin was degraded via pathways including cleavage of the ester or diphenyl ether bond, hydroxylation at the 4-position of the phenoxy ring and hydrolysis of the cyano group to the amide and carboxyl groups. The main degradation route was hydrolysis of the ester linkage. The resultant products underwent further degradation to form $^{14}$CO$_2$ and bound residues (Fig. 2).

Resistance and susceptibility to pyrethroids

Susceptibility and resistance varies from animal to animal (even though they belong to same group) and chemical to chemical. It may depend on the route of administration of chemical, size and sex of the test species and presence or absence of the enzyme systems that degrade pesticide.
A strain of *Spodoptera littoralis* from Egyptian cotton fields showed resistance to permethrin (Holden, 1979) but was not resistant to cypermethrin. Resistance to permethrin and fenvalerate has been reported in *Heliothis virescens* from the imperical valley, California (Twine and Reynolds, 1980). Adult fish, *Tilapia mossambica* was more susceptible to fenvalerate than that of juvenile (Yellamma et al., 1989). High levels of resistance have been noted for several species of noctuid larvae and for aphids and cattle ticks. Earlier work on houseflies, mosquitoes, and cotton pests has demonstrated pyrethroid resistance of various types (Farnham, 1973; Farnham and Sawicki, 1976; Farnham, 1977; Devries and Georghiou, 1980; Fullbrook and Holden, 1980; Priester and Georghiou, 1980). Malinowski Lenryk (1986) reported that when the houseflies (*Musca domestica*) were subjected to artificial sectors for development of resistance to pyrethroid pesticides, after 20 generations, resistance to deltamethrin increased 42-fold, resistance to cypermethrin increased 35-fold, and fenvalerate resistance 23-fold. Larvae and adult *Drosophila melanogaster* were more susceptible to cypermethrin than fenvalerate, and adult males were more sensitive than females to both insecticides (Bataste-Alentorn et al., 1987).

Of several types of resistant factors, kdr factor, i.e., resistance to knockdown is found to be the most important in pyrethroid resistance in houseflies (Elliott et al., 1978). The kdr form of resistance has been demonstrated to be due to site insensitivity factor (Miller et al., 1979; Osborne and Hart, 1979). More recently, a "super-Kdr" strain has been isolated from a multiresistant housefly strain (Sawicki, 1978). This strain shows considerably higher resistance to pyrethroids than the kdr strain when compared to susceptible cooper strain. Nicholson et al (1980) reported that
neither penetration nor metabolic degradation was significantly different between the susceptible and kdr strains suggesting that the resistance factor involves an insensitivity at the site of action in the nervous system.

Present study

Pesticides are now used extensively for eradication of various pests and to protect the crops. Among several factors that contribute to disturbance and imbalance of ecosystem, the pesticides are noteworthy. Excessive and indiscriminate use of pesticides in field operations made them to translocate into the aquatic environs directly or indirectly. Most of the pollutants in the aquatic organisms are reported to be in the form of particulate matter. These particles are absorbed into the surface of the plankton which in turn are ingested by the higher aquatic edible species like fishes, crabs and prawns. This indicates that impact of pesticides is bidirectional i.e., in one way they control the pests and in other way they affect the non-target animals (including man) through biomagnification.

Pyrethroids are also widely used for general pest control in agriculture because of their excellent insecticidal properties combined with remarkably low mammalian toxicity. Their insect (topical)-mammalian (oral) toxicity ratio is much better than for the majority of other organic insecticides. The pests which have already developed resistance to pesticides are susceptible to pyrethroids. Even though there is much work on the toxic effects of pesticides, little attention was given to study the effects of pyrethroids on non-target animals. Hence, in the present investigation, studies were made to understand the effect of a synthetic pyrethroid, Cypermethrin on a non-target field crab, Oziotelphusa senex senex in view of its
widespread use for the control of all types of insect pests. The rationalae behind selecting the field crab as test animal are that these crabs are commonly inhabitating in the paddy fields, where the pesticides are generally liberated into the aquatic system, these crabs are particularly relevant test species because of their economic value as highly proteinaceous food, and they may be adversely affected by direct toxicity or indirect damages caused by these toxicants.

Since the α-cyano group pyrethroids are well known for their action on the central nervous system and highly innervated tissues like muscles are more sensitive, it was appropriate to carry out all the investigations on the central nervous system (CNS) and pedipalpal muscle (PM). As it was reported that pyrethroids block neurotransmission by inhibiting the concerned neurotransmitter enzymes, a study was conducted on cholinergic system, estimating the AChE, BuChE activity and ACh content. In order to correlate the biochemical changes and physiological status of the animal a study on some aspects of protein metabolism was also conducted. To record the pattern of energy breakdown under toxic stress, energy source enzymes like ATPases and phsophatases were also estimated.
Material & Methods
SECTION - I

A. EXPERIMENTAL ANIMAL:

The fresh water field crab, *Oziotelphusa senex senex* (Fabricius) was used as the experimental non target species model in the present investigation.

Classification:

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Arthropoda</th>
</tr>
</thead>
<tbody>
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<td>Sub phylum</td>
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</tr>
<tr>
<td>Class</td>
<td>Crustacea</td>
</tr>
<tr>
<td>Sub class</td>
<td>Malacostraca</td>
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<td>Eumalacostraca</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Section</td>
<td>Brachyura</td>
</tr>
<tr>
<td>Genus</td>
<td>Oziotelphusa</td>
</tr>
<tr>
<td>Species</td>
<td>Senex</td>
</tr>
<tr>
<td>Variety</td>
<td>Senex</td>
</tr>
</tbody>
</table>

B. PROCUREMENT AND MAINTENANCE OF THE TEST SPECIES:

*Oziotelphusa senex senex* is an edible fresh water crab, normally inhabiting the paddy fields and irrigation canals of South India. It makes burrows in soft mud along the edges of the paddy fields and canals. It lives in burrows which are partially filled with water. These animals can survive longer periods on land, but they do not inhabit brackish or saline water. They
are carnivors, feeding on worms, insects etc., and are also cannibals, feeding on younger crabs.

Crabs were collected from paddy fields and irrigation canals in and around Tirupati. They were brought to the laboratory and maintained in large polythene troughs containing tapwater. They were exposed to natural day-night cycles. The temperature of water was 26 ± 1°C in winter and 31 ± 1°C in summer. The differences in the results due to these variations, if any, were not taken into consideration. They were fed with pieces of meat ad libitum. Feeding was stopped 1 day before the commencement of the experiment to avoid metabolic variations, if any, due to diet. Healthy male crabs weighing approximately 27 ± 2g were selected for the investigation.

C. SELECTION OF PESTICIDE:

The insecticide used in the present investigation was Cypermethrin, which comes under the category of α-cyano phenoxy benzyl containing group of synthetic pyrethroids. It is an extensively used insecticide to control a wide range of sucking and chewing insect pests. Cypermethrin is one of the best insecticides among the synthetic pyrethroids for the control of a large variety of pests which cause ravages to health and agricultural sectors.

Technical grade pyrethroid, cypermethrin (92% w/w min) obtained from Bharat Pulverising Mills Private Limited, Bombay, was used as the test insecticide. The compound has the following physico-chemical properties.
Sl.No  | Property                      | Details                                                                 
-------|-------------------------------|-------------------------------------------------------------------------
1.     | Common name                   | Cypermethrin (Ripcord)                                                  
2.     | Chemical name                 | (RS)α-cyano-3-phenoxy benzyl (IRS) -cis, trans-3-(2-2-dichloro vinyl)-2-2-dimethyl cyclopropane carboxylate. 
        |                               | (IUPAC) cyano(3-phenoxy phenyl) methyl 3-(2,2-dichloro ethenyl)-2,2-dimethyl cycle propane carboxylate (CA). 
3.     | Structural formula            | ![Structural formula](image)                                            
4.     | Empirical formula             | $C_{22}H_{19}Cl_2NO_3$                                                  
5.     | Molecular weight              | 416.3                                                                   
6.     | Appearance                    | Yellow brown viscous liquid                                             
7.     | Density                       | 1.12 at 22°C approximately                                               
8.     | Vapour pressure               | $5.1 \times 10^{-8}$ at 70°C                                             
10.    | Stability                     | Wide stability in neutral and weakly acidic media, only slight photo chemical decomposition. 

D. SELECTION OF SOLVENT:

Solubility of any insecticide varies from group to group and chemical to chemical. Some insecticides are soluble in water but some are insoluble. The accumulation of pesticides in animals system is characterised by their low solubility in water. Hence, some organic solvents like acetone, ethanol, benzene etc. are needed to solubilize the insecticides. In the present study acetone was selected as the solvent after due trials with diaxon, acetone, alcohol, chloroform and benzene. From these preliminary studies it was clear that acetone was the least toxic of all them, causing no perceptible toxic effects on the crabs, and the concentration of acetone used had no toxic effects on the physiology of the animal.

E. TOXICITY EVALUATION OF CYPERMETHRIN:

(i) Determination of LC$_{50}$:

Static bioassay experiments were conducted as suggested by Doudoroff et al., (1951). Male crabs of approximately equal size and weight (27 ± 2 g) were used in all the tests. A stock solution of 100 ppm (mg/litre) was prepared in acetone and mixed in water to make required dilutions. Fresh stock solutions were used for each exposure. The medium in which the animals were maintained was replaced for every 24 h with fresh water in order to prevent the accumulation of excretory products of animals and possible biodegradation products of pesticides.

Different concentrations (0.5, 1, 2, 3, 4 and 6 ppm) were used and for each concentration 10 crabs were exposed in two litres of test solution. Six replicates were maintained for each concentration. The number of crabs died in each concentration was recorded after 48 h of exposure. The
concentration that kills 50% of the crabs in 48 h duration (LC$_{50}$) was determined by probit analysis (Finney, 1964). The average mortality at each concentration was taken to determine LC$_{50}$ by graphic plots of probit mortality versus log concentrations.

(ii) Selection of sublethal concentration:

It is well known that acute exposure of animals to high concentrations was always lethal, causing death. Death may occur under lethal exposures before behavioural manifestations of toxicity could be observed. As such observation of such behavioural changes could be possible only during sublethal exposures. It is reported that long-term sublethal exposures may be dangerous to the organism (Anderson and Paterson, 1969). Although the test species looks normal due to apparent development of tolerance during the course of time, continuous exposure may give rise to many behavioural abnormalities. Further, it has been pointed out by Edwards (1973) that sublethal (low) concentrations of pesticides offer an excellent opportunity to observe behavioural and physiological changes in animals due to pesticides from close angles over a prolonged period. Hence, in the present study, 1/4 of LC$_{50}$ has been selected as the sublethal concentration.

(iii) Time-concentration dependency:

The effect of any pesticide is mainly related to the time of exposure and its concentration. In view of this, experiments were conducted using both sublethal and lethal concentrations, and a time course study was also done to evaluate the effect of the pesticide in relation to the time of exposure. All the experiments were carried out at 3, 6, 12, 24 and 48 h at both sublethal and lethal concentrations.
Besides the above ambient exposure studies in vitro experiments were also carried out to evaluate the direct effect of cypermethrin on some selected enzyme systems. For in vitro studies, the concentrations of cypermethrin selected varied from 5 to 200 μ moles viz., 5, 10, 50, 100, 150 and 200 μ moles.

F. FACTORS LIKELY TO INFLUENCE TOXICITY:

The following factors are likely to contribute to several variations in toxicity. Measures were taken in the present study to nullify them to the extent possible.

(i) Species specificity:

Since the toxicity of synthetic pyrethroids is known to vary for several species of fish (Marking 1974; Marking and Mauck, 1975) and for salmoids (Mauck et al., 1976), only one species of crabs, viz., Oziotelphusa senex senex was chosen for the present investigation.

(ii) Size:

The size of the test species is one of the important factors contributing to the differential toxicity. The susceptibility is known to differ with size of houseflies and mousquities (Page et al., 1949). The LC_{50} values obtained for fish, *Tilapia mossambica* also varied with size (Yellamä et al., 1989). So in the present study, male crabs of almost equal size, weighing about 27 ± 2g were used throughout.
(iii) Sex:

Sex of the test species also influences the toxicity of pesticides and plays an important role in toxicity evaluation (Chan et al., 1982). Since the reproductive cycle of females may induce some changes in normal metabolism, male crabs were chosen for experimental analysis in the present study.

(iv) Temperature:

The toxicity of pesticide is known to alter with fluctuations in temperature (Chadha et al., 1964; Herzberg et al., 1980). It was reported that pyrethroids show negative temperature coefficient (i.e., increased toxicity at low temperature), Narashahi (1971 a,b). Hence the toxicity studies were conducted at moderate cool conditions (30°C).

(v) Nutritional status:

It also contributes to differential toxicity to the test chemicals (Das and Garg, 1981). So crabs were fed regularly with pieces of meat ad libitum for one week before using them for experimentation.

G. PREPARATION OF DIFFERENT CONCENTRATIONS OF PESTICIDE:

A stock solution (100 mg/ml) of cypermethrin was prepared in acetone and appropriate amounts were aliquoted from this stock solution to prepare various concentrations of the chemical in water. Thus, 1 ml of stock solution contained 100 mg, 0.1 ml contained 10 mg, 0.01 ml contained 1 mg of cypermethrin. All these required concentrations were prepared just before experimentation.
H. SELECTION OF TISSUES FOR BIOCHEMICAL ANALYSIS:

Pyrethroids are found to be potential neurotoxic substances. Since highly innervated tissues like brain and muscle are more sensitive to pesticides, it was appropriate to carryout all the investigations on the central nervous system (CNS) and muscle. The CNS consists of brain, thoracic ganglia and abdominal ganglia and the muscle tissue selected in the present investigation was pedipalpal muscle (PM). All the behavioural, biochemical and physiological observations were done at 3, 6, 12, 24 and 48 h of exposure of crabs to both sublethal and lethal concentrations of cypermethrin.

I. ISOLATION OF TISSUES:

To isolate the central nervous system (CNS), crabs were dissected from the dorsal side and the CNS consisting of the brain, thoracic ganglia and abdominal ganglia was carefully isolated from each crab. The pedipalpal muscle (PM) tissue was obtained by cutting open the exoskeleton of the chelate leg and scraping the muscle from the interior with a scalpel. In all the experiments tissues were pooled from atleast six animals, and each experiment was repeated six times. The tissues were blotted and weighed in an electrical balance to the nearest milligram. After weighing, separate homogenates of the required percentage were prepared for estimating the enzyme activities. The entire process of preparation of tissue sample was carried out in few minutes.
1. Acetylcholinesterase (AChE) (Acetylcholine acetylhydrolase, E.C. 3.1.1.7) activity:

AChE activity was estimated by the method of Metcalf (1951) as given by Augustinsson (1957).

1% homogenates of CNS and PM were prepared in 0.25 M ice cold sucrose solution. The reaction mixture containing 1.0 ml of buffer substrate (contained 8 μ moles of acetylcholine - chloride) 100 μ moles of phosphate buffer (pH 7.2) and 0.1 ml of crude (uncentrifuged) homogenate of CNS or 0.4 ml of PM was incubated in hot water bath maintained at 37°C for 30 minutes. After incubation, the reaction was stopped by adding 2 ml of alkaline hydroxylamine hydrochloride followed by shaking and then filtered. To the clear filtrate, 0.5 ml of ferric chloride solution was added and the brown colour developed was measured against a blank at 540 nm in a spectrophotometer. The enzyme activity was expressed as μ moles of acetylcholine hydrolysed/mg protein/hour.

2. Acetylcholine content (ACh):

Acetylcholine content was estimated by the method of Metcalf (1951).

The CNS and PM tissues were isolated and weighed accurately. They were transferred into pyrex glass tubes and exposed to boiling water for 5 minutes to inactivate the enzyme, AChE. The tissues were then homogenised in 1 ml of distilled water. To the homogenate 2 ml of alkaline
hydroxylamine hydrochloride was added followed by 1 ml of 1:1 hydrochloric acid. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5 ml of ferric chloride solution was added and the colour developed was read at 540 nm against the blank. The acetylcholine content was expressed as \( \mu \) moles of acetyl choline present/g wet wt. of tissue.

3. Butyryl cholinesterase (BuChE) (Acylcholine acylhydrolase, E.C. 3.1.1.8) activity:

BuChE activity was assayed by the method of Metcalf (1951).

1% homogenate of CNS and 10% homogenate of PM were prepared in 0.25 M cold sucrose solution. The reaction mixture contained 1 ml of CNS or 2 ml of PM crude homogenate, 1.0 ml of substrate buffer solution containing 8 \( \mu \) moles of butyrylcholine chloride and 100 \( \mu \) moles of phosphate buffer (pH 7.2) was incubated in hot water bath at 37\(^{\circ}\)C for 30 minutes. After incubation, the reaction was stopped by adding 2 ml alkaline hydroxyl amine hydrochloride followed by 1 ml of 1:1 hydrochloric acid. The contents were thoroughly mixed by shaking and then filtered. To the clear filtrate 0.5 ml of ferric chloride solution was added and the brown colour developed was measured against blank at 540 nm in a spectrophotometer. The enzyme activity was expressed as \( \mu \) moles of butyrylcholine hydrolysed/mg protein/h.

4. Total proteins:

Total protein content was estimated by the method of Lowry et al., (1951).
1% homogenates of CNS and PM were prepared in 10% trichloroacetic acid and they were centrifuged at 1000 g for 15 minutes. The supernatant part was discarded and the residue was dissolved in 1 ml of 1N sodium hydroxide. From this, 0.1 ml was taken and 4 ml of alkaline copper reagent and 0.4 ml of folin phenol reagent (diluted with distilled water in 1 : 1 ratio) was added. After 30 minutes the developed colour was read at 600 nm in a spectrophotometer against a reagent blank. The amount of proteins present in the sample was calculated using a standard prepared from bovine albumin, and the values were expressed as mg/g wet wt. of the tissue.

5. Soluble proteins:

Soluble proteins were estimated by the method of Lowry et al., (1951).

1% homogenates of CNS and PM were prepared in 0.25 M sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant was used to estimate the sucrose soluble protein fraction. 2 ml of 10% trichloroacetic acid was added to the supernatant and centrifuged for 15 minutes at 1000 g. The supernatant was discarded and the residue was dissolved in 1 ml of 1N sodium hydroxide. To 0.1 ml of this, 4 ml of alkaline copper reagent solution was added followed by 0.4 ml of 1 : 1 folin phenol reagent. After 30 minutes the developed colour was read at 600 nm in a spectrophotometer against a reagent blank.

The amount of soluble proteins present in the sample was calculated with the help of a bovine albumin standard, and the values were expressed as mg/g wet wt. of the tissue.
6. Free amino acids:

Free amino acid content was estimated by the method of Moore and Stein (1954) as described by Colowick and Kaplan (1957).

1% homogenates of CNS and PM were prepared in 10% trichloroacetic acid and centrifuged for 15 minutes at 1000 g. To 0.4 ml of the supernatant, 2 ml of ninhydrin reagent was added and kept in boiling water bath for 6 1/2 minutes and then cooled. The contents were made up to 10 ml with distilled water. The intensity of colour developed was read at 570 nm in a spectrophotometer against a reagent blank. The free amino acid content was expressed as μ moles of tyrosine equivalents/g wet wt. of the tissue.

7. Protease activity:

Protease activity was estimated by the method of Davis and Smith (1955) with slight modification.

5% tissue homogenates were prepared in cold distilled water and centrifuged at 1000 g for 15 minutes. The supernatant fraction was used as enzyme source. The reaction mixture in a final volume of 2.0 ml contained 100 μ moles of phosphate buffer (pH 3.0) 15 mg of heat denatured haemoglobin protein and the supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was stopped by adding 2.0 ml of 10% TCA. The contents of incubated samples were filtered and the amino acid levels were determined in the filtrates. To 0.5 ml of aliquot of the filtrate, 2 ml of ninhydrin reagent was added and the
contents were heated in a boiling water bath for 6 1/2 minutes and cooled. The volume was made up to 10 ml with distilled water and the absorbance of the colour was measured at 570 nm against a reagent blank in the spectrophotometer. The proteolytic activity was represented as \( \mu \) moles of tyrosine equivalents/mg protein/h.

8. Assay of aminotransferases:

The activity of AAT and AIAT was determined by the method of Reitman and Frankel (1957) as described by Bergmeyer and Bernt (1965).

1% homogenates of CNS and PM were prepared in 0.25 M ice cold sucrose solution. The homogenates were centrifuged at 1000 g for 15 minutes. The supernatant thus obtained was used for aspartate and alanine aminotransferase assay.

a. Aspartate aminotransferase (AAT) (L-aspartate 2-oxoglutarate aminotransferase : EC 2.6.1.1) activity:

The reaction mixture of 2.0 ml contained: 100 \( \mu \) moles of phosphate buffer (pH 7.4) 100 \( \mu \) moles of L-aspartic acid, 2 \( \mu \) moles of \( \alpha \)-ketoglutarate and the supernatant as enzyme source. The contents were incubated at 37°C for 30 minutes. The reaction was stopped by addition of 1 ml of 2,4-dinitrophenyl hydrazine solution (0.001M) prepared in 0.01N HCl. This is called ketone reagent. After 20 minutes, 10 ml of 0.4N sodium hydroxide was added to all the tubes. The colour developed was read at 545 nm in a spectrophotometer against a reagent blank. The enzyme activity was expressed as \( \mu \) moles of pyruvate formed/mg protein/hr.
b. Alanine aminotransferase (A1AT) (DL-alanine 2-oxoglutarate aminotransferase: E.C. 2.6.1.2) activity:

The reaction mixture of 2 ml contained: 100 μ moles of L-alanine, 100 μ moles of phosphate buffer (pH 7.4), 2.0 μ moles of L-ketoglutarate, and supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 1 ml of 2,4-dinitrophenyl hydrazine solution prepared in 0.1 N HCl. The remaining procedure was same as that of aspartate aminotransferase.

9. Glutamate dehydrogenase (GDH) (L-glutamate and oxidoreductase: E.C. 1.4.1.3) activity:

Glutamate dehydrogenase activity was estimated by the method of Lee and Lardy (1965) with slight modification.

5% homogenates of CNS and PM were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 g for 15 minutes. The supernatant was used as the enzyme source. The reaction mixture of 2 ml contained: 40 μ moles of sodium glutamate, 100 μ moles of phosphate buffer (pH 7.2) 0.1 μ moles of NAD, 4 μ moles of INT and 0.5 ml supernatant. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was stopped by adding 5 ml of glacial acetic acid. Zero time controls were maintained by adding 5 ml of glacial acetic acid prior to the addition of homogenate. The formazan formed was extracted overnight in 5 ml of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as μ moles of formazan formed/mg protein/h.
10. Adenosine triphosphatase (ATPase) (ATP phosphohydrolase: E.C. 3.6.1.3) activity:

ATPase activity was assayed by the method of Fritz and Hamrick (1966) as reported by Desaiah and Ho (1979) with slight modification.

1% homogenates of tissues (CNS and PM) were prepared in cold 0.32 M sucrose containing 1.0 mM EDTA and 10 mM imidazole (pH 7.5). The homogenates were centrifuged at 1000 g and the supernatant obtained was used as enzyme source. The reaction mixture in a volume of 3.0 ml contained 3 mM ATP, 3 mM MgCl₂, 100 mM NaCl, 20 mM KCl, 135 mM imidazole-HCl buffer (pH 7.5) and 10-30 µg of protein. The reaction mixture was incubated at 37°C for 30 minutes and stopped by the addition of 0.1 ml of 50% trichloroacetic acid. Samples were then assayed for inorganic phosphate using the method of Lowry and Lopez (1946) as modified by Phillips and Hayes (1977). The colour was read at 800 nm in spectrophotometer.

Mg⁺² ATPase activity was measured in the presence of 1 mM ouabain, a specific inhibitor of Na⁺-K⁺ ATPase (Mc Ilwain, 1963). Ouabain sensitive Na⁺-K⁺ ATPase activity was obtained by the difference between total ATPase and Mg⁺² ATPase activity. The enzyme activity was expressed as µ moles of inorganic phosphate/mg protein/h.
11. Alkaline phosphatase (Orthophosphoric-monoester phosphohydrolase : E.C. 3.1.3.1) activity:

Alkaline phosphatase activity was estimated by the method of Bodansky (1932).

3% homogenates of CNS and PM were prepared in 0.25 M ice cold sucrose solution and centrifuged at 1000 g for 15 minutes and the supernatants were used for enzyme assay. The reaction mixture in a final volume of 9.5 ml contained: 0.5 ml of supernatant, 9.0 ml of alkaline phosphate substrate (Alkaline phosphate substrate was prepared by adding 3.0 ml of petroleum ether, about 80 ml of distilled water, 0.5 g of sodium $\beta$-glycerophosphate, 0.424 g of sodium diethyl barbiturate and water to make up the final volume to 100 ml, (pH 9.0). The reaction mixture was incubated at 37°C for 1 hour. The reaction was stopped by adding 2.0 ml of 30% trichloroacetic acid and the contents were centrifuged. Inorganic phosphate of the supernatant was estimated by the method of Fiske and Subba Rao (1925). The enzyme activity was expressed in $\mu$ moles of inorganic phosphate formed/mg protein/h.

12. Acid phosphatase (Ortho phosphoric - monoester phosphohydrolase : E.C. 3.1.3.2) activity:

The procedure followed was exactly the same as that of alkaline phosphatase except the substrate (sodium $\beta$-glycerophosphate) pH was adjusted to 5.0 with dilute acetic acid. The enzyme activity was expressed as $\mu$ moles of inorganic phosphate formed/mg protein/h.
Estimation of inorganic phosphate:

The inorganic phosphate content was estimated by the method of Fiske and Subba Rao (1925).

To 1.0 ml of the filtrate 1.0 ml of ammonium molybdate solution was added and mixed well and 0.4 ml of 1, 2, 4, aminonaphthol sulphonic acid (ANSA) reagent was added. The contents were made up to 10 ml with distilled water and allowed to stand for 5 minutes. The blue colour was measured at 660 nm in a spectrophotometer against a reagent blank. The blank contained 1.0 ml of distilled water, 1.0 ml of ammonium molybdate and 0.4 ml of ANSA was made up to 10 ml with distilled water.

SECTION - III

VALIDITY OF EXPERIMENTAL PROCEDURES

1. Aliquots for assay: Aliquots were selected for the assay such that the initial rates were approximately as near as possible, yet providing sufficient product of all in a convenient range for spectrometric measurement.

2. Enzyme units: The soluble protein content of the tissue homogenates (enzyme source) was estimated using Folin-phenol reagent (Lowry et al., 1951). This was used for expressing the enzyme activity. Enzyme activities were expressed in standard units, i.e., μ moles of product formed or substrate cleaved per mg protein per hour.
3. **Substrate requirements:** All the enzyme activity levels were determined at saturating substrate concentrations i.e., zero order.

4. **Lambert-Beer law:** All most all the products of the reactions were measured by using colorimetric procedure, in which the optical density (absorbance) of the resulting coloured complex was proportional to the concentration of the reaction products.

   Thus standard graphs were prepared for each colorimetric estimation between concentration of substance (either product or substrate) and the optical density from which the activities of enzymes of quantities of substances were calculated.

5. **Enzyme nomenclature:** The nomenclature of the enzymes followed in this dissertation is according to the report of the commission on the enzymes of the international union of bio-chemistry (Pergaman Press, Oxford, 1966).

6. **Assay of GDH by using INT:** Tetrazolium salts constitute unique classes of oxidation reduction indicators in the study of dehydrogenases. The advantage of using tetrazolium salts as electron acceptors are:
   a) the tetrazolium salts give a stable colour on reduction,
   b) they are highly insoluble in aqueous solution,
   c) they can be reduced both aerobically and anaerobically,
   d) they have high redox potential which makes the reduction easier,
   e) they can be easily permeable through membranes.
Various tetrazolium salts receive electrons from various sites of electron transport system (Nachlas et al., 1960). This is due to inherent difference in the redox potentials of various tetrazolium salts. Introduction of P-nitrophenyl group in $N_2$ phenyl region was observed to increase the efficiency of the dye by increasing its redox potential. Karmaker et al., (1959) reported that INT was superior to most of the tetrazolium salts as an electron acceptor for the assay of dehydrogenases.

7. Statistical treatment of the data: The mean, standard deviation (SD) and test of significance of student's "t" test was calculated following the method of Pillai and Sinha (1968). The formula used for calculating SD was

$$\frac{Ex^2 - (Ex)^2}{n}$$

$$n-1$$

Where, $x^2$ is the sum of square of deviation from the mean.

$n$ number of individual observations.

The significance of the deviations from normal was calculated by calculating student's "t" test by using the following formula.

$$t = \frac{m_1 - m_2}{\sqrt{\frac{SD_1^2 + SD_2^2}{n_1 + n_2 - 2}}}$$

Where, $m_1$ is the mean of first set of observations.

$m_2$ is the mean of second set of observations.

$SD_1^2$ and $SD_2^2$ are square of standard deviations of the first and second set of observations.

$n_1$ and $n_2$ are number of observations of first and second sets.
Chapter - 1
TOXICITY EVALUATION AND BEHAVIOURAL OBSERVATIONS
SECTION - A

TOXICITY EVALUATION

Introduction:

The life style of modern man has been greatly improved by chemicals. Through the use of chemical substances, we can increase food production, cure diseases and control the pests. As has been said "without chemicals, life would not be the same". Every corner of the world and every part of our life has been benefited by chemicals. With the development of new formulations, the synthesis of pesticides has come into the picture replacing the lower toxic chemicals. Every type of pesticide used is accompanied by inherent risk. Pesticides regularly strike at the species for which they are intended as well as the non-target species. Moreover, pesticide applications cannot always be confined to the intended area. Nor does their effectiveness cease after the pest population has been sufficiently reduced, often with unpredicted results.

Following decades of reckless and unconscious handling of chemicals, which have resulted in several disastrous incidences of pollution, man has recognised the need for better control of the present use and the future development of chemicals. This need has turned into a demand from the public to have chemicals tested and retested before they reach the consumers. It has now become a normal practice to test all new chemicals for their toxicity.
Toxicity is the property of a pesticide which upsets the normal activities of an organism when it is used. The assessment of lethality or toxicity would help in knowing the potentiality of the chemicals so that new and more powerful formulations may be speeded up in the manufacture of pesticides. Toxicity of a chemical can be influenced by physical factors (Herzberg et al., 1980) and biological factors (Braginskii et al., 1979; Jayantha Rao, 1981), nutritional status (Das and Garg, 1981; Pal and Kushwah, 1981), species specificity (Gouda et al., 1981; Li and Chao, 1981; Shanta Satyanarayana, 1981; Jacob et al., 1982) and chronobiology (Uttaman et al., 1979) of the animal. It also depends on the developmental stages of the animal (Salama et al., 1980) and also duration to which the animal is exposed (Abel, 1980; Gouda et al., 1981; Jacob et al., 1982). Hence, in order to arrive at a precise dosage level, it is necessary to plan a systemic model to conduct toxicity studies (NIN Special Report, 1982; NTP, 1982).

Toxic interactions of a chemical to any given biological system are dose related. The dosage of any compound is always a decisive factor in determining its effects (Hayes Jr, 1975) and hence it is important to collect information regarding dosages of pesticides that are tolerable and those that cause illness and death. Different investigators adopted different methods to assess the lethality of pesticides. There are various parameters like lethal concentration, sublethal concentration, median lethal concentration, median tolerance limit, median effective dose and safe concentration etc. to be computed for evaluating the toxicity of a pesticide. But it is customary to represent lethality of a pesticide to a particular animal species in terms of mortality and time. Most of the investigations in the assessment of toxicity of a pesticide involved in the determination of LC50 i.e. the concentration
which will kill 50% of the test species. Based on the habitat of the test animal the lethality is expressed in terms of lethal concentration in case of aquatic animals and lethal dose for terrestrial animals. It is well known that there is a direct relationship between the weight of the test animal and the dose of the pesticide. So lethal dose is always expressed in terms of micro or milligrams/kg body weight of the animal. If the pesticide is used in water, its concentration is expressed as parts per million (ppm) or parts per billion (ppb) or mg/litre. The lethality of the pesticide is also dependent on its concentration and time of exposure. Thus it is customary to express the concentration of pesticide that kills a certain percentage of test species, say 50% as LC$_{50}$ (X grams/Y hours). It represents the time required to kill 50% of the animals at a certain dose or concentration. In some instances, mostly in insects knockdown rate (KD$_{50}$) becomes an important criterion for assessing the potentiality of insecticides. In rare cases where killing or knockdown does not constitute the desired criterion the effective dose (ED$_{50}$) or effective concentration (EC$_{50}$) are used. In some cases where the availability of test species is limited, another term lethal time (LT$_{50}$) is frequently used.

Following the organochlorides (OC), organophosphates (OP) and carbamate insecticides, synthetic pyrethroids have come and their multiple beneficiary qualities have attracted the people to use them in agricultural and public health sectors. Extensive work has been done on the toxicity of pyrethroids on different animals. Pyrethroids have been reported to be highly potent insecticides as demonstrated by their excellent action on malathion-resistant and susceptible strain on Oryzaephilus surinamensis (Saleem, 1986). Synthetic pyrethroids have long been reported to be extremely toxic to fish
Murali Mohan et al., (1989) reported the LD$_{50}$ for cypermethrin to cockroach, *Periplaneta americana* as 1.99 µg/g body wt. and Stephenson et al., (1984) reported that cypermethrin is more toxic to fish, *Tilapia nilotica* and the LC$_{50}$ was found to be 2.0 µg/litre. Pyrethroids have a very low acute toxicity to birds (Elliott et al., 1978; Glickman and Casida, 1982). Permethrin possesses relatively low mammalian toxicity and LD$_{50}$ estimated for rats was 800 mg/kg (Miyamoto, 1976).

The foregone account indicates the importance of toxicity evaluation studies to assess the effects of pesticides on non-target animals and to take precautionary steps to minimise day by day increasing environmental pollution. Further, it also indicates that the toxicity of pesticides to various organisms varies from species to species and chemical to chemical. Since the biotic and the abiotic factors influence the toxicity of pesticides, in the present experiments all the factors are maintained consistent to the maximum extent possible, and wherever not possible suitable controls were maintained.

RESULTS:

In the present study the mortality rates for crabs to different concentrations of cypermethrin were determined under ambient exposure. The concentrations ranged from 1.2 ppm to 2.8 ppm (LC$_{50}$). The concentration at which 50% mortality could be obtained for 48 hours exposure was determined following the probit method (Finney, 1964). The data are tabulated in Table 1. No mortality was observed at 1.2 ppm, but the mortality started from 1.4 ppm. 10% mortality at 1.4 ppm, 20% mortality at 1.6 ppm, 30% mortality at 1.8 ppm, 50% mortality at 2 ppm, 70% mortality at 2.2 ppm, 90% mortality
at 2.6 ppm and 100% mortality at 2.8 ppm was observed. This indicates that the per cent mortality was increased with increase in concentration of cypermethrin. These concentrations were transformed to log concentrations and converted to probit kill and per cent kill, and then the corresponding graphs were plotted.

The per cent mortality when plotted against log concentrations showed a sigmoid curve (Fig. 4). The $LC_{50}$ value was found to be 2 mg/litre. When the probit mortality was plotted against log concentration, it showed a straight line (Fig. 3) and the $LC_{50}$ value obtained was found to be 2 mg/litre. Thus it is clear that the $LC_{50}$ values from both the graphs were exactly the same, indicating the accuracy of experimentation.

**DISCUSSION:**

From the above results it is evident that the lethal concentration ($LC_{50}/48$ h) of cypermethrin to field crab, *Oziotelphusa senex senex* was 2 ppm/litre. This $LC_{50}$ value is far less when compared to those of other pesticides like organochlorides (OC) and organophosphates (OP) to *O. senex senex* (Table 2). This clearly indicates that *O. senex senex* is very sensitive to cypermethrin exposure. If we look at the $LC_{50}$ values of various other pesticides for *O. senex senex* (Table 2), it is also evident that cypermethrin caused greater toxicity than many other pesticides. The $LC_{50}$ values of OC compounds like endosulfan (Kallapur et al., 1986) and BHC (Geetanjali, 1985) for *O. senex senex* were found to be 30 ppm and 28 ppm respectively. Fenvalerate (a synthetic pyrethroid) is about 100 times more toxic than DDT to *P. indicus*. It is due to fast degradation of pyrethroids, while DDT is stable and persistent (Glickman et al., 1979; Ramesh Babu et
al., 1987). The LC$_{50}$ values of OP compounds like methyl parathion (Nagaratnamma and Ramamurthi, 1981) and malathion (Bhagyalakshmi, 1981) to O. senex senex were found to be 3 ppm and 23 ppm respectively.

Neeraja Kumari (1986) reported the LC$_{50}$ value of fenvalerate (pyrethroid) on O. senex senex as 6 ppm/litre (Table 2), but in the present investigation the determined LC$_{50}$ of cypermethrin to same animal was 2 ppm/litre. Thus cypermethrin seems to be more toxic than fenvalerate though they belong to the same group. This observation finds support in an earlier report by Syed Babu (1991) showing that cypermethrin is more toxic than fenvalerate to the cockroach, Periplaneta americana. Further it was found that cypermethrin is more toxic than its analogues such as allethrin, permethrin and fenvalerate to German cockroach (Scott and Matsumura, 1983).

The toxicity and susceptibility vary with the type of pesticide compound and test species. Synthetic pyrethroids are found to be highly toxic to fish (Ghosh and Chatterjee, 1987; Richardson, 1988) and other aquatic invertebrates (Gupta, 1986; Sing and Agarwal, 1987). The LC$_{50}$ of fenvalerate to Tilapia mossambica was found to be 0.045 mg/litre (Radhaiah and Jayantha Rao, 1987). The C-cyano group containing pyrethroids like fenvalerate and cypermethrin were reported to be toxic to cockroach (Murali Mohan et al., 1988; Venkataramana Reddy and Yellamma, 1991) and the LD$_{50}$ values determined were 6.98 $\mu$g and 1.99 $\mu$g/g body wt. respectively. Yamono (1986) reported the toxicity of permethrin to 5th instar larvae of silkworm, Bombyx mori as 0.038 $\mu$g/g and 0.43 $\mu$g/g for topical application and subcutaneous injection respectively. Permethrin possesses relatively low
mammalian toxicity i.e., 800 mg/kg body wt. and it was extremely toxic to fish (Miyamoto, 1976; Zitko et al., 1977).

A number of factors contribute to the toxicity of a particular compound. These are like chemical composition, solubility, partition coefficient of the pesticide and species specificity of the animal (Yang and Sun, 1977). Neeraja Kumari (1986) reported that toxicity also depends on the size and weight of the animal. She reported that body weight of the crab exerts a marked effect on the ability of crab to withstand exposure to the toxicant. Gradual increase in the resistance was observed with the increase in the body weight of the crab, O. senex senex. Similar dose-weight dependance was observed for mice, where the mortality increased with the decrease in body weight in both male and female mice when fed with dietary concentration of 1250 ppm of fenvalerate (Parker et al., 1983). It has also been reported that the route of administration of chemical and the presence or absence of the enzymes which may degrade the pesticide contribute to wide variation in the levels of toxicity (Nagarathnamma and Ramamurthi, 1981). With the age of the test species also, the toxicity of a pesticide varies (Burdick, 1964). For example juvenile fish were more resistant than adults (Yellamma et al., 1989). This feature could be of a great survival value for the species as a whole. From all the above observations it is inferred that the LC$_{50}$ values are not constant for a group of insecticides or any particular group of animals and vary from species to species with alteration in the chemical and physical factors.

Moreover, the quality and quantity of the solvent used for dissolving the test chemicals also play a significant role in toxicity
evaluation. The effects of the solvent on the toxicity of pesticide and cuticle damage have been reported (Sun, 1970; Saxena and Saxena, 1982). To ascertain the effects of acetone, control experiments were conducted with acetone alone and it was confirmed that the concentration used was not toxic by itself to test animal *O. senex senex.*
Table 1: Mortality of crabs, *Oziotelphusa senex senex* at different concentrations of cypermethrin at 48 h of exposure in ambient medium. (Mortality expressed in both per cent kill and probit kill.)

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Concentration of cypermethrin in ppm</th>
<th>Log concentration</th>
<th>No. of Crabs Exposed</th>
<th>No. of Crabs Dead</th>
<th>Per cent kill</th>
<th>Probit kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.2</td>
<td>0.0791</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>1.4</td>
<td>0.1461</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>3.72</td>
</tr>
<tr>
<td>3.</td>
<td>1.6</td>
<td>0.2041</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>4.16</td>
</tr>
<tr>
<td>4.</td>
<td>1.8</td>
<td>0.2552</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>4.48</td>
</tr>
<tr>
<td>5.</td>
<td>2.0</td>
<td>0.3010</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>5.00</td>
</tr>
<tr>
<td>6.</td>
<td>2.2</td>
<td>0.3424</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>5.52</td>
</tr>
<tr>
<td>7.</td>
<td>2.4</td>
<td>0.3802</td>
<td>10</td>
<td>9</td>
<td>90</td>
<td>6.28</td>
</tr>
<tr>
<td>8.</td>
<td>2.6</td>
<td>0.4149</td>
<td>10</td>
<td>9</td>
<td>90</td>
<td>6.28</td>
</tr>
<tr>
<td>9.</td>
<td>2.8</td>
<td>0.4471</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>7.33</td>
</tr>
</tbody>
</table>
Table 2: Acute toxicity of different pesticides on crab, *Oziotelphusa senex senex*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the chemical</th>
<th>Animal Species</th>
<th>Period of Exposure</th>
<th>Concentration LC$_{50}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Endosulfan</td>
<td><em>Oziotelphusa senex senex</em></td>
<td>96 h</td>
<td>30.0 ppm</td>
<td>Kallapur et al., (1986)</td>
</tr>
<tr>
<td>2.</td>
<td>Endosulfan</td>
<td>&quot;</td>
<td>96 h</td>
<td>15.14 ppm</td>
<td>Rajendra Prasad (1990)</td>
</tr>
<tr>
<td>3.</td>
<td>B H C</td>
<td>&quot;</td>
<td>48 h</td>
<td>28.0 ppm</td>
<td>Geetanjali (1985)</td>
</tr>
<tr>
<td>5.</td>
<td>Malathion</td>
<td>&quot;</td>
<td>48 h</td>
<td>23.0 ppm</td>
<td>Bhagayalakshmi &amp; Ramamurthi (1981)</td>
</tr>
<tr>
<td>6.</td>
<td>Fenvelerate</td>
<td>&quot;</td>
<td>24 h</td>
<td>10.0 ppm</td>
<td>Neeraja Kumari (1986)</td>
</tr>
<tr>
<td>7.</td>
<td>Fenvalerate</td>
<td>&quot;</td>
<td>48 h</td>
<td>6.0 ppm</td>
<td>Neeraja Kumari (1986)</td>
</tr>
<tr>
<td>8.</td>
<td>Cypermethrin</td>
<td>&quot;</td>
<td>48 h</td>
<td>2.0 ppm</td>
<td>Present investigation</td>
</tr>
</tbody>
</table>
LEGEND

Fig. 3: Graph showing the straight line relation between log concentration of cypermethrin and probit mortality of the crab, *O. senex senex* at 48 h in the ambient medium.

Fig. 4: Sigmoid curve showing the relation between log concentration of cypermethrin and per cent mortality of the crab, *O. senex senex* at 48 h in the ambient medium.
SECTION - B

BEHAVIOURAL OBSERVATIONS

Introduction:

Behaviour includes all those processes by which an animal senses the external world and internal state of its body, and responds to changes which it perceives. Many of such processes will take place inside the nervous system and may not be directly observed but reflected through the behaviour of the animal. When a toxic compound is administered, some changes occur from the normal behaviour, and they can be observed externally. These behavioural changes would be caused by the changes in the nervous system caused directly or through metabolic or physiological activities.

Earlier experiments on fiddler crabs (Barnwell, 1966; Arechiga et al., 1974), cray fish (Rice and Armitage, 1974) and slugs (Ramamurthi and Sainathjanak, 1973; Pavanakumar, 1976) have demonstrated a good correlation between physiological activities, metabolic changes and behaviour of these animals. Leake (1977) reported an interesting correlation between poisoning symptoms and aberrant electrical activity in the central neurons of the leech, Hirudo medicinalis, during S-bioallethrin poisoning. Narahashi and Lund (1980) reported that hyperactivity in the central nervous system of the crayfish, Procambus clarki was correlated with overall behavioural excitation from pyrethrum treatment and the paralysis was associated with the block of impulse conduction in the nervous system. Behavioural responses involve the most complex processes of sensory input, central processing and motor output points to neurotransmitters systems, as key components of these molecular
events (Russell, 1978). In recent times, increasing attention has been paid to
the neurotransmitter systems and their role in the behaviour of intact
organisms as they react to, and manipulate their external physical and social
environment.

Behavioural toxicology has assumed a key place of increasing
importance in evaluation of toxic compounds which affect not only target
animals but also non-target animals, as it confirms the potentiality of the
compound and also the tolerance limit of the animal. It was reported that
alterations in the chemical composition of the natural aquatic environment
usually affects the behavioural and physiological system of the inhabitants (O' Brien, 1967; Edwards, 1973). Fishes and other aquatic organisms, including
crustaceans, are sensitive indicators of the quality of aquatic environment,
since they are susceptible to low concentrations of several insecticides. It is
known that insecticides have profound physiological and biochemical effects
on crustaceans, the magnitude of which vaires with the concentration and
duration of exposure.

The first step in collecting information on the site and mode of
action of insecticides is the categorization of poisoning symptoms. It was
found that there are two types or classes of pyrethroids based on the
symptoms produced (Barnes and Verschoyle, 1974). The type-I poisoning
syndrome or "T" syndrome is characterized by restlessness, incoordination,
prostration and paralysis in the cockroach (Gammon et al., 1981) and
aggressive sparring behaviour, elevated startle response, whole body tremors
and prostration in the rat (Verschoyle and Alridge, 1980). Type-II poisoning
syndrome or "CS" syndrome is characterized by incoordination, convulsions
and intense hyperactivity in cockroach (Gammon et al., 1981, 1982), whereas in rats, burrowing behaviour, tremors, clonic seizures and profuse salivation without lacrimation (Verschoyle and Aldridge, 1980) are observed. Type I or "T" syndrome was produced by pyrethroids without α-cyano substituent affecting the central nervous system, whereas Type-II or "CS" syndrome is produced by compounds with α-cyano group, exerting its action only on the central nervous system (Barnes and Verschoyle, 1974). Pyrethroids were equally toxic whether applied topically or injected and no differences were found in their relative overall toxicities (Wouters and Van den Berken, 1978). Burt and Good Child (1974) have suggested that injection of pyrethroids knocks the insects down much more rapidly than applying the insecticides topically. Thus the central nervous system is the primary site of action for knockdown, rather than the peripheral nervous system.

These poisoning symptoms are apparently not due to a primary interference with a single inhibitory transmitter such as γ-amino butyric acid (Smith, 1980; Cremer et al., 1980). There is little evidence to suggest a selective action on any of the other central neurotransmitters since a number of centrally acting pharmacological antagonists produce little modification of the poisoning syndromes (Staatz et al., 1982).

The literature available on behavioural changes of aquatic animals with reference to pyrethroids toxicity is scanty though the synthetic pyrethroid insecticides like cypermethrin, fenvalerate, decamethrin etc. are extensively used in agricultural sectors. The reports of Bradburg et al., (1986), Radhaiah and Jayantha Rao (1988) and Yellamma et al., (1989) showed significant behavioural changes in fish such as hyperactivity, jerky
movements and frequent opening of operculum following fenvalerate administration. Neeraja Kumari (1986) also reported similar behavioural changes like impaired locomotion and hyperactivity in crabs under fenvalerate intoxication. But the reports regarding the effects of cypermethrin and decamethrin on aquatic animals seem to be very less. Hence in the present study an attempt has been made to analyse the behavioural changes in crabs to cypermethrin intoxication under both sublethal and lethal exposures.

RESULTS:

Cypermethrin intoxication caused significant behavioural changes in the field crab, Oziotelphusa senex senex at both sublethal and lethal concentrations. The more obvious changes included restlessness for some time followed by impaired locomotion and oozing of dark frothing fluid from mouth after 1 hour of exposure. During initial phase of exposure, crabs exhibited hyperactivity moving about the trough with a sense of urgency as if to avoid something. After this excited phase, they stayed at one place for some time (few minutes) but continued to beat their walking legs one after the other probably in a bid to regain the original locomotor rhythm. At this stage excess mucous exudation was observed from the mouth.

Under lethal concentration, these behavioural changes were noticed at 1 h after exposure. The crabs did not feed, and exhibited weak response to external stimuli like disturbances including sound, touch and pricking. Dark fluid started coming out of the mouth and the limb movement was stopped after some time and the animal reached a state of flaccid paralysis. Locking of the pedipals within one another and elevated posture on
the tips of walking legs was also observed, and they gradually fell into a state of lethargy. Some of these anomalies were shown in Plate I.

At sublethal concentration, the crabs responded in a different manner. The behavioural changes were noticed at 3 h after exposure. The onset of knockdown was delayed. Snapping of mandibles and regurgitation of mouth were specifically observed. All these were slowly intensified during later hours, reached peak level at 12 h. Thereafter, these behavioural changes subsided and the animal started showing the tendency towards normalcy attaining full recovery at 48 h after exposure.

The behavioural signs observed in Oziotelphusa senex senex during sublethal concentration of cypermethrin were not as severe as those produced at lethal concentration.

DISCUSSION:

The observations made in the present study on the behaviour of Oziotelphusa senex senex following exposure to cypermethrin include irregular movements, impairment of locomotion, weak response to external stimuli and paralysis. These behavioural changes indicate the disturbance in the central nervous coordination. Animal behaviour is a neurally regulated phenomenon mediated by the brain and neurotransmitters (Bullock et al., 1977). Pyrethroids are neurotoxicants which act directly on excitable membranes and interfere with membrane ionic conductance in target organisms (Wouters and Van den Bercken, 1978; Lund and Narahashi, 1981). There are reports that the presence of α-cyano substituent in pyrethroids exerts its effects on the central nervous system (Barnes and Verschoyle, 1974). In the present study
irregular movements, weak response to external stimuli and paralysis are some of the behavioural symptoms which are indicative of the influence of cypermethrin on the central nervous system.

Type II compounds such as cypermethrin, fenvalerate, decamethrin etc. act on the central nervous system. Any disruption in the central nervous system will affect the general behaviour of animal. Reports are there to signify the effects of pyrethroids on the locomotor activity of insects. Topical application or injection of cypermethrin results in hyperactivity, restlessness, ataxia and paralysis in the cockroach, Periplaneta americana (Murali Mohan et al., 1989; Sesha Reddy, 1990). In the present study, irregular movements of crabs on cypermethrin intoxication indicate the impairment of the nervous system by the pesticide. Supporting evidence comes from the studies of Neeraja Kumari (1986) who reported similar defects in locomotor activity of crabs upon exposure to fenvalerate. Further it was observed that contact or oral administration of pyrethrins to honey bees resulted in (1) hyperactivity, restlessness running and frantic flying; (2) ataxia with zigzag flight and locomotion; (3) paralysis ascending from legs to still the buzzing wings; (4) complete paralysis with opisthotonus of abdomen (Botteher, 1939). These disorders were suggested as due to certain changes in sodium channels by the pesticide (Narahashi, 1984).

In the present study a progressive deterioration in feeding habit was observed in crabs during lethal exposure to cypermethrin. Similar reports were made in crabs with the pyrethroid fenvalerate (Neeraja Kumari, 1986), organophosphate methyl parathion (Bhagyalakshmi, 1984) and organochloride endosulfan (Rajeswari, 1990). In addition to the above, reports are available
showing passive feeding in different animals such as cockroach (Komala Kumari, 1988; Shesha Reddy, 1990; Syed Babu, 1991), rat (Lakshmirmajyam, 1991) and fish (Radhaiah and Jayanth Rao, 1988) under pyrethroid toxicity.

In the present study oozing of dark fluid from the mouth of crab during cypermethrin intoxication was noticed. Similar behaviour was observed on crabs during sumithion (Bhagyalakshmi, 1991), fenvalerate (Neerajakumari, 1986) and endosulfan (Rajeswari, 1990) intoxication. Mucous secretion from the foot in fresh water snail, *Pila globosa* was also reported (Ramana Rao, 1978) under exposure to sumithion. Besides these, hemorrhages were observed in the form of tearing of the tissues in crab and also appearance of coagulated film on the mantle and the interior visceral parts. These may be attributed to the histological damage caused to the tissues due to cypermethrin intoxication. Radhaiah *et al.*, (1988) reported histological changes in gill, intestine, liver and kidney in *Tilapia mossambica* upon fenvalerate exposure. Hemorrhages were observed at the basis of dorsal and ventral fins in *Cyprinus carpio* (Nagaratnamma, 1982). All these reports signify histological damages as well as intolerance of the animal to pesticide treatment.

It is well known that the behaviour of the animal reflects the physiological status of the body (Arechiga *et al.*, 1974; Rice and Armitage, 1974). The observed changes in the behaviour of crabs, in the present study upon exposure to cypermethrin might reflect its effects on the enzyme system (AChE, transferases and ATPases), electrical activity, energy metabolism and other physiological factors, which contribute to the incoordination (Dahm, 1971; Weiden, 1971). Further evidence comes from the
studies of Neeraja Kumari (1988), Radhaiah, (1988), Yellamma et al. (1989) and Venkataramana Reddy and Yellamma (1991) who demonstrated that pyrethroid compounds induced significant changes in cholinesterase system, ATPases, electrical activity etc. in different animals.

An interesting observation made in the present study was that the behavioural changes were more pronounced under lethal exposure than sublethal exposure. These changes were only short-lived and within 48 h the crabs recovered from these effects under sublethal exposure only, but no recovery was made under lethal exposure. It may be suggested that for any pesticide to produce a desirable effect, it might be applied in adequate concentration at its site of action. The most relevant factor is the amount of a chemical administered. Others include the extent and rate of absorption from the area of administration into blood stream, distribution to various parts of the body from the blood, binding of localization in tissues and mechanism of inactivation. Further, non-recovery of behavioural normalcy under lethal concentration may be due to the high concentration of pesticide, which might have caused irreversible damage to the functioning of the nervous system. The recovery under sublethal concentration is presumably due to the detoxification mechanism developed by the animal. Which were probably not as efficient under lethal concentration and hence the animals could not recover from the effects even by 48 h of exposure.

Clements and May (1977) concluded that characteristic neurophysiological effects of pyrethroids, particularly those of the peripheral sensory structures, indicated that structural rather than physical characteristics were more important in determining the speed of knockdown
and the potency of the pesticide in particular. The general ill-health and overall decline in physical ability due to disorders in central nervous system, in combination pose a great threat on the survival of the crabs.
LEGEND FOR PLATE-I

Some behavioural changes in *Oziotelphusa senex senex* following exposure to sublethal and lethal concentrations of *Cypermethrin* in the ambient medium.

A. Normal crab.

B. Crab exposed to sublethal concentration showing snapping of mandibles. Other changes such as impaired locomotion, oozing of dark frothing fluid from the mouth etc. could not be depicted in the photograph.

C. Crab exposed to lethal concentration, lying on its dorsal side, indicating a moribund disposition. Other changes such as oozing of dark frothing fluid from the mouth, locking of the chelae of pedipalps etc. could not be depicted in the photograph.