ABSTRACT

The thesis describes the effect of an organophosphate (OP) insecticide, fenitrothion on the certain aspects of the physiology of castor semilooper *Achaea janata* a major defoliator of castor *Ricinus communis*. The main objective was to provide evidences on the physiological perturbations produced by the topical application of the insecticide.

In order to exert its characteristic effects seen *in vivo* an insecticide must arrive at the site(s) of action (target) in appropriate concentration for its interaction. Toxic interactions of insecticide on biological system are dependant on dose and rate of penetration. Penetration of OP compound through insect cuticle is affected by variety of factors including the temperature. The effect of temperature was investigated on fenitrothion toxicity using 5th instar larvae. Both sublethal and lethal doses of fenitrothion demonstrated positive temperature coefficient, wherein as the temperature increased the toxicity of the insecticide was also increased. The LD50 value was considerably reduced at higher temperature (38°C) compared to the normal temperature (28°C). The toxic effect of fenitrothion was manifested into various types of poisoning sequences, viz., hyperactivity, tremors, convulsions, prostration/paralysis and finally the death. These observations provided the first indication that the primary action of fenitrothion was on the central nervous system (CNS). Hyperactivity tremors and convulsions were characterized by vigorous body movements. The oxygen consumption of the treated larvae was significantly affected. The succinate oxidation (an index of oxidative metabolism) by the poisoned fat body was increased significantly during the first 4 h and reduced at 5 h. Increased oxygen uptake during the first 4 h indicated the higher energy
yielding activity of the fat body. Insect fat body is highly aerobic organ and fenitrothion affected the respiratory mechanism at the organism level as well as at the tissue level.

The effect of fenitrothion on the metabolism of fat body, hemolymph and musculature was further investigated by determining the fuel reserves of different tissues. The glycogen and acylglycerol reserves of fat body and trehalose as well as free fatty acid contents of the hemolymph were significantly depleted at the prostration stage of poisoning. Depletion of the fuel reserves suggested that they were used by the larvae during their intense muscular activity such as hyperactivity, tremors and body convulsions which occur during poisoning sequences. In insect, the fat body metabolism is controlled by the hormones secreted from the neuroendocrine glands of the head region. It was believed that the fenitrothion effect on the fat body metabolism was mediated through the indiscriminate release of neurohormones. The fat body of the head-ligated larvae (in which case the release of neurohormones from the anterior region was eliminated) showed significant increase in glycogen and acylglycerol with concomitant depletion of free fatty acids and trehalose from the hemolymph. The significant build up of glycogen and acylglycerol in the fat body might be due to the rapid uptake of precursors from the hemolymph. The fenitrothion treatment to the head ligated larvae caused significant depletion only in the carbohydrate content of the different tissues. These results emphasized the metabolic adjustments of the castor semilooper larvae under two different physiological treatments which produce "stress" effect.

These findings suggested that fenitrothion disrupted the fat body metabolism. Uptake of labeled palmitic acid and leucine were investigated to probe further into the poisoning effect of the insecticide on the fat body metabolism.
The insecticide inhibited *in vivo* incorporation of labeled palmitic acid into tri- and diacylglycerol fractions. The incorporation of labeled leucine into fat body proteins was also inhibited. The release of diacylglycerol from the fat body into the incubation medium was also inhibited in the presence of fenitrothion. It was suggested from these findings that fenitrothion, a lipophilic insecticide exerts some of its toxic effect on the membrane lipids and disrupt the permeability of the cell membrane. It has already been established that fenitrothion inhibited respiratory mechanism which may prevent glyceride synthesis in the fat body cells.

The larvae treated with fenitrothion showed regurgitation of body fluid through the mouth. This means that the insecticide is acting on the gut wall as well. In castor semilooper larvae midgut is the main site of digestion and absorption. The activities of four digestive enzymes were determined following the lethal dose of fenitrothion treatment. All the four enzymes, protease, lipase, amylase and invertase of the midgut were significantly inhibited when assayed at the prostration stage of poisoning sequence. *In vitro* studies revealed that invertase, protease and lipase were inhibited with sublethal dose of insecticide also. It was suggested that the regurgitation of the body fluid would probably affect the gut pH and ionic imbalance of the hemolymph. The observed depletion in the activities of the enzymes might also be due to the starvation of the larvae during the experimental period. It was concluded from these observations that in the 5th instar larvae fenitrothion acts at several levels of digestion processes and disrupt the mechanism of digestion and absorption.

Generally, it has been accepted that insecticides act directly on the CNS and disrupt normal ion permeabilities in the nerve membrane that are implicit in the generation and conduction of nerve impulses. Transport of alkali metal
cations into and across the nerve membrane is catalysed by Na\(^+\)-K\(^+\), Mg\(^{2+}\) and K\(^+\) stimulated ATPases. Distribution of these various ion stimulated ATPases and their activities following fenitrothion treatment was investigated in the nerve cord, midgut and fat body. Both Na\(^+\)-K\(^+\) and Mg\(^{2+}\) stimulated ATPases were significantly higher in the nerve cord compared to fat body and midgut. K\(^+\) stimulated ATPase was found to be significantly less in the midgut than those of nerve cord and fat body. The quantitative differences in the activities of these various species of ATPases among the three tissues reflect on the functional role of these enzymes. Studies from other organisms suggest that Na\(^+\)-K\(^+\) ATPase is involved in Na\(^+\) transport across the nerve membrane, while Mg\(^{2+}\) stimulated one appears to be involved more in the energy regulation of the cell. K\(^+\) stimulated ATPase is involved in the transport of K\(^+\) ion across the cell membrane. Fenitrothion inhibited Na\(^+\)-K\(^+\) and Mg\(^{2+}\) stimulated ATPases in the nerve cord and midgut tissues. Fenitrothion had no inhibitory effect on K\(^+\) stimulated ATPase in all the three tissues. This has been attributed to the presence of high concentration of potassium in the hemolymph of the castor semilooper larvae.

The inhibition of acetylcholinesterase (AChE) is a very evident biochemical consequence of insect poisoning by OP compounds. The activity level of AChE was measured in the nerve cord, mid and hindgut tissues following the fenitrothion treatment. The endogenous enzyme content was very high in the nerve cord compared to mid and hindgut tissues. Fenitrothion treatment produced greater inhibition of the enzyme in the midgut than the hindgut and nerve cord. ACh is a major neurotransmitter in the insect nerve cord. Further experiments are required in the direction of purification of ACh receptors from the midgut region before attributing any functional significance to this finding. The inhibitory effect of fenitrothion on the nerve cord ACh was found to be dependant upon both age and dose. The older larvae treated with sub-lethal dose of insecticide showed tendency for recovery from AChE inhibition. This was attributed to the increase in the biodegradation of the fenitrothion due to the induction of glutathione S-transferase, an enzyme involved in the detoxication of xenobiotics, by the insecticide.
One of the key enzymes involved in the detoxication of xenobiotics is the glutathione S-transferase (GSH S-transferase). The conjugations that are formed due to the catalysing activities of GSH S-transferase are often less toxic and are easily eliminated from the body. The role of GSH S-transferase is considered as important factor in insect resistance to OP insecticides. Sublethal and lethal dose of fenitrothion treatments were investigated on induction of the enzyme. Sublethal treatment produced greater induction of the enzyme than the lethal dose. Carcass compared to midgut showed greater enzyme induction at both the doses of insecticide treatment. The results suggested that the carcass was more responsive to the insecticide treatment than the midgut. The difference between the two tissues may be due to high intrinsic level of the enzyme in the midgut. The effect of sublethal dose of fenitrothion on the GSH S-transferase activity in the three developmental stages revealed that the 5th instar larvae compared to pupae and adults showed greater induction of the enzyme. The greater production of the GSH S-transferase in response to insecticide treatment is of considerable importance to the larvae, since they are more vulnerable to the xenobiotics originating either from the host plant or insecticide sprayed on them. Phenobarbital has been shown to induce the GSH S-transferase in insect tissues. The larvae pretreated with sodium barbitone showed significantly higher LT_{100} at different doses of insecticide treatment. The results thus suggested that sodium barbitone induced drug metabolizing enzymes (GSH S-transferase) in the castor semilooper larvae.

In the final analysis, the present investigation has provided several lines of evidences to demonstrate that fenitrothion, in addition to its anticholinesterase property, produced biochemical lesions which augment the toxic effects of the insecticide.