

**STUDIES ON THE EFFECT OF AN
ORGANOPHOSPHORUS INSECTICIDE, FENITROTHION
ON THE ACTIVITY OF ACID PHOSPHATASE AND
ACETYLCHOLINESTERASE IN THE SELECTED TISSUES
OF THE DOMESTIC PEST, Periplaneta americana**

By

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Introduction

I INTRODUCTION

Quality of life refers to our health and happiness, both related to the quality of environment. Those which are beneficial for the maintenance of balance of the environment in the long run are to be conserved, others are to be checked. Man and his surroundings are to be well protected from the annoying insects which are of great menace to him by the introduction of certain pesticides. These pesticides, occupying a unique position among the many chemicals were sometimes used for the purpose of killing or injuring some form of life and leaving man unharmed.

The Cockroach, Periplaneta americana, a well known house-hold pest is the most obnoxious one to man. Being omnivorous in diet it lives on a wide variety of vegetable and animal matter and non-living materials like leather, paper, cloth etc. They found in various places such as bakeries, restaurants, distilleries, refineries, ware houses, hot houses and sewers, where there is warmth, dampness and plenty of organic food to devour. It avoids light and active during night i.e., nocturnal (David. R. Ragge, 1965).

Because of its disgusting character the cockroach is considered to be a repulsive intruder which has attained the pest status. To mankind, the cockroaches in the home and place of work are psychologically disturbing and causing mental stress by their increasing numbers of population if unchecked and their active, speed movement with a characteristic fowling odour of the excrement which spill over the food and surfaces with which they come in contact (. . . Cornwell, 1968).

Cockroaches carry many disease causing viruses, protozoans, parasitic worms and also typhoid producing bacteria in sewers, latrines and cesspit hence claimed to be a vector. Sybil P. Parker (1984) showed the evidence of allergies particularly asthma in man by cockroaches.

Hence in recent years there has been considerable research towards both insecticides and repellants to fight against this wide spread, persistant and offensive pest, the cockroach (Winchester, 1989).

Currently, organophosphorus insecticides are widely used for cockroach control. They are known to

be the offending agents. The popularity of organophosphorus insecticides derives from their effectiveness against insects resistant to organochlorines and pyrethroid compounds. The organophosphorus compounds selected with maximum beneficial effect, low acute toxicity and with the best possible insecticidal life are to be used in the control of cockroaches. The biological activity of the organophosphate is generally believed to be derived from their interference with a vital enzyme (acetylcholinesterase) important to nerve function in insects and mammals. The insecticides owe their potency inside the body to conversion or activation, to compounds which are direct inhibitors of this enzyme. The organophosphates most often used for cockroach control are Diazinon, Dichlorvos, Malathion, Chlorpyrifos, Fenitrothion, Iodofenphos, Trichlorphon which differ markedly in speed of action and efficacy (Cornwell, 1968).

Among the currently used insecticides fenitrothion is the best and most promising one in cockroach and many other insect control which was discovered by Sumitomo, (1959) with its trade name sumithion. It is a relatively new and low toxicity

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organophosphate for humans which is presently known as Bilfol. Like other organophosphorus insecticides, fenitrothion does not accumulate in the body but is degraded. The mammalian and insecticidal toxicity of fenitrothion mainly derives from its inhibitory action on cholinesterase not directly, but by oxosumithion, formed by enzyme oxidation of fenitrothion in the body.

Other organophosphorus compounds such as Diazinon and Dichlorvos are less effective in cockroach control in the sense that they are not giving good initial kill followed by good residual action on treated harbourages for more than a few days, but are toxic to human beings, where as the Dichlorvos and Malathion are less toxic to cockroaches (Cornwell, 1968). Chlorpyrifos is resistant to cockroaches (Michael K. Rust, 1991) whereas ~~iodo~~fenphos and Trichlorphon are less toxic to adult female cockroaches than the males.

Low doses of fenitrothion applied repeatedly to 'Periplaneta americana' at one or two day's interval cause an initial rise in the activity of phosphatase which may be involved in the metabolism of the insecticide (Cornwell, 1968). So the

organophosphorus insecticides in addition to its primary effect on acetylcholinesterase also have a secondary effect on acid and alkaline phosphatases. Biochemical evidences show the occurrence of acid phosphatase enzyme activity in ovary. This enzyme is reported to be involved in various physiological functions like nucleic acid metabolism, phospholipid metabolism and active transport of materials through phosphorylated intermediates (Goodman and Roth Stein, 1957), carbohydrate metabolism and glycolytic cycle (Miller and Crane, 1961).

since the mode of action of organophosphorus insecticide is the inhibition of the enzyme acetylcholinesterase, the determination of the acetylcholinesterase activity is the very important aspect in this study. The highest concentration of acetylcholine and the enzyme acetylcholinesterase is present in the brain and nerve ganglia. The organophosphate compounds act by binding acetylcholinesterase (AChE) (Corbett, 1974) because of these compounds, hyperexcitability will result. Death may also occur due to effects of neurohormones released by the massive neural activity (Granett and Leeling 1971; Samaranayaka, 1974).

In view of all these evidences and necessities the present study was undertaken to evaluate the impact of a versatile organophosphorus pesticide on acetylcholinesterase activity in the brain and acid phosphatase activity in the ovary of the cockroach during a sublethal exposure period of 24 hours.

Review of Literature

II REVIEW OF LITERATURE

The occurrence of cockroach in the Southern parts of United States and tropics were earlier described by Herbert. H. Ross (1965). Three cosmopolitan domestic species which are most abundant like German cockroach 'Blatella germanica' the larger oriental cockroach 'Blatta orientalis' and the American roach 'Periplaneta americana' were studied (David R. Ragge , 1965).

Cornwell (1968) made an attempt to study the pest status of cockroach. Further, worthwhile contribution on the disease carrying capacity of cockroach was provided along with human reactions like allergies and mental stress (Sybil. P. Parker,1984).

PESTICIDES IN COCKROACH CONTROL:

The study was conducted on the different pesticides especially the organophosphate, organochlorine and pyrethroid compounds which were used for the control of cockroach and the resistance developed to pyrethroid compounds were recorded (Kimitoshi Umeda,1988).

Recent evidences show that the cockroach has developed resistance to many pesticides except fenitrothion, diazinon, propoxur and methoxadiazon (Kimitoshi Umeda, 1988). Subsequently Cornwell (1968) explained the importance of fenitrothion in the control of adult female cockroach.

LETHAL DOSE OF PESTICIDES IN COCKROACH CONTROL:

Studies were undertaken to find out SD_{50} (dose required to sterilize 50% of the cockroaches) by treating the juvenoids, fenoxycarb and hydroprene topically as a control measure (King, 1989). Okamoto (1989) examined the daily mortality of cockroaches by exposing them to U.V. radiation. Control of Heliothis armigera on chickpea by droplet application of nucleur polyhedrosis virus in combination with endosulfan and boric acid was reported (Rabindra, 1987). The inhibition of reproductive activity in Blattella germanica by applying hydroprene 's' in food was reported by Bradley (1989). James·E. Baker (1971) in his publications showed the effects of 1,4 naphthoquinones and several sulphhydryl reagents on the CNS activity of Periplaneta americana. The inhibition of ATPase activity on the ventral nerve cord of Periplaneta americana by the localization of DDT was studied (Schal et.al. 1987).

The details regarding the effects of sub-lethal doses of DDT on the CNS of Periplaneta americana were examined by Elia et.al. (1990). Diflubenzuron when administered by feeding or injecting showed an inhibitory effect on the larval-adult development was recognised by Uljena wuttig et . al. (1991). Contributions of Le.pastourel et.al. (1990) showed that the deposits of amorphous silica on Blattella germanica under laboratory conditions proved to be lethal.

German cockroaches were tested for their susceptibility to 12 insecticides by topical application (Kimitoshi Umeda,1988). Through his work Appel (1990) determined the topical and oral toxicity of sulfluramid on german cockroach. Steele (1954) in his studies showed the differences between a resistant and non-resistant strain of the german cockroaches when he applied the insecticides chlordane, Indane and DDT. Temperature plays an important role in the pesticidal toxicity of Melanopsis dufouri as reported by Almar (1988). An attempt was made to study sublethal effects of chlorpyrifos methyl compounds on the reproduction in female cockroaches by Robert .L. Hamilton (1990).

Recent evidence shows that there is distinct increase in the resistance to lindane, pyrethrin, PBO, trichlorfon, dichlorvos and propoxur in cockroaches (Klunker, 1990). The contributions of Bose and Chanda (1989) determined the toxicity of Malathion and Carbaryl by topical application on the body weight of common cockroaches, Periplaneta americana. Hamilton (1990) showed evidences of the significant reduction in the longevity and lifetime fecundity in 2 days old female german cockroaches after topical application of chlorpyrifos. Clarias batrachus (Linn) when exposed to 2 organophosphorus insecticides showed changes in carbohydrate metabolism (Joyce Shobhan rani et.al. 1989).

Spray et. al. (1987) by their contributions reported the effects of aerial applications of fenitrothion on bird populations of a scottish pine plantation.

ACID PHOSPHATASE ENZYME ACTIVITY:

The occurrence and the importance of growth and differentiation of acid phosphatase in the embryonic and post-embryonic stages of insects were reported by

various authors (Pasteur and Costes 1974, Roussel 1974, Butter 1973). Through the contributions of Wilkenson (1970) it was understood that the multiple form of acid and alkaline phosphatases occur based on the optima in the hemocytes of cockroach, Periplaneta americana. Verkuil (1978) reported the activity of protease and acid phosphatase enzymes in different stages of ovary and fat body of the insects.

LETHAL DOSE OF PESTICIDES ON OVARIAN ACID

PHOSPHATASE:

Bio-transformation of the insecticides Fenitrothion along with an initial rise in the activity of phosphatase was reported in 'Periplaneta americana' when low doses was administered and repeatedly at one or two day's interval (Cornwell, 1968).

The effect of LD concentrations on the nervous system of female cockroaches applied by benediocarb and monochrotophos was studied by Rajender (1987). Diflubenzuron treated 4th instar larvae of mosquito 'Culex pipiens fatigan' show a decrease in Alkaline phosphatase and Acid phosphatase activity which impeded the growth and pupation (WU et al., 1990).

An increase in the level of alkaline phosphatase activity in the liver functioning of rabbit was reported as an effect of subacute DDT (Yati Chugh, et. al. 1990).

Reddy et.al. (1988) and various authors have made an attempt on Penaid prawn to show an inhibitory effect of both acid phosphatase and alkaline phosphatase on the addition of aldrin even in low concentrations. Immense researches have been undertaken by Rajender (1988) on the toxicity of 2 different insecticides on the haemolymph and nervous tissue esterases, phosphatases and proteins of adult female cockroaches.

ACETYLCHOLINESTERASE ENZYME ACTIVITY:

Acetylcholinesterase is of nerve cell origin, transported to axon for its functioning was studied under electronmicroscope on the mammalian nerve cells.

The enzymes first appeared on the insect eggs and a progressive increase was observed during hatching when the nervous system was fully developed. Acetylcholine in some appear before the presence of acetylcholinesterase and in other\$ later.

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Acetylcholine a 'nerve transmitter' varies greatly in different insect species and insects of the same species. Mikalonis and Brown observed 70 $\mu\text{g/g}$ of AcetylCholine for isolated ventral nerve of Periplaneta americana whereas Roader reported a value of only 37 $\mu\text{g/g}$. The occurrence of both Acetylcholine and AcetylcholineEsterase largely in the brain and nerve ganglia was reported by Cornwell (1968).

Hyperexcitability results by the binding action of acetylcholinesterase with toxic compounds (Corbett, 1974). The release of neural hormones due to the massive neural activity results in death, was evidenced by Granett and Leeling 1971, Samaraneyaka 1974).

Dubois et.al. (1949) demonstrated the mechanism of toxic action of parathion on the nervous tissue of rats which showed a marked reduction of cholinesterase activity and an increase in the free acetylcholine.

Further researches have revealed that other organophosphorus insecticides in sufficient doses [↓] inhibited.

Acetylcholinesterase invivo. Several other reviews and monographs of O'Brien 1960, Heath 1961, Cohen and Oosterbaan 1963, are available to study in detail the inhibitory mechanism of cholinesterase. Much of the reviews discuss the toxic action of organophosphorus insecticide in man (Holmstedt 1959, Grob 1953).

LETHAL DOSE OF PESTICIDES ON BRAIN ACETYLCHOLINESTERASE ACTIVITY:

Excessive activity of organophosphorus and carbamate insecticides are seen at the synaptic region where the enzyme acetylcholinesterase hydrolyses acetylcholine which results in the hypersensitivity, convulsions paralysis and eventually death.

Smitsaert et al. (1970) demonstrated the Acetylcholinesterase activity in organophosphate resistant and susceptible spider mites. Carbamate compounds like the organophosphorus compounds cause the poisoning by affecting Acetylcholinesterase was reported by Cornwell (1968). O'Brien (1967) and Corbett (1974) have contributed to the study of malathion on the oscillation of acetylcholine and acetylcholinesterase content of several tissues of the common carp 'Cyprinus carpio' which is suppressive and can be recovered without suffering lasting

effects. Cornwell (1968) demonstrated the activation of phosphorothionates, malathion, diazinon and fenitrothion into other chemical forms in the insects body which actually cause the poisoning. The invitro effects of trichlorfon, malathion or methyl parathion on kinetic constants of acetylcholinesterase in fresh water teleost 'Claries batrachus' (Linn) was demonstrated by Rani (1989). The resistance of organophosphorus insecticides, chlorpyrifos, temphos, and malathion was strongly associated with the presence of esterases A and B (Wirth et.al. 1990).

Conclusions were made that the resistance to carbamates and organophosphates are associated with a mutant form of acetylcholinesterase giving specific insensitivity to fenthion (Zhen-Hua Tang et.al. 1990). The toxic dose of dichlorvos administration to rats showed inhibition only on acetylcholinesterase activity (CH. Ellinger, 1985). Through the contributions of Rajendar (1986) it was understood that the block of acetylcholinesterase activity and suppressed synaptic function of nervous tissue of the american cockroach was associated with the application of Sumicidin. Frontali et.al. (1883) revealed the localization of acetylcholinesterase in the brain of Periplaneta americana.

The brief reviewed literature on the application of the insecticides and the findings of LD₅₀ concentration, activity of the enzymes acid phosphatase and acetylcholinesterase on different tissues of different organisms provide sufficient information for a beginner to understand the effect of pesticides for the control of cockroaches. Hence the present study has been undertaken.

Materials and Methods

III MATERIALS AND METHODS

For the present study, the commonly occurring important domestic pest, the cockroach Periplaneta americana' was selected because, it is easily available and highly adaptable to the laboratory conditions.

COLLECTION AND ACCLIMATIZATION OF THE INSECTS:

The female adult cockroaches were collected from market areas and brought to the laboratory for the experimental analysis. Equal sized cleaned bottles of one litre capacity were selected with the lids perforated for aeration. A batch of about ten cockroaches were introduced in each bottle and was allowed to acclimatize to the laboratory conditions for about 3 - 4 days, when the temperature was 32°C.

The cockroaches were fed with a mixture of powdered biscuit and roasted bengal gram in the mornings. A cotton swab moistened with water was placed inside each bottle for the purpose of getting moisture. The containers with cockroaches were cleaned at regular intervals.

STUDY OF LD₅₀ IN COCKROACHES:

The toxicity of insecticides to a particular organism is usually expressed in terms of LD₅₀. LD₅₀ is the dose of the chemical expected to cause a 50% mortality in an animal population tested. It is expressed as mg or mg/larva or adult.

Topical application was the method used for administering the insecticide on to the cockroach. Topical application means placing the insecticide directly on to the insect's cuticle. The apparatus used for this purpose is a microapplicator syringe of 2 μ l capacity. The cockroaches were anesthetized by keeping in a freezer for 7 - 10 minutes and were treated with acetone solution of technical organophosphorus insecticide "Fenitrothion". A measured droplet was applied to the penultimate abdominal sternite through the micro-applicator. Controls were kept by applying 1 μ l of acetone to the penultimate abdominal sternite. Different concentrations of fenitrothion were applied to each group of 30 cockroaches separately. The treated cockroaches were then transferred to glass bottles and were

provided with food and water. The number dead and moribund were counted after the exposure period of 24 hours. Percentage mortality between 10 and 80% were observed and analysed by probit analysis.

The advantage of using topical application method is to ensure the entry of equal amount of toxicant to the test insect.

ACID PHOSPHATASE ACTIVITY:

Preparation of the sample:

The ovaries of the cockroaches were removed homogenized with distilled water, centrifuged at 3000 rpm for 10 minutes and the supernatant was used as the enzyme source.

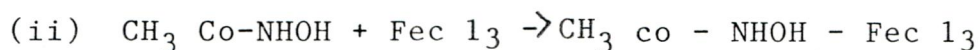
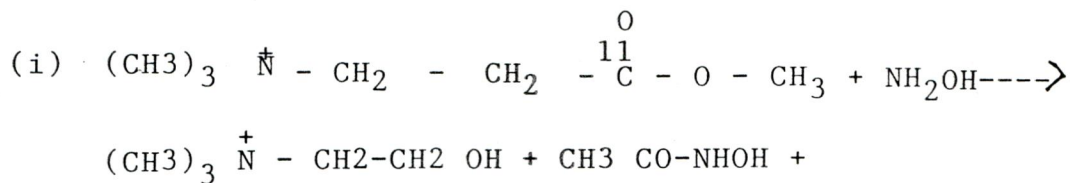
Activity of the acid phosphatase was determined colorimetrically using the King's method as described by Varley (1967) using the substrate disodium Phenyl Phosphate.

ACETYLCHOLINESTERASE ACTIVITY:

Principle:

The acetylcholine remaining after hydrolysis is converted to a hydroxamic acid by treatment with

alkaline hydroxylamine. Hydroxylic acid with ferric chloride in acid solution gives a red-purple complex. The intensity of colour is proportional to the concentration of acetylcholine present in it.



REAGENTS:

- a) Buffer: Dissolve 16.72 g clear crystals of $Na_2 H PO_4 \cdot 12 H_2O$ and 2.72 KH_2PO_4 in one litre of distilled water and adjust ^{the} PH to 7.2.
- b) Acetylcholine Standard Solution:
Dissolve 0.111g Achcl in 100ml of buffer solution prepare before use and add a drop of toluene.
- c) Hydroxylamine hydrochloride solution:
($NH_2 OH - HCL:0.2M$)
Dissolve 1.398 g of $NH_2 OH HCL$ in 100 ml of distilled water and keep it in refrigerator.
- d) Ferric Chloride Solution:
(0.37M)
Dissolve 10g Ferricchloride ($Fecl_3 \cdot 6H_2O$) in 100ml of 0.1N Hydrochloric acid.

e) Sodium Hydroxide Solution:

(3.5M)

Dissolve hydrochloric acid (SP-gr 1.18) with 2 volumes of distilled water.

PREPARATION OF THE SAMPLE:

The brain of about 25 cockroaches weighing 100mg was removed in insect ringer's solution and homogenized with 8.0ml of 0.2M sucrose solution. The homogenate was centrifuged for about 15 minutes and the supernatant was transferred to a test tube. This enzyme source was used for the analysis.

PREPARATION OF THE STANDARD CURVE:

Aliquots of 0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 ml of acetylcholine chloride solution was pippered out into a series of test tubes. Then the volume of each tube was made upto 1.0ml with phosphate buffer and incubeted at 37° C for 30 minutes. After incubation 2.0ml of alkaline hydroxylamine solution was added to all the test tubes. After this, 1.0ml of dilute hydrochloric acid was added. To each test tube 1.0ml of ferricchloride was added, and was shaken

vigorously. The intensity of the colour was measured at 540nm in the spectrophotometer. This was proportional to the concentration of the acetylcholine present.

CHOLINESTERASE ACTIVITY:

The enzyme (AChE) was taken in each tube at the rate of 0(Control), 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0ml and the volume was made upto 1.0ml with distilled water. Then aliquots of 1.0ml of acetylcholine chloride (0.004M) substrate solution was added to each tube and incubated at 37°C for 30 minutes. Then 2.0ml of alkaline hydroxylamine solution (0.2M hydroxylamine and 3.5M sodium hydroxide mixed in equal ratio) was added followed by the immediate addition of 1.0ml of dilute hydrochloric acid (1 concentration HCL.2distilled water V/V). To this 1.0ml of ferric chloride (0.37M) was added and the test tubes were shaken vigorously. The absorbance was measured, at 540nm in the spectrometer (Spectronic 2000, Bausch and Lomb New York) and plotted against acetylcholine standard (Koch-light laboratories England). The quantity of acetylcholine showed the enzyme activity.

Results and Discussion

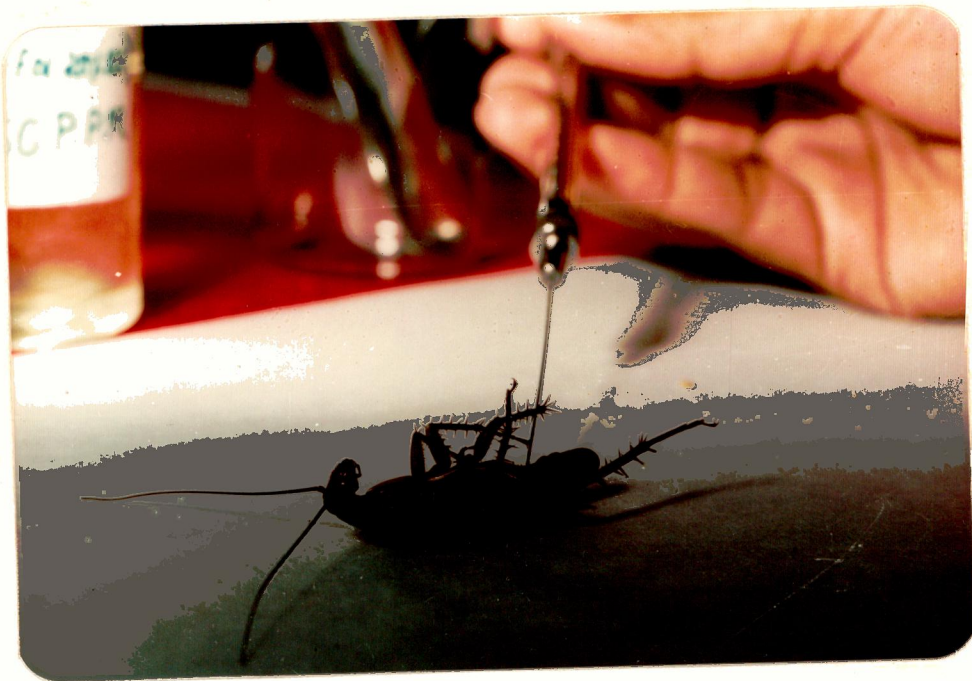
RESULTS

The results pertaining to this study, Effect of an organophosphorus insecticide, Fenitrothion on the activity of Acid Phosphatase and Acetylcholinesterase in the selected tissues of the domestic pest Periplaneta americana has been discussed and presented in this chapter.

The results obtained in the present investigations gained much importance in determining the effect of the organophosphorus insecticide on the nervous tissue and ovary of the cockroach, an aeriually adapted organism. The organophosphorus insecticides act as potential inhibitors and play an effective role in cockroach control. The treated cockroaches exhibited changes in the enzyme levels and activity.

LD₅₀ in Cockroach:

The acclimatized female adult cockroaches were treated topically with fenitrothion (Fig. 1) and the LD₅₀ value was found to be 3.65µg/adult. 10-80% mortality of cockroaches at different lethal doses were also observed during this study (Table 1) LD₁₀ value - 3.0µg/adult; LD₂₆ value - 3.5µg/adult; LD₃₃ value - 3.6µg/adult; LD value-3.7µg/



TOPICAL APPLICATION METHOD

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TABLE I

PERCENTAGE MORTALITY OF Periplaneta americana
 AT DIFFERENT DOSES OF FENITROTHION FOR AN EXPOSURE
 PERIOD 24 HOURS

Dose in μg	Log concen- tration of dose	No. of insects exposed	No of insect dead	Percen- tage mortality %
2.5	3.4343	30	0	-
3.00	3.4771	30	3	10
3,50	3.5441	30	8	26
3.60	3.5563	30	10	33
3.65	3.5623	30	15	50
3.70	3.5682	30	20	66
3.75	3.5740	30	26	86
4.00	3.6021	30	30	100
4.50	3.6532	30	30	100

adult; LD₈₆ value - 3.75 μ g/adult. The LD₅₀ value was computed in a standard graph. (Fig. 2).

Behavioural changes in the treated cockroaches:

Even for the slightest disturbance of the container the treated cockroaches showed fast, hapazard movements.

No positive approach towards food and water was shown when supplied to them.

An agitated condition was shown, may be due to lack of oxygen supply. The insects were assembled near the lid surface and tried to escape.

No behavioural changes and mortality were observed in the normal cockroaches.

Acid Phosphatase activity;

Ovarian acidphosphatase activity was done by King's method. Low doses of fenitrothion applied repeatedly to Periplaneta americana at one to two day intervals, cause an initial rise in the activity of phosphatases which may be involved in the metabolism of the insecticide (Cornwell, 1968). The ovarian acid phosphatase activity of

ESTIMATION OF LD₅₀ VALUE AT 24 hrs

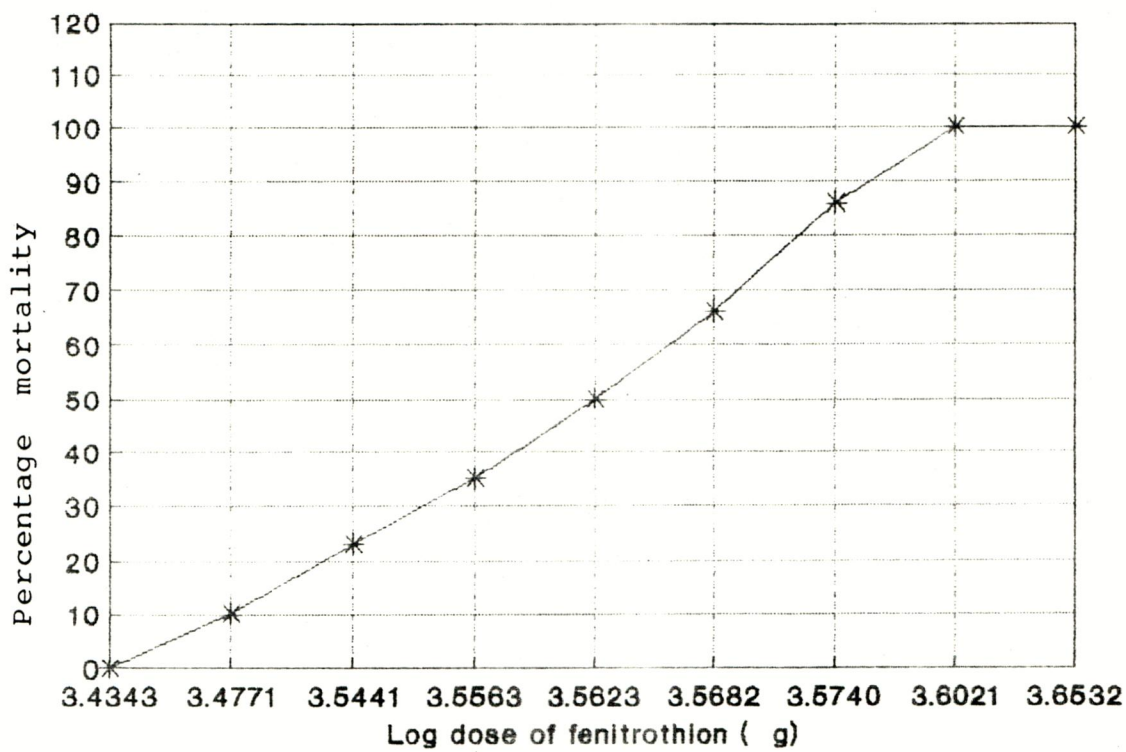


FIGURE : 2

TABLE II

S.No	Enzyem	Control	Dose in (μ g)					
			3.0'	3.5	3.6	3.65	3.7	3.75
1	Acid Phosphatase Activity	1.27	0.90	2.20	2.94	4.50	6.0	7.5
		± 0.21	± 0.23	± 0.73	± 0.82	± 0.31	± 0.01	± 0.43
	Percentage change		29.13	73.23	131.50	254.33	372.44	490.55

Table shows mean \pm S.D. of each value.

**EFFECT OF FENITROTHION ON ACID
PHOSPHATASE ACTIVITY IN THE OVARY
OF *Periplaneta americana***

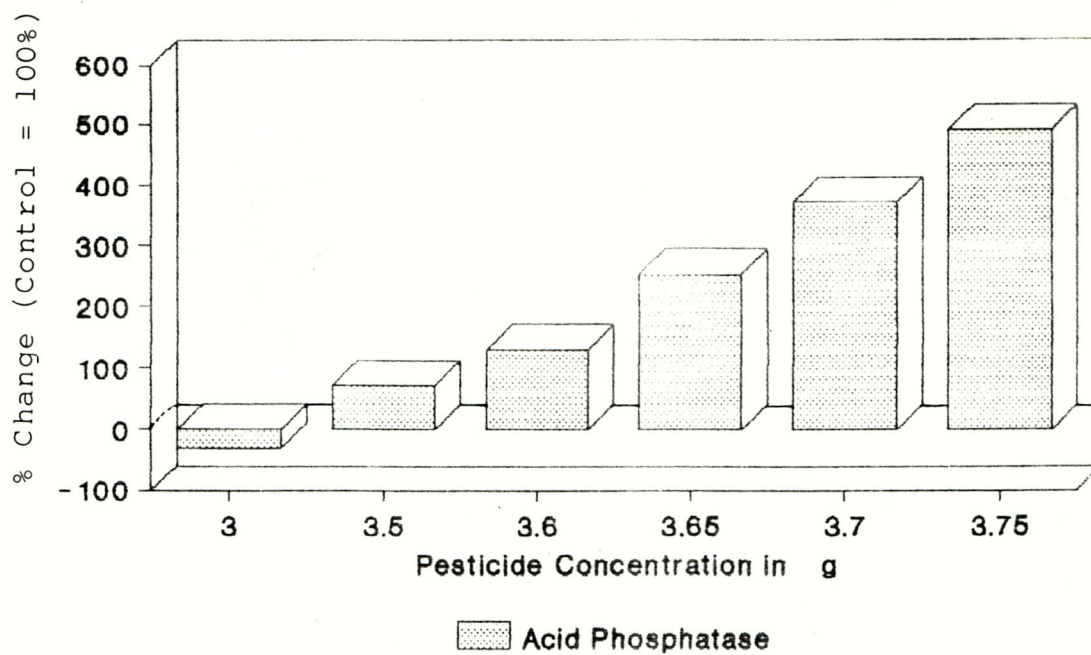


FIGURE : 3

the normal cockroach was determined as 1.12 K.A.units. An increase in the Acid phosphatase activity of 4.5 K.A. units was measured colorimetrically in cockroaches treated with LD₅₀ concentration of fenitrothion.

The acid phosphatase activity showed a varying levels of decrease in LD₂₆ and LD₃₃ and an increase in LD₆₆ and LD₈₆ than the value recorded in LD₅₀ as given in (Table 2; Fig 3).

The experiments were carried out in triplicates both in normal and experimental cockroaches and the results were statistically analysed

Acetylcholinesterase Activity:

Acetylcholinesterase activity was determined by Augustinsson's (1957) method. In the present study, the impact of the fenitrothion on acetylcholinesterase activity and the acetylcholine content was measured. Acetylcholine standard curve was drawn. The amount of unreacted acetylcholine was determined from the standard graph. By subtracting the amount of unreacted acetylcholine from the amount in the standard, the

51

TABLE III

EFFECT OF FENITROTHION ON BRAIN "AChE" ACTIVITY OF
THE COCKROACH Periplaneta americana

4

S.No	Enzyme	Amount of ACh hydrolysed in normal cockroach in (μ g)	Amount of Ach hydrolysed in fenitrothion treated cock roach in (μ g)
1	0.2	145	44.4
2	0.4	245	188.7
3	0.6	265	288.6
4	0.8	433	356.0
5	1.0	500	415.0

**EFFECT OF FENITROTHION ON
ACETYLCHOLINESTERASE ACTIVITY IN THE
BRAIN OF *Periplaneta americana***

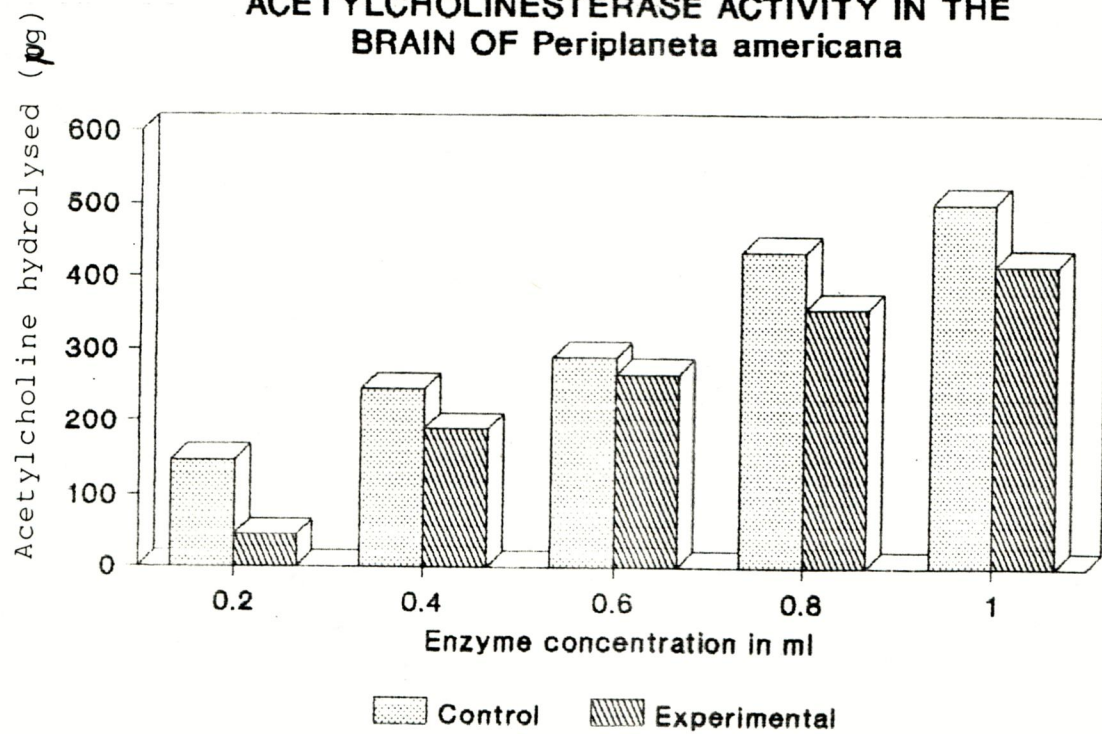


FIGURE : 4

amount of reacted acetylcholine was determined.

The acetylcholine hydrolysed by Acetylcholinesterase of the normal cockroach was found to be 500 $\mu\text{g/ml}$ of cockroach brain homogenate whereas that of treated cockroaches with LD₅₀ concentration of fenitrothion showed a value of 415 $\mu\text{g/ml}$ of cockroachs brain homogenate.

The result which shows the difference is an evidence for the inhibitory action of the insecticide on the target enzyme acetylcholinesterase (Table 3 Fif.4).

DISCUSSION

The development of new insecticides which are generally toxic paved way for destroying the pest insects and leaving man unharmed. Some of the organophosphorus insecticides from their effectiveness against insects are used as control.

The first insecticidal organophosphate compound was synthesized in the year 1854, and the potent insect killing properties was recognised little later. During World War II the insecticidal action of organophosphate was discovered in Germany. Oral LD₅₀ (rat) described by using Schredan in 1941 and Parathion in 1947 (cornwell, 1968).

Lethal effects of organophosphate pesticides have gained importance in the past few years (Brungs et al., 1978; Spehar et al., 1979).

Fenitrothion was considered to be one of the best suited and superior Organophosphate compounds in the control of Cockroaches for varying reasons such as safer to use with maximum effective benefits and low toxic to human which can be applied topically. Because of its good

insecticidal action and far better kill than the other organophosphorus compound it has been selected for the present study. It will not accumulate in the body.

Since rearing of cockroaches in the laboratory will take many months, it was difficult to provide proper assessment in details about the LD₅₀, enzyme activities during this short period of study. However care was taken to observe the activities of acid phosphatase in ovary and acetylcholinesterase in the brain of fenitrothion treated cockroache and the obtained results were tabulated.

King (1989) determined SD₅₀ values (dose required to sterilise) for male and female German cockroaches when administered with various insecticides such as fenoxycarb, hydroprene as 39.82 $\mu\text{g/g}$ body weight in male and 86.64 μg /body weight in female. The topical LD₅₀ of sulfuramid in cockroaches was found to be 176.6 $\mu\text{g/g}$ for adult males and 117.8 $\mu\text{g/g}$ for adult females. This compound had an inhibitory effect on the female reproductive system (Appel, 1990). Hamilton

(1991) observed when applied low concentration of pesticides the failure of first oothecal hatch in cockroach.

The 50 per cent mortality rate observed in the present investigation reached at a concentration dose level of 3.65 $\mu\text{g/g}$ when fenitrothion was applied under laboratory conditions. This revealed that the activity of fenitrothion is far superior to its other counterparts in terms of its efficiency in cockroach control.

The activities of Acetylcholinesterase, Acid phosphatase and Alkalinephosphatase on the different nervous tissues of brain, ventral nerve cord and ganglia by the application of pyrethrum were studied in the newly emerged adult male and female schizodachytus monostrosus. It was found that there was a significant increase in Acidphosphatase and ATPase activities and rapid decreased activity of Acetylcholinesterase (Banerjee et al., 1984).

Reddy et al., (1988) in their studies explained the increased Acidphosphatase activity when applied aldrin was due to the destruction of lysosomal membranes. Acid Phosphatase activity was stimulated in the kidneys of rabbits when treated with endosulfan was demonstrated by Tejendra et al., (1990). Cornwell (1968) stated that the low doses of fenitrothion applied to adult cockroaches *Periplaneta americana* repeatedly at 1 to 2 days² interval cause an initial rise in acidphosphatase activity which may be involved in the metabolism of insecticide.

In all the observations recorded regarding the Acidphosphatase activity and increase in the level was noted in the treated ones than in the control. In the present investigation there was a profound increase in acidphosphatase activity than the normal ones.

Contributions from many authors have revealed the inhibitory action of the organophosphorus and other insecticide on the nervous tissue of cockroaches.

Naqui (1986) has stated in its work that the acetylcholinesterase activity was reduced significantly in Musca domestica and Blatella germanica when treated the petroleum ether soluble neutral fractions of ripe neem fruits and neem leaves. A comparative study on Periplaneta americana by treating with two different insecticides was found by Rajendar (1987). His findings showed an inhibitory mechanism on acetylcholinesterase which act as a tool to support the present investigation.

When applied with sumicidin the american cockroach showed an inhibitory action of acetylcholinesterase and a decrease in the synaptic function of nervous tissue (Rajender, 1986). A powerful inhibitory effect was shown when low doses of carbaryl and malathion were applied to cockroaches (Bose, Chanda 1989).

Inhibition of Acetylcholinesterase activity was found in dichlorvos treated organisms (Ellinger, 1985).

Thus the acetylcholinesterase activity is inhibited in the CNS in treated cockroaches, where there is less hydrolysis, increase in the level of acetylcholine content and thereby blocking the synaptic transmission. In this way fenitrothion acts as an effective insecticide in checking the outnumbering cockroach population.

Due to want of time and chemicals the acetylcholinesterase activity could be recorded only for a period of 24 hours.

A more intensive investigation in the control of cockroaches by using new technologies will be highly available in the next few years in addition to the insecticidal use.

Summary and Conclusion

SUMMARY AND CONCLUSION

There has been considerable research towards improving both insecticides and repellants during recent years against cockroach. Because it is a widespread, persistent, offensive, disease causing pest (Winchester, 1989). Since they prefer to live in warmer parts of buildings near to food and water, they tend to end up in or near kitchens, hiding in dark crevices during the day and emerging at night in search of food and drink. There is strong evidence to suggest that cockroaches give rise to allergies in man, particularly asthma, typhoid and viral diseases etc (Busvine, 1991).

The organophosphorus compounds play an effective role in cockroach control than the organochlorine and pyrethroid compounds. Among the organophosphorus insecticides fenitrothion was selected for the study because of its maximum beneficial action and low toxicity towards man. It showed promising results on the control of cockroaches especially, females.

LD₅₀ concentration of fenitrothion was recorded to be 3.65 μg /adult cockroach *Periplaneta americana*. 100 per cent mortality was obtained in the dose of 4.0 μg /adult. Fenitrothion played an active role in the acid phosphatase activity of ovary. An increase in the level was recorded in the treated cockroaches than the normal ones. The elevated level of Acid Phosphatase was found to be due to detoxification of the insecticide. The result obtained in the present investigation is supported by various experimental evidences.

The acetylcholinesterase activity of the brain was decreased without any initial rise was recorded, showed the powerful inhibitory action of the organophosphorus insecticide fenitrothion on the nervous tissue of the common cockroach *Periplaneta americana*.

Hence, from the above cited experiment it can be concluded that the fenitrothion is the best suited one among the insecticides in cockroach control.

Newer tools or technologies using Baits and Insect growth regulators along with insecticides will become available within the next few years as advanced pest control devices for the wide spread cockroaches.

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