MATERIAL & METHODS
CHAPTER II

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Larvae of rhinoceros beetle, Oryctes rhinoceros were collected from coconut farms in and around Bangalore. The larvae are available throughout the year. The third instar larvae were selected for its abundance, and also for its presence in the upper most strata of the compost heaps or manure pits. This makes the easy accessibility of pesticides to the larvae. For this reason many people have carried out experiments involving the pesticide instar larvae. The larvae collected from the acclimatized to the laboratory conditions simulating their natural habitat and relative humidity were treated with two types of insecticides.

1. Chlorinated hydrocarbon insecticide - Benzene hexachloride (50% commercial grade)
2. Organophosphate insecticide - Malathion

1. Benzene hexachloride:

BHC is widely used in Indian agriculture. BHC contributes about 45% of total pesticide manufactured in India. It was first synthesised by Michael Faraday in 1825. Its structure was determined in 1836 as C₆H₆Cl₆.

\[ \text{Existence of four stereoisomers was demonstrated by Matsumura (1982 & 1985). This chemical is commonly known as} \]

\[ \text{BHC} \]

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benzene hexachloride, though it is a misnomer. The insecticidal properties of BHC was discovered by Duprine in France (1941) and Thomas in England (1962). Various isomers of BHC differs widely in their solubilities in organic solvents, generally in the order $\delta, \gamma, \varepsilon, \zeta, \xi$ so also their biological activity with $\gamma$ being the most (Ishida & Dahm, 1965).

Technical grade of BHC is a mixture of several stereo isomers $\zeta (70-72\%), \beta-(8-14\%), \gamma (11-18\%), \delta (6-10\%)$ and $\xi$ traces of hepta- and octachlorocyclohexane.

$\gamma$ - BHC is the insecticidal component and is also known as lindane. Though it is less persistent and biodegradable it has been replaced by technical BHC for making formulators because of its low cost, easy availability and its potency against a wide range of pests.

Due to unhindered and indiscriminate use of BHC this chemical is known to be present in almost all components of biosphere.

2. **Malathion**: S-[1,2-bis-(ethoxycarbonyl)ethyl]O,O-dimethyl phosphorodithioate,

\[
\begin{align*}
\text{CH}_3\text{O} & \xrightarrow{S} \text{P-S-CH-C-O-C}_2\text{H}_5 \\
\text{CH}_3\text{O} & \quad \text{CH}_2\text{C-O-C}_2\text{H}_5
\end{align*}
\]
Malathion is one of the safest organo-phosphate compounds used in controlling pests (Spiller, 1961). Malathion was declared as an insecticide in December 1950 by the American Cyanide Company, who claimed it to be less toxic to mice and rats. These claims have been largely substantiated, and malathion now enjoys wide as an insecticide. It is available in the form of emulsion. It is also possible to use malathion in the form of dust or suspension prepared from wettable powder.

There is no information on degradation of malathion by other insects than cockroaches but the fact that some materials of even low or negligible insecticidal activity potentiating the toxicity of malathion to houseflies, suggests the presence of detoxification mechanism. Most of the formulations and concentrations of malathion are non-injurious to plants.

**Experimental:**

For experimental studies 20-25 larvae of *O. rhinoceros* were introduced in 5Kg of compost in earthen pots. The insecticides BHC and Malathion were separately mixed with the compost for different sets of experiments to get the final concentration of 0.5, 1.0, 1.5 and 2.0 gms/kg. Though preliminary experiments the LD-50 value for BHC and Malathion were found to be 2gms. Hence the lower concentration than LD-50 were fixed for experimental studies. Sampling was done arbitrarily at intervals of 24,
48, and 72 hrs for each concentration for the analyses of 
organic components.

To study the effect of insecticides on different 
organs, the entire digestive system and body wall were 
chosen, since their effect on these tissues will be more 
conspicuous, since the larvae are voracious feeders.

Biochemical Analyses:

In order to follow the changes taking place in organic 
constituents, total protein, carbohydrates and lipids were 
estimated in digestive system and body wall of the normal 
larvae.

2ml. of haemolymph was syringed out (and 1% sodium 
citrate was added into the collection tube to avoid 
coagulation of the haemolymph) for analyses of the above 
mentioned constituents. The results are expressed in 
terms of micrograms/millilitre (µg/ml).

After treating the larvae with different concentrations 
and at various intervals the larvae were carefully dissected 
and digestive system and body wall were removed and 
transferred to the petri dishes. Few drops of 5% 
trichloroacetic acid were added to prevent enzyme 
hydrolysis. The treated tissues were dried in vacuo under 
HCl. The difference between wet and dry weights of tissues 
were taken as the water content of the tissue. The analyses 
of the total protein, carbohydrates and lipids were carried 
out on dry tissues and the results are presented in
percentage dry weight. While comparing the protein, lipid
and water contents of tissues under experimental conditions,
percentage of protein and lipids were recalculated to get
their percentage as wet weight. The organic constituents
were analysed as follows:

1. Protein: Lowry et al. (1951)
2. Carbohydrates: Dubois et al. (1956)
3. Lipids: Raymont et al. (1964)

Protein was further analysed by disc-gel
electrophoresis (Following the method of Davis, 1964) to see
the changes occurring at the fractional level. This study
was carried out for normal and experimental tissues (Digestive system and Body wall as well as haemolymph). The
gels were stained with amido-black and the intensity of
different protein fractions were read through the scanner
("Quick-Scan-Densitometer- Junior-Desaga, Germany") using red
filter.

To follow the rate of accumulation of the pesticides in
the larva, the residue analyses of malathion using different
concentration was investigated through the gas
chromatography. (Chemito – 8510) equipped with nitrogen –
phosphorous detector (NPD) and Shimatzu – C – RZAX
integrator.

Weighed tissues of digestive system and body wall were
blended in a blender and extracted twice with 100 ml of
chloroform. The blended samples were filtered through glasswool. The filterate was concentrated to dryness. To this, 50 ml of hexane and 100 ml of acetonitrile were added. This was transferred to 500 ml separating funnel for phase separation. The lower phase was collected into a round bottom flask. This procedure was repeated and then concentrated to dryness. To remove fat, if any, was treated with acetone, precipitating solution (2.5 ml of propionic acid and 1.25 gm ammonium chloride in 1000 ml of distilled water) and chloroform. They were concentrated to dryness and taken for quantifying in the gas chromatography (Anon 1985) The extracted samples were dissolved in hexane and injected in the column.