CHAPTER I

GENERAL INTRODUCTION
One of the most convenient means for controlling harmful organisms is the method of using various chemical compounds. The chemical compounds used to prevent, destroy, repel or mitigate any species of pests, are called pesticides. Depending upon the purpose for which they are used, the pesticides can be divided into various groups, viz. acaricides, algicides, antiseptics, arboricides, bactericides, mollouskicides, fungicides, herbicides, limacides, nematocides, zoocides and insecticides, etc. Depending upon their mode of action, these could be classified into various groups, such as contact poison, stomach poison, nerve poison, etc. On the basis of their chemical nature the pesticides can be classified into two basic groups:

(1) Inorganic pesticides
(2) Organic pesticides.
The organic pesticides can be further divided into three groups:

1. Organochlorine or chlorinated hydrocarbon pesticides,
2. Organophosphate or organophosphorus pesticides,
3. Carbamate pesticides.

The organochlorine insecticides are being used more extensively than organophosphate and carbamate pesticides are. The organochlorine pesticides such as DDT, Lindane, BHC, endrin, endosulfan, aldrin, chlordane, toxaphene, heptachlor and methoxychlor are being used widely in agriculture, public health, house-hold and forestry for the control of various harmful insects and pests. As a result of this the crop production is increasing, the insect vector born diseases such as malaria, filaria and elephantiasis, etc. can be controlled. However, the indiscriminate use of these chemicals has caused serious concern about the toxic effects of residues of these chemicals on non-target organisms.

Around 1960 many side-effects of these pesticides were noticed and reports started appearing which have been reviewed by Lal (1982) and Karanth and Anu (1988 a-b). This subject today constitutes one of the most serious challenges to the public health and environmental pollution because the residues of insecticides appear to be a permanent part of men's environment. The residues of insecticides are translocated in plants
water and animals and ultimately reaches to our body through food chain, often causing undesirable and unpredictable side effects.

The wide spread use of insecticides over the past 45 years has resulted in problems caused by their interaction with the natural biological system. In the context of agriculture, the excess application of insecticides to the agricultural soil, the major concern is the harmful effects of insecticides and their residues on soil microflora (Heinonen et al., 1985) and hence on soil fertility. Apart from causing environmental contamination and health hazards, they are now known to be mutagens and carcinogens (Corbett, 1974).

The environmental dynamics of pesticides are largely influenced by the various factors operating in the environmental properties of pesticides. The pesticides are found at detectable levels in many parts of the environment (Abbott et al., 1965), in the inhabited or non-inhabited areas of the world. The introduction of pesticides into these areas can occur in several ways and for a variety of purposes.

Alexander (1965) and Edwards (1966, 1973) have pointed out that the chlorinated hydrocarbons are more stable than organophosphate and carbamate insecticides which are readily metabolized by bacteria (Venkateswarlu and Sethunathan, 1985; Ziegler et al., 1980; Adhya et al., 1981) actinomycetes and fungi, however, the chlorinated hydrocarbons and their degradation
products persist for several years (Lal and Saxena, 1982; Edwards, 1973 and Menzie, 1978). Residues of DDT and other organochlorines have been found in farm soils (Harris et al., 1977; Miles and Harris, 1978; Maurer et al., 1984; Foxall and Maroko, 1984) rice seeds and plants (Rosario and Yoshida, 1976), Oligotrophic lake ecosystems and sediments (Anderson et al., 1982; Kveseth, 1981; Veith et al., 1977), aquatic environments (Bjerk and Brevik, 1980), marine ecosystems (Young et al., 1976) and in lagoons (Rosales and Escalona, 1983). Therefore, the use of organochlorine insecticides has now been restricted or banned completely in many countries. However, in India the chlorinated hydrocarbon insecticides are being used continuously.

The microbial metabolism is an important process for degrading pesticides in soil environment (Edwards, 1973 by Domsch, 1984; Kearney and Kellogg, 1985). The pesticide degrading microorganisms offer potential for development of low cost methods for the disposal of aqueous pesticide wastes, or for the clean-up of heavily contaminated soils (Friedman, 1984; Seiber, 1984; Chakrabarty, 1982; Kearney et al., 1987).

The major aspect of the microbes insecticide interaction is the microbial degradation. Once thought to be recalcitrant, molecules are now believed to be broken completely by microorganisms. Menzie (1969 and 1978) has reviewed the metabolism of pesticides in much detail. Various other review articles have also been published on the degradation of pesticides (Alexander, 1972, 1974, 1980 and 1981; Bollag, 1972, 1974 and
1982; Lal and Saxena, 1982; Munnecke, 1979; Fries, 1972; Matsumura et al., 1971; Matsumura and Krishnamurty, 1982; Sethunathan, 1982 and Meikle, 1972) which cover the microbial degradation of chlorinated hydrocarbons insecticides to varying degrees of thoroughness.

Some workers have demonstrated that the particular species of microorganism can degrade certain pesticides, but is much more common for breakdown of a pesticide to be non-specific with a wide variety of microorganism possessing the ability to degrade any particular pesticide. Many species of microorganisms seems to be able to degrade insecticide completely. There are some indications that microbial breakdown may be synergistic. Neither Arthrobacter nor Streptomyces can degrade diazinon alone, but together can metabolize larger quantities of this pesticide (Gunner and Zuckerman, 1968).

In general practice after the microbial attack the more persistent and toxic compounds converted to less persistent and/or less toxic, non-toxic compounds. However, there are several reports which indicate that some chlorinated insecticides become more toxic to pests and to other non-target organisms, after microbial attack. These include aldrin (become dieldrin) and heptachlor (become heptachlor epoxide) (Lichtenstein and Schulz, 1960), although DET, BHC, endosulfan, isobenzene, etc. converted to less toxic compound by microbial degradation.

Patil et al. (1970) tested 20 soil microbial cultures to study their ability for degrading aldrin. They found that 13 out of 20 microbial cultures were able to degrade aldrin and dieldrin
transdiol were the major metabolites. In a study Miles et al. (1969) have reported 15 species of Trichoderma, 11 species of Penicillium, 2 species of Aspergillus, 2 species of Phizopus, 1 species of Mucor, 16 species of Nocardia, 7 species of Streptomyces, 3 species of Thermoactinomyces, 12 species of Bacillus, 5 species of Arthrobacter and 1 species of Corynebacterium are found capable of degrading a chlorinated pesticide, i.e. heptachlor.

Microbial degradation of endosulfan was studied by Perscheid et al. (1973). These workers reported that the endosulfan alcohol was the main metabolite. Others reported the formation of endosulfan alcohol and endosulfan sulfate, after exposure of microbes to endosulfan (El Zorgani and Omer, 1973 and 1974). Martens (1972, 1976 and 1977) extensively studied the degradation of endosulfan by 87 soil microbes and found that 28 soil fungi and 50 bacterial strains were able to degrade endosulfan.

Ability of DDT and BHC degradation by several bacterial strains was reported by many workers (Largolos, 1967; Flimmer et al., 1968; Bedemeyer, 1966; Barker et al., 1965; Sethunathan and Yodia, 1973; Chacko et al., 1966; Patil et al., 1970; Francis et al., 1976; Focht and Alexander, 1971), however only a few workers have studied the degradation of chlorinated pesticides by fungi (Martens, 1972, 1976 and 1977; El Zorgani and Omer, 1973 and 1974; Miles et al., 1969; Anderson et al., 1970).

Breakdown of insecticides by microorganisms is a complex process and several mechanisms are known by which microbial
degradation of insecticides occur. It was originally assumed that the patterns of metabolism in microbial system were very similar to the higher animals. However, it has now become clear that the patterns of degradation in these two groups of organisms are very different (Matsumura, 1982). Microbial conversion of organochlorine insecticides via dechlorination, dehydrohalogenation, oxidation and/or isomerization are the common mechanisms and these reactions are described by several authors (Johnson, 1976; Kaufman, 1974; Kearney and Kaufman, 1972; Lal and Saxena, 1982; Matsumura, 1973 and 1982; Pfister, 1972 Ware and Roan, 1970; Hill and Wright, 1978; Khan, 1977; Matsumura et al., 1972 and Zabik et al., 1976) in detail.

Dehalogenation of chlorinated hydrocarbon insecticides by microorganisms is a reductive mechanism. The "dehalogenation" proceeds by replacing a halogen atom on a non-aromatic carbon with a hydrogen, the most well known case is the conversion of DDT to TDE (DDD). Other insecticides known to go through such reactions are Y-BHC (Ruvo et al., 1974; Beneset and Matsumura, 1973; Jagnow et al., 1977; Mathur and Saha, 1977; Babu et al., 1988) and endrin (Matsumura et al., 1971).

"Dehydrohalogenation" involves the removal of adjacent halogen and hydrogen atoms, i.e. the elimination of HCl and hence is called dehydrohalogenation. As a result of this the formation of a C-C double bond occur (Hill and Wright, 1978). The most familiar dehydrohalogenation reaction in microorganisms is the enzymatic conversion of DDT to DDE (Guenzi and Beard, 1968;
Matsumura and Boush, 1968; Fries et al., 1969; Miyazaki and Thorsteinson, 1972). Aerobic conditions favoured the reaction, although small amounts of the same products (DDT to DDE) are also formed under anaerobic conditions (Stenersen, 1965; Guenzi and Beard, 1967). \( \gamma \)-pentachlorocyclohex-1-ene, formed by dehydrochlorination of lindane (\( \gamma \)-BHC) in moist soil (Yule et al., 1967) was attributed to microbial activity.

Isomerization reactions are as important as other reactions. As a result of incidental metabolism of insecticides by microorganisms, such reactions take place, although it is not at all clear, why microorganisms go through such reactions. However, from the view point of environmental toxicology, such reactions are very important, since they give rise to other unsuspected environmental contaminants. The example of such a reaction is the formation of \( \alpha \)-BHC from \( \gamma \)-BHC as shown to occur in \textit{Pseudomonas putida} (Beneset and Matsumura, 1973) and by other microbes under anaerobic condition (Jagnow et al., 1977) Mathur and Saha, 1977). Another important isomerization reaction is the formation of \( \alpha \)-endosulfan from \( \beta \)-endosulfan, as shown to occur in mixed microbial cultures (Miles and May, 1979).

"Oxidative reactions" are frequent in microbial world than other biological groups. The oxidative reactions can be divided into four types:

1. Epoxylation: Microbially catalysed oxidation of unsaturated carbon-carbon double bonds results in the formation of an
Epoxidation of chlorinated cyclodiene insecticides such as aldrin and heptachlor to corresponding epoxide was reported by Lichtenstein and Schulz (1960), Patil et al. (1970), Miles et al. (1969) and (1971). Moy and Abbott (1973) have isolated an enzyme system from a pseudomonad, capable of converting alkenes to their corresponding epoxide in the presence of NADH and molecular oxygen.

(2) Oxidative Dealkylation: These reactions are mostly non-specific oxidations requiring mixed function oxidases and a hydrogen donor such as NADPH. The microbially mediated removal of an alkyl group from a nitrogen atom has been recorded for numerous pesticides and apparently proceeds via unstable hydroxymethyl intermediates (Geissbühler, 1969; Kaufman and Kearney, 1970).

(3) Ring Opening: One very important reaction that takes place only in the microbial world is the aromatic ring-opening mechanism. The system is operated by a series of oxidative ring hydroxylation (including epoxidation) reactions. The ring hydroxylation can occur even at the chlorine-attached aromatic carbon, in contrast to reductive dechlorination reaction on chlorinated
hydrocarbons (Focht and Alexander, 1970 a-b; Furukawa and Matsumura, 1975; Furukawa et al., 1978).

(4) Decarboxylation: In the decarboxylation reaction the carboxyl group is replaced by hydrogen or on the aromatic ring the carboxyl group is replaced by a hydroxyl group (Fig. 1a and b), and CO₂ evolved (Rao et al., 1969).

\[
\text{FIG.-1a} \quad \begin{array}{c}
\text{C} & \text{COOH} \\
\text{H} & \text{C} & \text{H} & \text{CO₂}
\end{array}
\]

\[
\text{FIG.-1b} \quad \begin{array}{c}
\text{COOH} \\
\text{1/2 O₂} \\
\text{CO₂}
\end{array} \xrightarrow{-} \begin{array}{c}
\text{OH}
\end{array}
\]

Other common oxidative reactions are β-oxidation, conversion of alcohols and aldehydes to acids and dehydrogenation, but they are much less frequently observed among metabolic activities on pesticides (Matsumura, 1982).

Another important microbial reaction on pesticidal chemicals is the hydrolysis. Hydrolytic activities are much more common in microbial system than in any other biological group. Perhaps the reason for such hydrolytic reactions being common in microbial world is that many of the microorganisms, particularly the fungi, produce hydrolytic enzymes outside the cell (exo enzymes). Nearly all the exoenzymes liberated by microorganisms seem to be related to the metabolism of insecticides. Various soils also contain different types of exoenzyme that are hydrolytic in nature. Thus most of the hydrolytic reactions beling to a type of metabolism, where incidental
degradation takes place by the broad-spectrum activity of enzymes (Matsumura, 1982).

Certain environmental factors, such as moisture, temperature, organic matter, redox conditions (Siddaramappa and Sethunathan, 1975), sorption-desorption (Wahid and Sethunathan, 1978, 1979a and 1980) and mineral constituents (Glass, 1972; Parr and Smith, 1974; Rao and Sethunathan, 1979; Wahid and Sethunathan, 1979b) can influence the rate of microbial breakdown of pesticides; the conditions that favour microbial activity. Large pH changes are often associated with microbial metabolism (Venkateswarlu et al., 1977; Seiber et al., 1978; Sethunathan and MacRae, 1969 and Siddaramappa and Sethunathan, 1975) together with changes in nutritional sources.

Some workers have demonstrated that the microbial breakdown of pesticides often progress faster under anaerobic condition (Guenzi and Beard, 1968; Kearney et al., 1969; Yoshida and Castro, 1970; Tsukano and Kobayashi, 1972). The disappearance of 1,2-BHC from flooded soil was studied by Tsukano and Kobayashi (1972).

Sethunathan and coworkers have extensively studied the degradation of organophosphate and carbamate insecticides in soil under flooded and non-flooded conditions (Sethunathan et al., 1977; Adhya et al., 1981, Sethunathan and Siddaramappa, 1978; Reddy and Sethunathan, 1983, Barik et al., 1982 and 1984). In this regard, however, very little work has been done on chlorinated hydrocarbons except BHC (Siddaramappa and Sethunathan, 1975).
Raghu and MacRae, 1966; MacRae et al., 1967; Castro and Yoshida, 1974; Sethunathan et al., 1969; Heritage and MacRae, 1977, 1979).

Biodegradation of α- and β-BHC in a soil slurry under different redox conditions has been studied by Bachmann et al. (1988).

It seems important to evaluate the overall importance of microorganisms particularly the soil fungi in the degradation of chlorinated hydrocarbons insecticides. However, this is difficult, because we still have insufficient informations, but some idea can be obtained by comparing the rate of disappearance of more persistent chlorinated hydrocarbon insecticides in sterile and non-sterile soils.

Thus, as revealed from the foregoing account, a good number of microorganisms have been evaluated and many yet to be evaluated, for their ability to degrade a number of insecticides and it could be believed that some of the microbial strains would be selected in future for the field trials (Kearney and Kellog, 1985; Friedman, 1984; Seiber, 1984; Chakraborty, 1982 and Kearney et al., 1987). However, due to lack of concentrated efforts in exploring the potential of microorganisms, there appears to be an urgent need of isolation and evaluation of good microbial agents from soils, particularly useful for degrading chlorinated hydrocarbons insecticides, which could be used for the disposal of these chemicals from the heavily loaded areas. According to a survey made in SAGAR District 95% of the farmers preferred to use DDT, BHC, aldrin and endosulfan in place of
malathion, carbaryl and other organophosphate and carbamate insecticides because of their low cost and effectiveness. Hence the present problem on the role of microorganisms in the degradation of chlorinated hydrocarbon insecticides in soil was undertaken and the experimental attempts briefly covered the following objects:

(1) A survey of the residues of chlorinated hydrocarbon insecticides in soils of different localities.

(2) Isolation of soil fungi from different field soils containing insecticide residues.

(3) Role of isolated soil fungi in the degradation of BHC, DDT and Endosulfan.

(4) Effect of BHC, DDT and Endosulfan on the growth of some soil fungi.

(5) Degradation studies of DDT, BHC and Endosulfan by some soil fungi on PDA media and in soil.

(6) Degradation studies of DDT, BHC and Endosulfan in soil under flooded, non-flooded and sterile conditions.

(7) Degradation of P'P'-DDT, Y-BHC and Endosulfan (Tech) by a soil bacterium Pseudomonas sp.

Microbial degradation studies are being carried out in only a few laboratories in India. There is no report on the microbial degradation of chlorinated insecticide in soil from M.P. The present investigation has been undertaken to study the
role of fungi and bacteria in the degradation of DDT, BHC and Endosulfan which are used most commonly by Indian farmers.