CHAPTER 1

INTRODUCTION

1.1 GENERAL

In India, agriculture provides a principle means of livelihood for about 60% of country’s population. It is the largest economic sector in India which contributes to about 22% of Gross Domestic Product (GDP) of India. Total area coverage under food grains in 2007-2008 was about 124.10 million hectares which recorded a crop production statistics of 230.67 million tonnes. In this target achievement plant protection plays a major role especially against various pests. Over the recent years, there was a considerable increase in plant protecting chemicals like pesticides due to increase in cultivable area and fast changing cropping pattern. The average pesticide consumption is about 0.5 Kg/ha in India. On global level, pesticide consumption in India occupies tenth place. According to Central Insecticide Board (section of 9(3) of the insecticides Act 1968, amended on 20th August 2014) 246 pesticides were registered for use in India. In general among the registered pesticides, insecticides consumption covers about 69.39%, fungicides 19.06%, herbicides 16.75%, and others by 2.80% (Shetty et al, 2008). In developing country like India where agriculture forms the major economic output, dependence on cheaper crop protection agents like pesticides will be more. Hence, the probability of persistence of pesticide residue in various ecosystems will be more in a country like India. Moreover, India has varied climatic conditions and also receives high rainfall which contributes to nonpoint source pollution of environment. Indian History has witnessed many
tragic events related to pesticide usage. Therefore, research in various aspects of pesticide like monitoring of its residues, its toxic effect and its remediation has got its own significance in India.

In India use of synthetic pesticide has started from 1948 and its production started in 1952 with the setting up of BHC production plant at Rishra in Kolkata. The first two pesticides used in India were DDT and BHC. With this began the emergence of many large and small scale industries in India producing different formulations of pesticides. At present, in India there are about 125 basic pesticide producing industries and 500 formulation industries. Among the formulations produced, major share goes to dusting powder (85%). In India about 246 pesticides are registered for use under section of 9(3) of the insecticides Act 1968 and of which 13 are restricted in usage. Organochlorine pesticide has found maximum application in India which is followed by organophosphates. Regarding the type of usage, insecticides come first, which is followed by herbicides and fungicides. On global level herbicides are the leading which comes to about 50%, in India its consumption is 15%. Among the various organochlorine pesticides used, endosulfan is the most extensively used insecticide in India. This insecticide has got wide range of application against various crop pests.

Endosulfan is a pesticide belonging to the organochlorine group of pesticides, under the Cyclodiene subgroup and belongs to the class of organochlorine insecticides. It was introduced in the 1950 and it emerged as a leading chemical used against a broad spectrum of insects and mites in agriculture and allied sectors (Harikrishnan & Usha 2004). Endosulfan is a broad spectrum contact insecticide and acaricide registered for use on a wide variety of vegetables, fruits, cereal grains, and cotton, as well as ornamental shrubs, trees, vines, and ornamentals in agriculture. Commercially it is available in two different formulations- 35% EC and wettable powder in the
name of endosulfan, thiodan, Thionex, Endosan, Farmoz, Nufarm etc. Chemically endosulfan (1,2,3,4,7,7 hexachlorobicyclo-2,2,1-heptene–2,3-bishydroxy methane-5,6-sulfite) is a mixture of stereo isomers of α and β endosulfan in the ratio 7:3. Physically it is a cream to brown coloured solid that may appear crystalline or in flakes. It has a distinct odour similar to turpentine. Its water solubility is 0.33 mg/L and hence its half-life is more than hundred years.

The U S Environmental Protection Agency (EPA) classifies endosulfan as Category Ib – Highly Hazardous. The European Union also rates it Highly Hazardous. World Health Organization (WHO) classifies endosulfan in Category II – Moderately Hazardous. The classification by WHO was found to be inappropriate considering the classification followed in many countries and the available toxicity information. It has been alleged that the classification is based mainly on LD50 value for acute toxicity generated by the producer company (Paul et al 1995). The Industrial Toxicological Research Centre (ITRC) in Lucknow classifies endosulfan as Extremely Hazardous (Anon 1989).

Endosulfan is acutely toxic and has been implicated in many cases of poisoning and fatalities. It has been identified with a range of chronic effects, including cancer and impacts on hormonal systems, exhibiting similarities with its predecessors in the organochlorine class. Many studies related to its acute and chronic toxicity in laboratory reveals that Endosulfan is highly toxic to rats and mice (Romeo 2000, Susan & Sania, 1999). Some studies revealed its teratogenic (Anon 2001) and carcinogenic properties (Reuber 1981) on rats and mice. Endosulfan also damages the endocrine system, nervous system, circulatory, reproductory, respiratory and excretory systems (Anon 1997, 2000, Romeo 2000, Paul et al 1995, Sinha et al 1995, Naqavi & Vaishnavi 1993, Reuber 1981, Susan & Sania 1999). The National
Institute of Occupational Health, Ahmedabad have linked the higher prevalence of neurobehavioral disorders, congenital malformations in female children and abnormalities related to male reproductive systems to the continuous exposure to endosulfan spray in one of the villages in Kasargod District in Kerala.

The fate of endosulfan released in the environment is different for the two isomers and also depends on the medium it gets deposited. Beta endosulfan is more persistent than alpha isomer. Endosulfan sulfate is the main degradation product of both isomers, which is equally toxic and is itself more persistent in the environment than its parent compounds. Endosulfan can be broken down by photolysis, hydrolysis and bio degradation. Endosulfan diol, endosulfan lactone, endosulfan ether etc. are some of the other byproducts. Although the isomers are fairly resistant to photolysis, the break down products is susceptible. On plant surfaces endosulfan rapidly degrades to its metabolites. Endosulfan is fairly immobile in soil and is highly persistent. Major products in soil are endosulfan diol, endosulfan sulfate and endosulfan lactone. Residues of endosulfan were detected from air, water (surface and ground water) and soil in India, water and sediments in Ghana (Mensah et al 2011), marine water and sediments in India, shallow ground water in Pakistan (Jabbar et al 1993), river water in China (Hongliang et al 2012), lagoons in Spain (Ruzafa et al 2000) surface and ground waters in Portugal (Cerejeira et al 2003), ground and well waters in the Philippines (Cerejeira et al 2003), coastal, estuarine and river sediments in Israel, water in Benin, Malawi, Nigeria and from drinking, ground, surface and marine waters in South Africa (Fatoki et al 2004), soil in Benin, Nigeria, Sudan and Zambia, sediments in Benin and Nigeria, vegetation in Madagascar, Zambia and Ghana, Paddy fields in Mediterranea, water from remote mountain lakes in Europe and river and sea water in South East Asia etc. (Pozo et al 2004).
Endosulfan is a banned insecticide which was in use in India after DDT. It is the third largest selling insecticide worldwide (George et al 2012). The usage of this insecticide had resulted in many tragic events in India due to its high toxicity and persistence in environment. It is one of the persistent organochlorine pesticides having a water solubility of 0.33 mg/L and hence its half-life is more than hundred years. Endosulfan is a persistent pesticide which is ubiquitous in environment mainly in soil and water. Less solubility of endosulfan results in persistence of endosulfan and its major metabolite endosulfate in soil (Manickam et al 2007).

Endosulfan and its isomers are proved to be biodegraded by many bacteria, fungi in laboratory condition, but still in the field its bioremediation is limited due to its less bioavailability. Endosulfan degradation by microbes was investigated by many researchers and those studies reveal that endosulfate, endosulfan diol, endosulfan lactone, endosulfan monoaldehyde are some of the metabolites of endosulfan (Supriya & Dileep 2009). In soil environment, endosulfan is degraded to endosulfate, which is less soluble and more toxic than its parent compound. Most of the biodegradation pathway in soil will produce endosulfate and it is the major residue detected in soil (Goebel et al 1982). Moreover, endosulfate is bioaccumulative in nature and was detected in plants and animals (Beck et al 1966, Stewart et al 1974). Less solubility of endosulfate makes it less bioavailable and is adsorbed to soil particles leading to its persistence. The toxicity of endosulfate may decelerate the biodegradation of their parent compound.

Many studies were conducted on biodegradation of endosulfan and its biochemical pathway in aqueous system, but studies on endosulfan and endosulfate degradation in soil is less and most of them are on its biodegradation in culture media. Many microbes are capable of utilizing endosulfan and other pesticides in the optimized laboratory condition and
showed higher efficiency. But when they are brought to field condition, there was considerable reduction in degradation. Soil microbial community will be also affected by the soil characteristics (soil type, organic matter content, pH and salinity) and environmental factors like relative humidity, evapotransmission, and temperature (Cycon & Piotrowska 2007, Fang et al 2009). A study addressing those limitations in the field is needed for successful bioremediation of pesticide contaminated site. Hence, the present study targets to enhance the bioavailability of soil bound endosulfan and some other pesticide residues using microbial catalyst which can solublize the soil sorbed pesticide residues and thereby enhanced biodegradation with the following objectives.

1.3 OBJECTIVES OF THE PRESENT STUDY

The objectives of the present study are

1. To study the physicochemical characteristics of agricultural soil.

2. To enrich endosulfan degrading bacteria from pesticide contaminated soil.

3. To screen the bacterial isolates for endosulfan and endosulfate degradation and for biosurfactant production.

4. To develop a consortium using the promising bacterial isolates.

5. To enhance the bioavailability of pesticide in soil by
   - using a biosurfactant producing bacterial consortium.
   - studying the biodegradation of pesticides in different layers of soil (at different depths)
6. To characterize the bacterial strains in the selected consortium through biochemical and molecular techniques.

1.4 STRUCTURE OF THESIS

The thesis is presented in five chapters.

Chapter 1 gives an overview of pesticides, its consumption in India, endosulfan and its toxicity, persistence in environment and its biodegradation. The scope and objectives of the present research are outlined in this chapter.

Chapter 2 presents a detailed literature review on pesticide usage in India, its toxicity, fate of pesticide in environment, pesticide residues in soil, water, sediment, flora, fauna and food, bioremediation of pesticide contaminated soil, bioavailability of pesticides, factors affecting bioremediation process, biodegradation of endosulfan in soil and water, influence of synthetic and biosurfactants in enhancing the bioavailability of pesticides.

Chapter 3 describes about the various methods employed during the present investigation.

Chapter 4 presents the major results and discussions of the study in the light of available literature.