INTRODUCTION
INTRODUCTION

Grape (*Vitis vinifera*) is believed to have originated in Armenia from the regions between south of the Black and Capsian seas in Russia as well as subsequently spread both in temperate in tropical areas to the west and east. Grape was introduced into India in 1300 AD by the Moghul invaders. It is highly remunerative and most assured horticulture crops of tropical India.

In India, grape production is done in an area of 111 thousand hectare including a 1,235 thousand tons of total production and yield of 11 tons/ hectare. In our country, the productivity of grapes is comparatively more amongst all countries in the world which produce grapes, because in India special training systems are provided by government and used by farmers for grape cultivation. In the whole country Maharashtra is a main contributing state in grape production. Grape is usually grown under different climatic conditions and in various agricultural-climatic regions containing a variety of soil. Hot tropical region spread over Solapur, Sangli, Nasik, Satara, Pune, Osmanabad and Latur districts of state and this main viniculture region accounts for about 70% of the region under grape cultivation of India. Regarding the agricultural land in grape production or grape cultivation, Sangli and Nasik districts are at a head in the state in a scientific manner.
Sangli district including Walwa Tehsil, Miraj Tehsil, Palus Tehsil, Vita Tehsil and Khanapur Tehsil are one of the more grape producing areas in Maharashtra. Government of Maharashtra has started Krishna Valley Wine Park 30km far from Sangli to elevate a wine industry. The District is actually well published for high quality and high yielding grapes. It also has various cold storage facilities as privately owned and state level. This grape wine park covers about 1.42 km² (350 acres) area and has been started at Palus, 30 km from Sangli city.

In Maharashtra, region under grapes is 86 thousand hectare with its productivity approximately 774 thousand tons per annum. During 2011-12, total transport of grapes to abroad is 108.58 thousand tons profiting of Rs.602.88 crores from India. From above transport approximately 80% is exported from Maharashtra. According to Food and Agricultural Organization report (2010), India has secured eighteenth position in the world as far as high grape productivity concerned.
In India, there is rise in production of grapes with respect to acreage. This increase is from 47.5 to 111.4 thousand hectare from 2001-2002 to 2010-2011 in acres respectively and similarly also the productivity has raised.

Table No.1: Productivity of grapes in India according to Area and Production

<table>
<thead>
<tr>
<th>Year</th>
<th>Area (000'hectare)</th>
<th>Production (000'tons)</th>
<th>Productivity (tons/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001-02</td>
<td>47.50</td>
<td>1184</td>
<td>25</td>
</tr>
<tr>
<td>2002-03</td>
<td>52.10</td>
<td>1248</td>
<td>24</td>
</tr>
<tr>
<td>2003-04</td>
<td>57.80</td>
<td>1475</td>
<td>25</td>
</tr>
<tr>
<td>2004-05</td>
<td>60.50</td>
<td>1565</td>
<td>26</td>
</tr>
<tr>
<td>2005-06</td>
<td>66.00</td>
<td>1649</td>
<td>25</td>
</tr>
<tr>
<td>2006-07</td>
<td>65.00</td>
<td>1685</td>
<td>26</td>
</tr>
<tr>
<td>2007-08</td>
<td>68.00</td>
<td>1735</td>
<td>25</td>
</tr>
<tr>
<td>2008-09</td>
<td>80.00</td>
<td>1878</td>
<td>23</td>
</tr>
<tr>
<td>2009-10</td>
<td>106.40</td>
<td>881</td>
<td>08</td>
</tr>
<tr>
<td>2010-11</td>
<td>111.00</td>
<td>1235</td>
<td>11</td>
</tr>
<tr>
<td>2011-12</td>
<td>116.00</td>
<td>2221</td>
<td>19</td>
</tr>
<tr>
<td>2012-13</td>
<td>116.50</td>
<td>2604</td>
<td>22</td>
</tr>
</tbody>
</table>

(Source: National Horticulture Board, Government of India)

According to National Horticulture Board (NHB), Government of India, Maharashtra state is at top list in grape production (774 thousand tons) after that Tamilnadu (53 thousand tons), Andhra Pradesh (27.6) and Karnataka (330.3) as southern states.
**Table No.2: Grape producing states of India along with their productivity of grapes in last 3 years**

<table>
<thead>
<tr>
<th></th>
<th>2008-09</th>
<th></th>
<th></th>
<th>2009-10</th>
<th></th>
<th></th>
<th>2010-11</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area ‘000 ha</td>
<td>Production ‘000’ MT</td>
<td>Pdy. ha/ MT</td>
<td>Area ‘000’ ha</td>
<td>Production ‘000’ MT</td>
<td>Pdy.</td>
<td>Area ‘000’ ha</td>
<td>Production ‘000’ MT</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>55.7</td>
<td>1415</td>
<td>25</td>
<td>82.0</td>
<td>440</td>
<td>5</td>
<td>86.0</td>
<td>774</td>
</tr>
<tr>
<td>Karnataka</td>
<td>14.9</td>
<td>268</td>
<td>18</td>
<td>17.4</td>
<td>318</td>
<td>18</td>
<td>18.1</td>
<td>330</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>3.1</td>
<td>91</td>
<td>30</td>
<td>2.6</td>
<td>44</td>
<td>17</td>
<td>2.7</td>
<td>53</td>
</tr>
<tr>
<td>Andhra Pradesh</td>
<td>3.0</td>
<td>62</td>
<td>21</td>
<td>1.4</td>
<td>30</td>
<td>21</td>
<td>1.3</td>
<td>28</td>
</tr>
<tr>
<td>Other</td>
<td>3.0</td>
<td>41</td>
<td>14</td>
<td>2.9</td>
<td>49</td>
<td>17</td>
<td>3.3</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>79.6</td>
<td>1877</td>
<td>108</td>
<td>106.4</td>
<td>881</td>
<td>78</td>
<td>111.4</td>
<td>1235</td>
</tr>
</tbody>
</table>

(Source: NHB, Government of India)

**Insect Pests of Grapes and their Management**

Grape is an important commercial fruit crop in India, which receives frequent application of large number of agrochemicals that is pesticides, throughout the cropping season for management of various pests and diseases. Indian grape is under constant scrutiny of the environment and health protection agencies worldwide, as in India, the cultivation of grapes receives frequent application of large number of pesticides and further, grape is mostly consumed as fresh fruit in intact form without any processing. Therefore, pesticide residues left on the grapes and during harvest can be carried through into the wine.

The most important and frequently occurred insect pests on grapes in India are as follows-

**Flea Beetles**: Mature flea coleopterans take buds and after each trimming finish totally. Then these defected buds fail to germinate. Various pesticides which kill insects are used
on this such as Carbaryl at 0.15gm/L, Quinalphos at 0.05gm/L, Dichlorvas at 0.1gm/L or Phosalone at 0.05gm/L are applied until the growth of leaves.

**Thrips:** These act on the berries and suck sap from them. Thrips causes scab formation on the berry surface which damages the ovaries and such damaged berries are not acceptable. Thrips are most specifically overcome by applying Phosphamidon at 0.05gm/L, Carbaryl 0.125gm/L, Phosalone at 0.05gm/L or Malathion at 0.05gm/L.

**Mealy bugs:** Nymphs and imago take sap from shoots resulting in growth stunting. These mealy bug infected bunches are not suitable for marketing. Only Dichlorvas 0.1gm/L mixed with Neem oil 0.2gm/L or Tridemorph 0.1gm/L is sprayed.

**Leaf hopper:** Young and mature nymphs of these insects take sap entirely from the leaves. For its control of these pests Carbaryl at 0.15gm/L, Fenitrothion at 0.04gm/L Phosalone at 0.05gm/L or Quinalphos at 0.05gm/L are applied.

Basically, India is an agricultural country consisting of about 60-70% of its growing population based on this sector. Now a day, agriculture sector in India going through two major challenges, the production of massive amount of food to fulfill the need of growing population which is in the near future that is by 2020 crossing 1.4 billion (Kanecker et.al., 2004) and prevention of environmental contamination. One of the major problems to increased food productivity is damage of crops due to crop pests including diseases, insects, nematodes, rodents etc. and about 18% of crop losses caused by insect pest and diseases. These annual crop losses due to pests were valued at Rs. 45,000 million. The reduction in these losses is the definite scheme for increased food supply.

In this sector, quality level like pest-free agricultural products are being increasingly important with respect to market liberalization and raising importance of agricultural transports. This insect control method has traditionally meant the use of chemical pesticides. These chemical pesticides have played significant role to overcome the attack of pests in protection of crops. These chemicals like insecticides and other chemicals in foreseeable future will still continue to play a significant role in Indian agriculture to fulfill our increasing requirement of food. These pesticides are used to overcome the action of pests which damages crops, to eradicate parasites and insects pests.
of farm animals, and continuously maintain good and germ free conditions in production department or during product storage. Also it has played a significant role in enlarging easy supply of large amount of product like grapes for consumers, because it allows growers and handlers to raise the productivity. The effects of this chemical use and it also recognized that some foods contain these pesticides and their residues which are predominantly important to the consumers. Such residue containing food can easily reach the growing population and it more specifically concerned with the possible hazards of human health.

Because the safety of human is the front most consideration in food production lines, international systems of legal control have been developed a strategy for prevention of residue contaminated foods from becoming the part of human food supply. To ascertain food safety there is a more and expanding interest to use Gas and Liquid Chromatography Mass Spectrometry (GC-MS) in control of residues of pesticide in food.

The trend of these synthetic chemicals consumption would show that their consumption has risen from 43,400 million tones technical grade in 1954 to about 50,000 tones today. There has been a significant argument on the need and role of pesticides because these synthetic organic chemicals (pesticides) are biological substances which cause harm to human and none of these are entirely specific, hence when released in to the environment, they have harmful side effects. The level of this chemical residue in Indian environment is also an issue of great importance and surveys in the country have reported that food products are highly contaminated with these poisonous pesticides and their residues. Their possible effects are direct toxicity to handlers and consumers, pesticide resistant pests development, revival of pests, occurrence of secondary pests that are no longer controlled by their natural enemies and a mass of harmful residues on edible crops, human, farm animals, wildlife and the ecosystems including soil and water.

The occurrence of more amounts of pesticides and their residues in our environment is somewhat alarming for us because many of our food commodities contain these persistent chemical residues. Because of widespread use of these insecticides in the modern ‘Industrial Agriculture’ has undoubtedly controlled many diseases of our food crops. However, their extensive use for more than 100 years has now brought serious
environmental pollution and has caused many diseases to the human beings. A study on pesticide exposures in livestock and farm families from the Agricultural Health Study reported that, farmers, who applied these agricultural chemical insecticides in their farms, they faces health related problems like increase in headaches, dizziness, fatigue, hand tremors and also other neurological symptoms.

As these pesticides are defined as chemical substances which are used for eradicating, preventing, attracting, repelling and to overcome various pests including unwanted plants such as weeds, ectoparasites or other animals during the cultivation, storage, transportation, sorting and any processing of the food, or agricultural commodities and applied by administering to the animals for their safety. There are different types of pesticides out of that more frequently used pesticide types are insecticides for killing insect pests, rodenticides for killing rodents, herbicides for killing weeds, and fungicides for control of fungi and molds.

Every year, in the USA, nearly 5.1 billion pounds of chemical pesticides are used. These chemicals are used since ancient times by farmers and scientists, and they were treated with chemicals like elemental sulphur, lead and arsenic on commonly grown crops. In 1900s, study was concentrated on more natural and easy methods consisting of components made with chrysanthemums and tropical vegetable roots. In the year 1939, DDT was found to be highly effective, popular and predominantly used insecticide throughout world within a short period. After about twenty years, hazardous problems of the human safety and also biological effects of DDT have led to ban its use in 86 countries. There are various pesticides which are banned now because these are concerned with public health and also have related to a variety of disorders or diseases. Out of these chemical pesticides used, many pesticides are known to cause poisoning, respiratory failure, collapse the nervous system, infertility or birth defects and possibly can lead to cancer.

About 66% of US grain and 37% of the worlds grain is applied as farm animal’s feed. This huge amount of feed is produced by advanced farming methods and cultivation methods that apply mass amount of these chemical pesticides. While cultivating these grains, many constraints arise like pesticide tolerance capacity in target insects and
unwanted plants, and pollution of regional aquatic bodies and soil with above toxic chemicals. Also most of these grain crops are modified genetically; hence these plants are produced with capacity as to contain pesticides with their whole genetic makeup or to tolerate direct use of chemicals as pesticides and herbicides. Also, while growing these grains with these chemicals and then given to farm animals, fed pesticides and their residues concentrate in animal’s muscle tissues. When human consume these dairy products or meat containing these chemicals, they had contact with the toxic residues of chemical pesticides and also they are at high risk of producing health hazards as a result of consumption. Moreover, the more toxic and most direct way to exposure of pesticides is through orally by ingesting product containing pesticide residues.

Although, it is most widely accepted and understood that contact of these toxic chemicals is very much harmful to human being. Previous report has given that many exposed people in USA carry exceeding concentrations of these pesticides in their muscles and bodies. According to date collected by Centers for Disease Control and Prevention, an American child between ages six to eleven carries in their bodies four times more the acceptable limits of pesticides called organophosphorus insecticides which are more toxic and also known for leading to nerve damage. Peoples, who are studying the impacts of chemical pesticides on human, they have reported that exposing human to even small doses of these residues or poisons during the foetal stage and during young stage can lead to long term damage.

Environmental Protection Agency (EPA), have investigated and granted pesticides for application in farms, and it also standardized maximum residue concentrations or ‘tolerances’, that limit the level of a given chemical which can safety remain in or on a food commodities. Food and Drug Administration (FDA) department is then responsible for the monitoring of pesticide levels on vegetables and fruits, while Department of Agriculture (USDA) is in charged with the responsibility of survey of pesticides and their residues in meat, eggs and dairy products. For example, extensive and excessive application of endosulfan has finally resulted in far flung residual contamination in that ecosystem. Endosulfan residues have been found in all types of ecosystem levels as atmosphere, sediments, soils, rain water, surface water and food stuffs. Adverse and severe
health hazards have been found on exposure to endosulfan, in several countries including Sub-Saharan Africa, India, South Pacific and South Asia.

Pesticides are applied both as pest control agents in public health programmes and in agriculture sector. Vector control programmes with agriculture and horticulture account for greatest use of pesticides. Also significant amounts are used in forestry and livestock protection.

These synthetic chemical pesticides can be differentiated in many types depending upon target pests, chemical structure of compounds used or degree of type of health hazards involved. Once released in environment, pesticides may be broken down by various ways as photolysis involving sunlight, hydrolysis, chemicals, or microbial degradation involving microorganisms such as bacteria, fungi etc. These degradation processes generally resulted in to formation of non-toxic or less harmful products. Some of these pesticides show resistance towards any kind of degradation and remain unaltered in that ecosystem for long periods of time.

As per IUPAC, pesticide is said to be persistent whenever its measurable quantity exists in some discernable chemical form. The most useful pesticide would be the one that has stability just sufficient to perform its functions with no persistent residues. The chemical which is most easily and rapidly degraded that has the shortest period of time to transfer or to have hazardous impacts on human or other organisms. As compare to these pesticides, persistent pesticides can move over far a long distances from application point and remain in that environment causing adverse effects. In the process of pesticide distribution and persistence, living organisms play significant role that is in the living organisms pesticides get stored in fat tissues, where they accumulate and their level increases over time. If a higher organism eats this organism its pesticide level becomes further higher. This type of accumulation is called bioaccumulation.

Food and Agriculture Organization (FAO) has described the residue of pesticide as any specific chemical substance in food, animal feed, agricultural commodities, soil or water, which is resulted from pesticide use. These long lasting pesticide residues may be desirable because pesticides are effective for long time as well as for long distances and
they usually not applied very often. However, these long-lasting residues on food sources pose health risk to consumers. These pesticide residues are unwanted even in smallest quantity because of their toxic nature. Therefore, these residues should be removed from food materials or environmental samples (such as soil or water) or should be converted into non-toxic products. Soil is the most difficult substrate to decontaminate from pesticides, as it has enormous capacity to store these pesticides.

Degradation of insecticides in soil system can be accelerated by using microorganisms such as bacteria, fungi or algae. These microorganisms degrade pesticides by utilizing them as energy source in the process of co-metabolism. Microbial cells and cell free extracts of microbial enzymes may be used for biodegradation of pesticides mainly organophosphates. In India, 75% of the pesticides used are insecticides like the organophosphates, organochlorines, carbamates, synthetic pyrethroids and other groups.

Table No.3: Percent consumption of diff. pesticides in India

<table>
<thead>
<tr>
<th>Agrochemical Market</th>
<th>Pesticide</th>
<th>Global (%)</th>
<th>Indian (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Herbicides</td>
<td>44</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Insecticides</td>
<td>22</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Fungicides</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Ag-biocides</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

(Manoj Maxine, 2010)
Now a day, peoples prefer chemical pesticides as most important tool to insure the production of required mass of crops with quantity and quality to fulfill the demand of an increasing human population. Regarding to this, agricultural experts believe that these food and fibre needs can be met, but to do so will require the increased use of pesticides. Initially, farmers use two main chemical groups of insecticides that are organochlorines (OCs) and organophosphates (OPs), and both were neurotoxic in action. Now a day, on the basis of chemical nature insecticides are classified in to four major groups as organochlorines (16% use), organophosphates (50% use), carbamates (4% use) and pyrethroids (19% uses).

Organophosphate is the second major group of insecticides. These organophosphates are divided into three main groups as Aliphatic Organophosphates, Phenyl organophosphates and Heterocyclic organophosphates and these insecticides kill insects by inhibiting cholinesterase which is the most common mechanism of this type of insecticides. Dichlorvas and Quinalphos are mostly used on the grapes to control various types of insects. Among the insecticides, monocrotophos, Quinalphos and chlorpyriphos are top the list of organophosphorus insecticides (Singhal, 2003). During World War II, similar to the chemical warfare agents produced, organophosphates (OPs) are some of the most common, and most toxic insecticides used today, heavily affecting the human nervous system.

This indiscriminate and unilateral use of chemical pesticides has resulted into several risks and effects such as ecological imbalance, human and animal health hazards, resistance developed in pests to pesticides, resurgence of minor pests and environmental pollution. Besides the destruction of natural enemies of pests, higher level of pesticides in water, soil, food as well as fodder have also been noticed. These pesticides can enter the atmosphere by a variety of routes, particularly from vaporization from soil and water or drifting. Usually a small part of these chemicals used on to crops act on their target, the remaining fall on the ground or taken up into the atmosphere by air currents or turbulence.

According to the survey of ICMR, approximately 51% of our food products were contaminated with residues of pesticide and out of that 20% of has the residues above maximum residue limit. This persistent problem of pesticide residues in the environment
raises severe health related issues threatens the marketing of many commodities such as food grain, wool, meat, fruits, vegetables, nursery plants etc. This major problem is highlighted by the mounting pressure from various public health groups and environmental agencies. This increased awareness in this field leads to significant rise in study on different methods that are employed for remediation of our contaminated environment. One of those strategies is the use of bacteria for decontamination of environment.

Living organisms in the biosphere have ability to obtain energy through the degradation of organic molecules and soil microorganisms are important in this process because they are capable of degrading organic substances that accumulate in the biosphere. These soil bacteria degrade different types of persistent pesticide compounds in consortium and return degraded elements to plants. These bacteria also has a significant participation in the dissolution of these persistent pesticides in soil system and in that especially bacteria have developed genetically determined system against toxicants due to their continuous exposure to such environmental stresses (Parsek et. al., 1995).

Many chemical pesticides, though they are biodegradable, still persist in environment for a long time because the catabolic capacity required for degradation is not present or because the microbial populations which bring about their degradation are not in sufficient number or are not active enough.

The scientists’ world over adapting strategies to address environmental issue caused by insecticides, the use of bioremediation which include biotransformation, bioaccumulation, bio mineralization, biodegradation, bio augmentation and co metabolism. There are many soil bacteria which have been enriched and isolated from various parts of the world with amazing property to degrade xenobiotic contaminants that is pesticides (Habe and Omori, 2003). Microorganisms are nature’s bio degraders. They are considered to be more powerful scavengers in nature, able to recycle many natural and toxic non-wanted materials into non-toxic byproducts, when exposed to a series of an increasing concentration of synthetic chemicals. These synthetic pollutants are removed from the soil by endogenous bacteria by their extraordinary capacity to utilize a variety of xenobiotic as their growth sources (Hemmingsen, 1993). Soil microorganisms are highly adaptive and have developed ability to decompose such recalcitrant insecticides by evolution of some
new genes, which encode enzymes that can use these compounds as their primary substrate (Parsek et. al. 1995, Suenaga et. al., 2001).

In cleanup of xenobiotic containing soil system, survival of organisms under insecticide stress can provide the efficient, cheaper and environment friendly solution (Arnett et. al., 2000). In these bacteria pesticide degradative genes have been observed to be present on transposons, plasmids or chromosomes. Genetically engineered bacteria have been developed for the purpose of decontamination of soil by studying their pathways for degradation and catabolic gene organization.

An important feature of previous studies on pesticide degradation have helped to discover a new technology for biological treatment of pesticide waste and the expanding field of biotechnology helped to strengthen the idea that microbial enzymes could be used to control the effects of pesticides on the environment. There are several results of the isolation and cultivation of cultures which use pesticides as their energy source of but it is rare indeed for this specific activity to be retained fully, following transfer of the culture into a natural soil environment.

**Soil microbial diversity**

Soil is the nature’s gift and which is considered as a complex environment colonized by tremendous diversity of microorganisms. It is fundamental and irreplaceable, it maintains biogeo-chemical cycles and it increases productivity of plants and fertility of soil in ecosystem, because microorganisms present in the soil decompose, nearly all organic compounds involving xenobiotic and natural phenolic compounds. The living organisms present in soil including macro fauna, micro fauna and micro flora and in this, review focused on interactions within bacterial diverse groups and their functions in soil by taking into consideration 80-90% of the soil reactions are microbe based reactions (Nannipieri & Badalucco, 2003; Coleman & Crossley, 1996,).

The tremendous type of diverse groups of microbes in top soil is depend upon presence of different content and a various types of organic chemical compounds in top soil as compare to below ground. For the study of soil microbial diversity, researchers generally investigate various functions of soil by detecting microbe process rate, either
studying their species level efficiently included in the respective process. The major problem of studying the relation between functions of soil and microbial diversity is to determine the link within their community structure and gene diversity and between structure of community and its function (O’ Donnell et. al., 2001).

This incredible diversity in soil microorganisms is a vast array of potentially useful organisms. Many scientists have interest in research of agriculture and their microbial diversity since past 25 years and it has been raised rapidly as microbiologists have become found interest in the importance of microbial diversity for many beneficial ecological processes as well as medical, agricultural as well as environmental applications of microbial diversity have developed.

This microbial world has extraordinary diversity in their morphology, physiology as well genetics and because of their bewildering diversity, it is essential to classify or arrange them into groups based on their mutual similarities. These soil microorganisms have very diverse mechanisms for degrading a variety of compounds because these microorganisms use numerous natural substances. These microbes are very much significant as almost all of the chemical transformations occurred in soil system includes active participation of microorganisms of soil. Particularly, they play a significant role in improving soil fertility which is resulted from their participation in nutrient cycling as carbon and nitrogen cycle. For example, these different types of microorganisms in soil are predominantly considered to have capacity for decomposition of carbon matter adding to environment as soil and therefore involved in the biogeochemical cycles of soil. Along with these organic matters soil organisms can decompose plant debris manure, pesticides, so as to prevent them from entering water and becoming toxic pollutants (Singh et. al., 2010).

As human activities like agricultural chemicals have polluted the environment with a wide variety of synthetic or processed compounds, many of these toxic substances can be detoxified by soil microorganisms. This is the basic mechanism behind the treatment of contaminated soils by biological remediation, which includes the application of microorganisms or their enzymes to remove contaminants of environment. So, soil microbiologists study the microorganisms, their metabolic pathways and optimizing
environmental conditions that can be applied to eliminate toxic pollutants from the soil environment.

The interrelationship amongst microbial biodiversity in soil and functioning of ecosystem is a point of predominantly important, specifically in view of Earths atmospheric alterations and human interference in environmental interactions. Different functions of ecosystem performed by soil micro flora that is very important for ecosystem stability maintenance. This soil microbial biodiversity has significant implications in productivity and stability of ecosystem. Soil microbes are an important component of soil responsible for various biogeochemical cycles in the ecosystem. It helps in maintaining soil health as well as microbial diversity is basic tool in preservation and careful maintenance of genetic resources on entire earth.

Among the different types of microorganisms present the soil; bacteria are most common and which found in great number. According to Manual of Bergey on Systematic Bacteriology, in soil system, majority of the predominant bacteria are taxonomically included under the three orders, Pseudomonadales, Eubacterials and Actinomycetales. Bacteria are differentiated depending upon their metabolic activities or way of nutrition consumption, specially a kind in which they assimilate their energy, carbon, nitrogen and many other nutritional demands.

This diversity pertains to the series of variations or distinction among a set of entity. Microbial diversification may be due to a number of factors which are morphological factors (cell shape, cell size, cell division, cellular flexibility, cell envelope, cyst formation etc.), associative relationship (co-operation and competition, symbiotic association, parasitic association etc.), physiological factors (relation to oxygen, light, UV rays, nutrition, pigments, temperature etc.) and genetic factors (isoenzymes, plasmids, rRNA sequencing etc.). Therefore, biological organization has following fundamental and hierarchically related levels viz. genus, species, ecosystem and agro-biodiversity, so diversity may define in these terms as-

1. Diversity in genetic makeup: It gives genetic variations in and between microbial populations. It includes variations in four base pair sequence which is main parts of genetic
material constituting the genetic code. This gene diversity may be at the level of quantity
of DNA per cell and number and structure of chromosome.

2. Diversity among species: Biodiversity is generally known as diversity among species.
The species level is commonly referred as the most basic which is considered as the whole
organism diversity.

3. Diversity in Ecosystem: It is frequently an appraisal of the measuring of the component
species diversity. This involves determination of the relation among number of different
species and types of species under consideration.

4. Agro-biodiversity: Agro-biodiversity (agricultural biological diversity) has been fast
emerging biodiversity as a strong, evolutionary divergent line from diversity, which deals
with the other life forms at large. It has been more specifically considered to differentiate
between ecosystems versus agro-ecosystems, wild forest fauna and flora versus agriculture
related plants, insects, reptiles, avian and microbes; in situ conservation of wild forms
versus on farm conservation of traditional cultivars and landgraves or ex situ preservation
of genetic resources of plants etc. (Shilpkar and Shilpkar, 2010).

The biodiversity of soil bacteria can be studied at the species level because this
bacterial species is specified as ‘a set of relevant organisms that is differentiated from same
groups by a combination of important phenotypic, ecological and genotypic
characteristics’. A common phenotype in one bacterial species may be determined by
morphological characteristics, antibiotic resistance and BIOLOG etc. Also a common
genotype in one bacterial species may be determined by restriction analysis of amplified r-
DNA, sequencing of 16s and 23s rRNA genes and r-DNA intergeneric spacer region
restriction analysis.

For the study of soil bacterial diversity, an enrichment culture technique is used
mostly. This classical enrichment culture technique is based on mimicking the organism’s
habitat and using natural selection to enrich the desired population over other members of
the community. But about 99% of these bacteria inhabiting many natural environments
may not be cultivated and isolated in the normal laboratory conditions and remain hidden
for their ecosystem functioning and unused for recent biotechnological applications.
Scientists found that the microbes which grow in dense pure culture may not be representative of most microbes in the environment and these studies also shown that the organisms which are cultivated from environmental samples are frequently minor components of the resident microbial population. There are a various ways discovered to search microbe diversity at genetic level which involves DNA-DNA as well as mRNA-DNA hybridization, its reassociation, DNA sequencing and cloning, and Polymerase Chain Reaction-dependent methods like DGGE, TGGE, ARISA and RISA (Kirk et.al., 2004).

**Figure No.2: Hybridization analysis** (De Los Reyes et. al., 1998)

**Importance of Microbial Diversity**
There are a number of microorganisms has been identified which have great potential to serve human being. It is due to the fact that each bacterium has its own metabolic activity to utilize various substrates and hence produces product of different properties. The importance of microbial diversity can be categorized in to-

- Role of microbial diversity in biodiversity maintenance: Microbial diversity plays a main and significant role in diversification and evolution of microorganisms as they contribute in various processes of the ecosystem. So these microorganisms maintain ecosystem structure because they act as biocontrol agents. For example, pathogenic microorganisms of plants can affect production of plants and endogenous microorganisms can also affect population of insects but these pathogens are controlled by other microorganisms and if such plants and insects play a critical ecological role in respective ecosystem then their losses, in absence of controlling microorganisms, can lead to major alterations in the ecosystem.

- Role of biodiversity of microbes in biosphere functions: According to many scientists the evolution of microorganisms and human was possible due to presence of microorganisms because earlier earth has a large quantity of carbon dioxide and no oxygen, then photosynthetic *Cyanobacteria* utilized this carbon dioxide and releases oxygen which helps in their evolution. Also soil microorganisms cause circulation of certain matters like carbon, nitrogen, sulphur, minerals etc. For example, algae, bacteria, fungi and protozoa causes weathering of rocks and produces minerals like carbonates, phosphates, oxalate, sulphate, silicate, sulphides, oxides of iron and manganese.

- Role of microbial diversity in sustainable development: Agricultural production can be increased by introduction of nitrogen fixing microorganisms like *Rhizobia* in legumes, introduction of *Cyanobacteria* inoculums like *Azolla* in rice, using microorganisms as biocontrol agents for insect pests, plant pathogens, disease vectors and noxious weed, use of *mycorrhiza* inoculums and cloning of useful genes producing certain metabolites or enzymes from microorganisms and their insertion into crop plants. Biodegradation of various toxic compounds as well as biodegradation of cellulosic and lignostic wastes from agriculture and industries can be done by microorganisms which convert them in to nontoxic compounds and animal feed stuff respectively. Microorganisms are also used in removal of toxic heavy metals as well as bioremediation process of oil spills at sea. A well-
known example of application of bioremediation as the beneficial solution to oil contamination is as in March 1989, tanker Exxon Valdez accidental oil spill which ran surrounding the Alaska Gulf Bligh reef, approximately 41000m$^3$ of crude oil spilling and contaminating approximately 2000km of coastline.

**Using Microbial Diversity for Environmental Remediation**

Many industrial activities produces a large amount of chemicals like herbicides, insecticides, dyes, plasticizers, solvents, agrochemicals, pharmaceuticals, fire retardants, hydraulics, halogenated compounds, which releases in environment and create countless numbers of contaminated sites. Removal of these xenobiotics from contaminated sites involves land filling, excavation, incineration like physical and chemical processes, which are very much expensive and many times difficult to apply.

As these microbes are considered to be original recyclers of nature, transforming toxic organic chemical compounds to non-toxic components, majority of the time carbon dioxide and water. Bioremediation is a degradation process by selective microorganisms in which xenobiotic contaminated sites are cleaned up. This uses bacteria and their biogeochemical mechanisms preferentially in-situ, which applies the microbial ability to reduce the amount and toxicity of pollutants. This bioremediation process utilizes the great diversity of microorganisms in nature and their catalytic enzymes. If effective biodegrading microorganisms exist but are not at the remediation site then inoculation with the desired microorganism can be attempted and the most easily managed bioremediation is when the pollutant serves as a growth substrate for the microbes.

Microbial diversity helps in biodegradation of a number of ‘difficult to degrade’ compounds including Nitroaromatic compounds (NAC’s), aromatic and aliphatic hydrocarbons, crude oil constituents, polycyclic aromatic hydrocarbons (PAH’s), halogenated organic compounds, halogenated aliphatic hydrocarbons, chlorinated aromatic compounds and chlorinated polycyclic hydrocarbons. Many of these chemical compounds have been discovered to be cancer causing, some are causing mutations and have immunological deficiency effects on humans, animals and plants. Successful bioremediation of these compounds requires knowledge about which microorganisms and
in how many numbers can decompose a specific compound and also knowledge about their pathways of degradation. Mostly these degradative pathways are catalysed by common enzyme types produced by common microbial groups.

These bacterial strains isolated from various agricultural fields i.e. grape wine yards can be employed in the microbe based bioremediation of insecticide contaminated environment. It is interesting to research on the biodiversity of these chemical pesticide resistant bacteria community in the grape wine yard soils. The present investigation will be useful for suggesting the use of efficient soil bacteria in the treatment process which successfully reduce most of the disorders related with the traditional methods applied for the decomposition of toxic compounds. There is no research so far on the environmental health and its biochemical aspects regarding grape cultivation from various developing countries.