EFFECT OF PESTICIDES ON BACTERIAL COMMUNITIES FROM COASTAL ENVIRONMENTS

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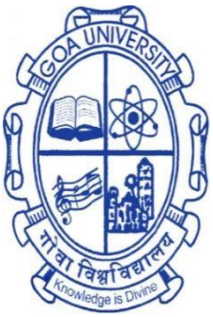

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COMPLETION CERTIFICATE

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It gives me immense pleasure to present my project entitled “Effect of Pesticides on Bacterial Communities from Coastal Environments”

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CHAPTER I

INTRODUCTION

1. **Introduction**

Pesticides are substances (natural or synthetic) used in different agronomic practices to manage pests, weeds, and diseases in plants. Pesticides include herbicides, insecticides, fungicides, rodenticides, nematicides, and other chemicals **(Dhaliwal et al., 2015).** Crop losses induced by insect pests are significant in both developing and developed nations. Reduced crop loss will be critical, and improved pest control, including diseases and weeds, will require tremendous effort. Pesticides have become an important tool in agriculture for plant protection and crop enhancement **(Sharma et al., 2019).** According to a study published in the journal Nature, Ecology & Evolution, pathogens and pests are causing global wheat losses ranging from 10% to 28%, rice losses ranging from 25% to 41%, maize losses ranging from 20% to 41%, potato losses ranging from 8% to 21%, and soybean losses ranging from 11% to 32% **(Savary et al., 2019).** The intensity of crop protection has increased considerably, as indicated by a 15-20-fold increase in pesticides used globally, in order to make agriculture more productive and profitable. Despite a clear increase in pesticide use over the last 40 years, crop losses have not decreased considerably **(OERKE, 2006).**

Agriculture must satisfy the rising demand for food, feed, fibre, biofuel, and other biobased products. Population growth in developing countries is expected to increase

food production demand by 70% due to shifts in dietary patterns towards high-quality food, such as increased meat and dairy consumption and increased use of kernels for livestock feed **(Popp et al., 2013;** During the twentieth century, the global population increased from 1.65 billion to 7.7 billion **Green, 2018; Nations, 2019).** Furthermore, the global population is expected to reach 8.5 billion by 2030, 9.7 billion by 2050, and 10.9 billion by 2100 **(Nations, 2019).**

Pesticides are used to improve agricultural productivity, but they are applied in an indiscriminate manner, destroying the biota. Pesticide transfer in the environment causes harm to non-target organisms. Some insecticides may be hazardous to human health and the ecosystem. Only about 0.1% of pesticides are thought to reach the targeted organisms, with the remainder polluting the environment and harming the environment **(Carriger et al., 2006; Gill and Garg 2014)**.

When we examine pesticide use more closely, we discover that we are using more pesticides and treating fields more frequently than ever before. According to the FAOSTAT database (Food and Agriculture Organization Corporate Statistical Database), global pesticide use (in tonnes of active ingredients) rose by 46% between 1996 and 2016 **(WHO, 2019).** Pesticides work by causing damage to the creatures they are designed to kill. Pesticides, on the other hand, do not work the same way on every species. They have an effect on non-target species as well. The most commonly used herbicides are organophosphate, carbamate, and pyrethroid insecticides **(Gilbert, 2012).** Currently, approximately four million tonnes of pesticides are used globally each year, the majority of which are herbicides (56%), followed by insecticides (19%), fungicides (25%), and other kinds such as rodenticides and nematicides **(FAO, 2018).**

1.1 INSECTICIDES

Most insecticides have multiple effects on the nervous system; they disrupt the membrane transport system of sodium, potassium, calcium, or chloride ions, inhibiting the selective enzymatic processes engaged in chemical transmission at nerve endings **(Correia et al., 2010).**

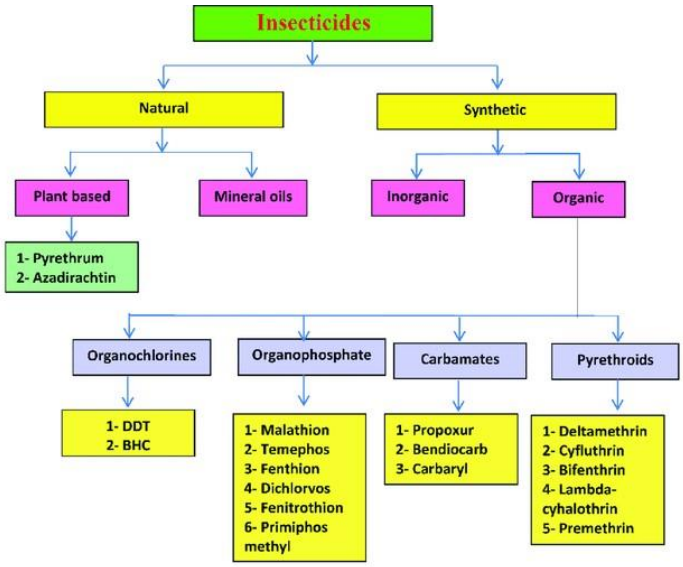

Fig. 1. Insecticides Classification (Source: **Yadav & Devi, 2017**).

1.1.1. Organochlorines

They are a class of insecticides that affect the nervous system as they are chemically unreactive stable compounds, which leads to long-lasting effects. DDT is the most studied pesticide among all insecticides, which inhibits the release of neurotransmitters. Endrine and lindane are other two organochlorine insecticides, in addition to DDT, which affects the nervous system **(LeBlanc, 2007).**

1.1.2. Organophosphates

Many nations previously banned some organochlorines (DDT), which were replaced with organophosphorus insecticides such as malathion and parathion **(Sankhla et al., 2018).** This class of insecticides is also neurotoxic, meaning that it inhibits the enzyme acetylcholinesterase (AChE), causing intoxication effects to last longer and be more severe **(Ecobichon, 1991).**

1.1.3. Carbamates

This type of insecticide also suppresses AChE by attaching to the enzyme's reactive site **(Nie et a., 2020).** It inhibits AChE in a brief and reversible manner **(Shefali et al., 2021).**

1.1.4. Synthetic pyrethroids

This is the most recent insecticide category, with two distinct acidic portions, chrysanthemic or pyrethric acids, resulting in type I and type II syndrome **(Correia et al., 2010).** Both of these syndromes impact sodium channels in nerve membranes, which cause repetitive neuronal discharge; this process is very similar to DDT action. Pyrethroid pesticides have multiple modes of action. Some of them include $Ca^{2+,}$ Mg^{2+} -ATPase inhibition, which interferes with calcium removal from nerve endings, triggering neurotransmitter release in the postsynaptic gap **(Shefali et al., 2021).**

1.2. THE MOST COMMON SOURCE OF PESTICIDES

There are numerous issues associated with extensive anthropogenic activities that have become a major source of water pollution, impacting the development of the aquaculture industry:

1) The effluents released by coal refineries, phenol manufacturing, nuclear power plants, pharmaceuticals, dying, petrochemical, pulp mill, and other industries include a

variety of chemicals such as petroleum hydrocarbons, phenols and their derivatives, heavy metals, radioactive substances, dyes and paints, oil spills, agricultural wastes (e.g., pesticides and fertilisers) that reach marine environments via land runoff and as industrial effluents **(Ravindra et al., 2019).**

2) Wastewater and solid residues from different industries, primarily phenols, hydrocarbons, dyes, and paints, are released by the chemical and pharmaceutical sectors. Detergents, disinfectants, metals, dyes, phenols, medicines, herbicides, and pesticides are all found in agricultural sources. These pollutants have a variety of negative effects on the health of aquatic organisms and people. As a result, it is critical to treat wastewater before it is released into the environment or enters into other water bodies **(Ravindra et al., 2019).**

1.3. PESTICIDES IN THE AQUATIC ECOSYSTEM

Farmers use pesticides to protect high-yielding crop varieties from pests because these crops are highly susceptible to pests and diseases, which can result in a 40% loss in crop production; thus, these pesticides are used to improve crop quality and quantity by protecting them from pests **(Fleeger et al., 2003; Stark et al., 2004; Park and Clough, 2004; Bagchi et al., 2009; Dias et al., 2020).** The use of pesticides harms the environment and also affects non-targeted organisms in addition to the targeted pest, which has been a major source of concern for decades because it adversely impacts several links in the food web **(Tsui & Chu 2003**; **Latijnhouwers et al., 2003).** Pesticide pollution has a negative impact on both the soil and aquatic ecosystems as they move from one to the other due to their specialised characteristics such as half-life, solubility, mobility, and degradation **(Pataik & Patra 2006; Boran et al., 2007; Jabali & ElHoz, 2020).**

Pesticides, among all the toxic substances that run off into the aquatic ecosystem, are of greatest concern because they are known to pose significant hazards to biological organisms, including humans. The pesticide enters the aquatic ecosystem via surface runoff, spillage, industrial effluent, vapourization to the atmosphere, agricultural returns, groundwater intrusions, pesticide-treated soils, adsorption, or plant uptake, which causes adverse effects on the health of aquatic organisms **(Pataik & Patra, 2006; Boran et al., 2007; Jabali & El-Hoz, 2020); Ansara et al., 2012; Uddin et al., 2013; Pico et al., 2020).** The majority of pesticides used in urban and agricultural environments are harmful to aquatic organisms such as birds, fish, and zooplankton **(Ware, 1980).**

Pesticides from agricultural areas typically runoff into reservoirs or drainage systems via rain or irrigation **(Best & Ruthven 1995).** Pesticides mainly affect aquatic organisms in three ways:

(i) **through the skin:** because aquatic organisms are in touch with water, pesticides produce harmful effects through dermal pores;

(ii) **through breathing:** because aquatic organisms breathe through gills, pesticides are directly absorbed through breathing.

(iii) **orally:** aquatic organisms are usually exposed to pesticides by eating pesticidecontaminated prey (this is also known as secondary poisoning; for example, if a fish feeds on pesticide-treated insects, the fish may be killed if a large amount of toxic compound is consumed by the insects) or by drinking contaminated water **(Shefali et al., 2021)**.

1.4. EFFECTS OF PESTICIDE ON THE MARINE ECOSYSTEM

Pesticides and herbicides are chemicals that are commonly used in agriculture and everyday living. These chemicals include organophosphorus, endosulfan, nitrophenols, morpholine, synthetic pyrethroids (SPs), and carbamates. These chemicals interfere

with groundwater and runoff, which eventually reaches the sea or ocean **(Lalithakumari, 2011)**. Invertebrates, plants, microorganisms, fish, and amphibians are among the creatures found in the aquatic environment. **(Shefali et al., 2021)**. βCypermethrin and SP, an insecticide, is a great threat to the survival of aquatic fishes and invertebrates. This compound has the potential to cause serious health effects, including reproductive, neurotoxicity, and developmental disorders **(Zhang et al., 2011)**. Excessive use of nitrogen- and phosphorus-rich fertilisers causes algal blooms and leads to hypoxia in the water, resulting in a loss of species diversity. These compounds, including dichlorodiphenyltrichloroethane (DDT), accumulate in the fatty tissues of fish and circulate on subsequent trophic levels due to bioconcentration and biomagnification. DDT has been identified as a cause of cancer and birth abnormalities **(Ravindra et al., 2019)**.

Pesticides can have a direct or indirect effect on these organisms; the direct effect involves physiological changes within the organism **(Singh and Mandal 2013; LópezPacheco et al., 2019; Acosta-Sánchez et al., 2020)**. For example, pesticide exposure to water flea results in their mortality, which can be considered a direct effect of pesticides, and it may contribute to a drastic rise in algae biomass due to grazing pressure release, which can be considered an indirect effect. Herbicide, primarily glyphosate, is used globally to control both terrestrial and aquatic weeds, and its use has increased drastically in recent years, and it is now known to adversely affect nontarget organisms in the aquatic environment **(Mensah et al., 2012)**. Originally, the mode of action was intended to affect only plants **(Larson, 2019)**, but in recent years, several reports have emerged indicating the negative impact of non-target organisms, which can be lethal or sub-lethal **(Mondal et al., 2020; Preston et al., 2002; Glusczak et al., 2007; Khalid et al., 2020)**. At the physical level, indicators for exposed

organisms include survival, development, and morphological/behavioural changes. Reproductive performance is frequently used to evaluate sub-lethal response, which includes sexual maturity, time taken to release the first brood, time taken for egg growth, fertility, and alterations in reproductive characteristics **(Shefali et al., 2021).**

Fish interact with the physical, biological, and chemical marine ecosystems, making them an essential component of the aquatic ecosystem. They are an important food source for other animals such as sea birds and marine mammals, and thus form a fundamental part of the marine food web. Several studies have linked the decline in the fish population to the toxic effects of pesticides **(Giesy et al., 2000; Salam et al., 2020).**

1.5. MICROBIAL DEGRADATION OF PESTICIDES

Pesticides pose a significant risk due to their bioaccumulation and prevalence. Microorganisms, particularly bacteria and fungi, consume and degrade these poisons. Furthermore, *Mycobacterium* sp. and *Arthrobacter* sp. have been used as bioremediators against endosulfan compounds, as have *Pseudomonas putida* against hexachlorocyclohexane (HCH), *Alcaligenes eutrophus* against 2,4-Dichlorophenoxyacetic acid, and *Dehalospirilum multivorans* against DDT **(Sinha et al., 2009).**

Pesticides are also biotransformed, which is carried out by a variety of bacterial populations. Even extremely persistent pesticides can be metabolised by pesticidedegrading bacterial cultures as a source of energy and nutrients or as a co-metabolism with other substrates for their development. Co-metabolism is the chemical conversion and degradation of most recalcitrant compounds by microbes that are not suitable for their growth. This ability of microorganisms to degrade pesticides can be used to accomplish sustainable agriculture while also reducing the negative impact on

farmers' health. Complete mineralization or biodegradation of pesticides or chemicals is more effective and rapid in microbial communities than in single microorganisms. Microbial metabolism is most likely the most persistent and widely recognised pesticide degradation process. However, the persistence of pesticides in soil is mainly determined by the extent of bacterial metabolism, which reduces pollutants in the environment. Despite the fact that most agrochemicals biodegrade with suitable microbes or microbe consortia, some agrochemicals are highly recalcitrant and cause severe residue problems **(Kumar et al., 2020).**

Actinobacteria, Bacteroidetes, Ascomycota, Basidiomycota, Chlorophyta, Cyanobacteria, Firmicutes, and Proteobacteria were discovered to be the greatest sources of pesticide degradation for various groups of microbes. (Carbamate, Organochlorine, Organophosphate and Pyrethroids). *Achromobacter* **(Karns et al., 1986)**, *Arthrobacter* **(Sandmann and Loos 1988)**, *Aspergillus* **(Ortega et al., 2011)**, *Bacillus* **(Liu et al., 2012; Onunga et al., 2015)**, *Burkholderia* **(Satapute and Kaliwal 2016)**, *Chlamydomonas* **(Cáceres et al. 2008)**, *Chlorella* **(Cáceres et al. 2008)**, *Methylobacterium* **(Wang et al. 2012)**, *Micrococcus* **(Patil et al. 1970)**, *Paenibacillus* **(Romeh and Hendawi 2014)**, *Penicillium* **(Ortega et al. 2011)**, *Pseudomonas* **(Hay and Focht 1998; Rayu et al. 2017)**, *Rhizobium* **(Rayu et al. 2017)**, *Trichoderma* **(Katayama and Matsumura 1993)**, and *Xanthomonas* sp. **(Rayu et al. 2017)** were isolated from various microbe groups, and pesticide degradation ability was demonstrated. Pesticides degraded include a wide range of extremely hazardous (Phorate), highly hazardous (Carbofuran), moderately hazardous (Endosulphan), and slightly hazardous (Malathion) pesticides **(Kumar et al., 2020).**

1.6. PESTICIDES USED IN THIS STUDY

1.6.1. Monocrotophos

Monocrotophos ($C_7H_{14}NO_5P$) is an organophosphorus compound that is used as a systemic pesticide and acaricide. It keeps pests away from a wide range of crops, including sugarcane, cotton, rice, fruits and veggies, and so on. Monocrotophos is widely used in agriculture in Pakistan to control boring, chewing, and sucking insects (such as aphids, Helicoverpaspp, caterpillars, moths, mites, jassids, budworm, scale and stem borer, and locusts) for a wide variety of crops. Unfortunately, the widespread use and ease of access to monocrotophos have led to increased utilisation for homicidal and suicidal poisoning cases **(Sayed, 2014).**

Monocrotophos is an extremely toxic compound that inhibits the enzyme cholinesterase. It is extremely dangerous through all pathways of exposure. Ingestion, inhalation, and cutaneous contact can all result in the absorption of Monocrotophos. Monocrotophos can harm the respiratory system, causing runny noses, tears, pain, chest soreness, coughing, localised sweating, involuntary muscle spasms, blurred vision, and pupil constriction **(WHO, 1993).** It is extremely poisonous to bees, birds, mammals, and aquatic invertebrates. Monocrotophos has an acute oral lethal dosage (LD_{50}) of 21 mg kg^{-1} in rats **(Janghel et al., 2006)**. Monocrotophos (120 mg) may be fatal to people, according to the World Health Organization **(WHO, 1993)**. Monocrotophos is classified as a highly hazardous pesticide (Class I_b) according to World Health Organization 2004 edition of the recommended classification of pesticides by hazard **WHO, 2004).**

1.6.2. Dimethoate

Dimethoate ($C_5H_{12}NO_3PS_2$) is a well-known systemic organophosphorus insecticide and acaricide **(Derbalah et al., 2021)**. It's also classified as a carbamate insecticide. In its pure form, it is a colourless crystalline substance that is soluble in chloroform, methylene chloride, benzene, toluene, alcohols, esters, ketones, and partially in the water **(Raghu et al., 2014).** Dimethoate has been widely used to manage a wide variety of insects since 1956, including aphids, red spider mites, pea midges, thrips, wheat bulby, sawy, suckers, and woolly aphids **(Deshpande et al., 2004; Pappas & Kyriakidis 2003)**. It is a pesticide that is widely used in the fields of avocado, cereal, citrus, cotton, mango, peanut, and pulse crops **(Lang et al., 2016; Yang et al., 2014)**. These insects have a negative effect on a wide range of crops **(Derbalah et al., 2021).** Dimethoate kills insects through contact and stomach action by interfering with acetylcholinesterase (AChE) activity, which is required for the normal working of both human and insect nervous systems **(Hassal, 1990; López-Carillo & López-Cervantes 1993).**

The World Health Organization (WHO) classifies dimethoate as a relatively hazardous compound that is stable in aqueous media with pH values ranging from 2 to 7. Dimethoate is highly toxic when ingested or inhaled. Although dimethoate poisoning can occur through the mouth, it is readily absorbed through the skin **(Al-Jaghbir et al., 1992)**. Dimethoate and its oxidised analogues have been found in soil, fruits, vegetables, and even cow milk **(Srivastava & Raizada 1996)**. Dimethoate exposure may be more dangerous for people who have respiratory problems, have recently used cholinesterase inhibitors, have impaired cholinesterase production, or have liver dysfunction **(Steingrímsdóttir et al., 2018; Huang et al., 2019; Wang et al., 2018).**

Traditional dimethoate residue detection techniques include colorimetric methods **(Xie et al., 2018)**, immunoassays **(Cao & McDermott, 2018)**, chromatography **(Farajzadeh et al., 2015)**, chromatography-mass spectrometry **(Santos et al., 2018)**, and high-performance liquid chromatography coupled-tandem mass spectrometry (LCMS/MS) **(Marques et al., 2018; Tu et al., 2020)**.

1.7. METHODS TO DETECT PESTICIDES

One method for reducing pesticide contamination is to implement an effective monitoring programme that involves sampling and chemical analysis **(Syafrudin et al., 2021; Saleh et al., 2020)**. Monitoring is also necessary to evaluate the effectiveness of pesticide contamination mitigation measures (such as bans and restrictions on use, safe handling practices, and integrated pest management) **(Oliveira et al., 2014; Pradhan et al., 2022; Chow et al., 2020)**.

The majority of the methods recommended by the Environmental Protection Agency (EPA) for detecting pesticides in water are analytical techniques such as highperformance liquid chromatography (HPLC) **(Aulakh et al., 2005; Harshit et al., 2017)**, gas chromatography (GC) **(Schwanz et al., 2019; Chiron et al., 1993; Ballesteros et al., 2004; Khetagoudar et al., 2019)**, micellar electrokinetic chromatography (MEKC) , enzyme-linked immunosorbent assays (ELISA), and gasliquid chromatography coupled with mass spectrometry (GC/MS, LC-MS) **(Campanale et al., 2021; Menezes et al., 2016)**. Despite their high precision and sensitivity, most of these methods necessitate sample preparation, costly equipment,

qualified personnel, labs, and time, and they are not portable, making their use extremely difficult **(Menezes et al., 2016).**

Electronic tongues (ETs) are promising analytical devices that can be used to identify, classify, or quantify chemical and/or biological families in complex matrices, delivering quick and accurate information at a low cost **(Cetó & Del Valle 2022; Shimizu et al., 2019).**

The review by Sopanrao et al., (2022) on nucleic acid-based aptamer sensors (singlestranded ribonucleic acids (ssRNA) or single-stranded deoxyribonucleic acids (ssDNA) for the molecular diagnosis of toxic chemicals in food, water, human fluids, and the environment **(Kadam & Hong 2022)**. reported on a novel aptamer-based disposable, flexible, and screen-printed electrochemical sensor (Aptasensor) for the rapid detection of chlorpyrifos (CPF) (**Inam et al., 2022).**

In another research, a DNA Aptamer sensor with a detection limit of 14 nM (3.88 ppb) was developed to detect fenitrothion (an insecticide in the organophosphate pesticide family). Aptamer-based biosensors are frequently used as an alternative because they provide a quick and simple way to identify contamination from various sources. They also have the benefit of detecting low concentrations that traditional chromatographic assays may fail to detect **(Xu et al., 2022)**.

Chromatographic methods combine separation abilities with mass system sensitivity, such as ion trap (IT), triple quadrupole (QqQ), and time of flight. (TOF) **(Menezes et al., 2016)**. Traditional extraction methods, such as solid phase extraction (SPE) **(Vukcevic et al., 2012; Vidal et al., 2000)** and liquid-liquid extraction (LLE), use toxic solvents in multiple stages and take a long period to complete **(Menezes et al., 2016)**.

Hollow fibre liquid phase microextraction (HF-LPME) **(Pinto et al., 2010; Sun et al., 2011; San Román et al., 2012; Tankiewicz & Biziuk 2011; Huang & Huang 2007)** created by Pedersen-Bjergaard and Rasmussen **(Pedersen-Bjergaard & Rasmussen 2008)**, as well as dispersive liquid-liquid microextraction (DLLME) **(Seebunrueng et al., 2014; Cortada et al., 2009)**, have been used for pesticide analysis concentration and clean-up in waters **(Menezes et al., 2016)**.

CHAPTER II

AIMS AND OBJECTIVES

1.8. AIMS AND OBJECTIVES

1.8.1. Aims

Pesticides are often used in agriculture. When used correctly, pesticides can kill or control pests such as weeds, insects, fungi, bacteria, and rodents. Chemical pest management has resulted in significant yield increases for the majority of important fruit and vegetable **(Block et al., 1992).** Pesticides have been proven to be effective in increasing agricultural output, but excessive or improper use has been linked to microbiological **(Mandal et al., 2020)**, ecological **(Koli et al., 2019)**, and environmental harm **(Cederberg et al., 2019)**. Microorganisms may use agrochemicals as a nutrient source after they have been applied. Following intake, these compounds are destroyed by bacteria, resulting in the creation of new metabolites that may be significantly more damaging to plants than the original molecules **(Magnoli et al., 2020)**. Excessive use of such agrochemicals, on the other hand, contributes to the development of chemical resistance among beneficial bacteria. To deal with these issues, different researchers have recovered pesticide-resistant bacteria that could be used as microbiological agents to improve crop yield in polluted soil **(Shahid and Khan, 2020; Khan et al., 2020; M. et al., 2019; Shahid and Khan, 2019)**.

Numerous *Bacillus* species have been observed to tolerate greater pesticide concentrations while also increasing agricultural production and productivity **(Radhakrishnan and Lee, 2016)**. The ability to tolerate pesticides at higher rates is a unique trait among microorganisms, including N2-fixers and phosphate solubilizers, and could be because of constitutive or induced mechanisms **(Kirubakaran et al., 2019)**. The ability of the PGPR to survive high levels of pesticides could be encoded by plasmid, mediated by chromosomal, or because of other mechanisms **(Shafiani and Malik 2003)**.

Therefore, the aim of this work is to study the **"Effect of Pesticides on Bacteria from Coastal Environments"**

1.8.2. OBJECTIVES

1. To compile a review of literature available on pesticides and their usage in India.
2. To isolate bacteria from different coastal locations, and investigate their sensitivity to different pesticides.

CHAPTER III

LITERATURE REVIEW

1.9. LITERATURE REVIEW

Pesticides are classified using a variety of factors, including their toxicity, pest organisms killed and their ability to function as pesticides, chemical composition and route of entry, mode of action, how or when it functions, formulations, and sources of origin **(I. Yadav and Devi 2017); (Akashe et al., 2018); (Freedman, 2018); (Hassaan and Nemr, 2020); (Nayak et al., 2020); (Tudi et al., 2021).**

Pesticide toxicity is determined mainly by two factors: dose and time. Thus, the quantity of this chemical (dose) involved and the frequency (time) the material is exposed to result in two types of toxicity, acute and chronic. WHO has classified different pesticides into four classes based on its toxicity. They are as follows: **(Nayak & Solanki, 2021)**

Class I_a- Extremely hazardous (Red Band)

Class I_b- Highly Hazardous (Yellow Band)

Class II- Moderately Hazardous (Yellow Band)

Class III- Slightly Hazardous (Blue Band)

Class IV- Unlikely to present acute hazard (Green Band)

1.9.1. Classification of pesticides based on the pest organism they kill and pesticide's functionality(Use): (Nayak & Solanki, 2021)

Pesticides are classified in this manner based on the pest organisms they kill and the functions they perform. Pesticides of various types are listed below:

Table 1. Pesticides based on the pest organisms they kill and their functions

Pesticide	Uses
Insecticides	chemicals that are used to kill insects and other arthropods
Fungicides	chemicals that kill fungi.
Acaricides	pesticides that kill mites and ticks
Algicides	chemicals that kill or suppress algae.
Herbicides	chemicals that are used to kill undesired plants.
Antifeedants	chemicals that stop insects and other pests from eating.
Avicides	poisonous chemicals used to kill birds.
Bactericides	substances that kill or inhibit bacteria
Larvicides	stop larvae from growing.
Repellents	substances that repel bugs based on their taste or odour.
Dessicants	work by drying the tissues of plants.
Virucides	antiviral agents

Ovicides	inhibits the growth of insect and mite eggs
Nematicides	chemicals that kill nematodes, which are plant parasites.
Termiticides	chemicals that kill termites
Chemosterillants	Chemicals that make an insect sterile and hence prevent it from reproducing
Plant growth regulators	substances that affect the expected rate of plant growth, flowering, or reproduction.

1.9.2. Classification of Pesticides according to Chemical Composition (Nayak & Solanki, 2021):

This is the most common and effective method of pesticide classification based on chemical composition. Insecticides, fungicides, herbicides, and rodenticides are also classified according to their chemical makeup, as shown below:

1.9.2.1. Insecticides: Insecticides are classed chemically as Carbamates (Carbaryl), Organochlorine (Endosulfan), Organophosphorus (Monocrotophos), Pyrethroids (permethrin), Neonicotinoids (Imidacloprid), various pesticides such as Spinosyns (Spinosad), Benzolureas (diflubenzuron), Antibiotics (abamectin)

1.9.2.2. Fungicides: Fungicides are categorised as aliphatic nitrogen fungicides (dodine), amide fungicides (carpropamid), aromatic fungicides (chlorothalonil), dicarboximide fungicides (famoxadone), dinitrophenol fungicides (dinocap), and others.

1.9.2.3. Herbicides: Herbicides include anilide herbicides (flufenacet), phenoxyacetic herbicides (2, 4- D), quaternary ammonium herbicides (Paraquat), chlorotriazine herbicides (atrazine), sulfonylurea herbicides (chlorimuron), and others.

1.9.2.4. Rodenticides: Rodenticides are classed as inorganic rodenticides (Zinc phosphide, Aluminium Phosphide) or organic coumarin rodenticides (bromadiolone, coumatetralyl).

2.0 Classification of pesticides based on Mode of Entry: (Nayak & Solanki, 2021) The various methods pesticides come into contact with or enter the target are referred to as pesticide modes of entry.

- Systemic pesticides: pesticides that plants and animals ingest and transport to untreated tissue. Systemic pesticides include 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and glyphosate.
- Contact (non-systemic) pesticides: They work on target pests when they come into contact with them. Contact pesticides such as paraquat and diquat dibromide are examples.
- Stomach poisons: These toxins reach the pest's body via the mouth and digestive system. One such case is malathion.
- Fumigants: Pesticides that kill or have the potential to kill specific pests by producing vapour and entering the pest's body via the trachea.
- Repellents: Repellents do not harm but are repulsive enough to keep them away **2.1.**

Classification of pesticides by mode of action: (Nayak & Solanki, 2021)

Pesticides are classified according to their method of action and are classified as follows:

- Physical poison: Pesticides have a physical impact on insects.
- Protoplasmic poisons: pesticides induce protein precipitation;

• Respiratory poison: chemical substances that are inactive respiratory enzymes; and

• Nerve poison: chemicals that inhibit impulse transmission.

• Chitin inhibition: Compounds inhibit the production of chitin in pests

2.2. Classification based on sources of origin: (Nayak & Solanki, 2021)

Pesticides are classified into two types based on their origin: biopesticides and inorganic pesticides.

Pesticides categorised as organochlorine, organophosphate, carbamate, and pyrethroid are further classified as organochlorine, organophosphate, carbamate, and pyrethroids. Bio-pesticides are pesticides derived from natural sources such as animals, plants, and microbes. (bacteria, viruses, fungi, and nematodes). They are classified into three groups:

• **Microbial pesticides:** These are pesticides created by microorganisms. Microbial herbicides contain active ingredients that are microorganisms such as bacteria, fungi, and protozoa. These pesticides destroy insects by infecting them or releasing poisons made by microbiological organisms **(Nayak & Solanki, 2021)**.

• **Plant-incorporated pesticides:** These pesticides are generated naturally by plants. Furthermore, genetic engineering is used to introduce the pesticide-production gene into the plant. As a consequence, plant integrated protectants refer to both the pesticide produced by such a plant and the genetic material injected. (PIPs) **(Nayak & Solanki, 2021)**.

• **Biochemical pesticides:** These are nontoxic insect control compounds derived from natural compounds. Biochemical pesticides include insect sex pheromones (which

prevent mating) and a range of fragrant plant extracts. (works by attracting insect pests into traps) **(Nayak & Solanki, 2021)**.

2.3. PESTICIDE USAGE PATTERN

There are 293 pesticides registered in India, and 104 pesticides are still produced/used in the country despite being banned in two or more countries around the globe **(Goi, 2021)**. In India, 50% of all insecticides used for pest control are redirected to cotton pest control **(Mooventhan et al., 2020)**. Many negative consequences, such as residue in plant parts, insecticide resistance, secondary pest outbreaks, pollution of natural resources, health complications for humans and wildlife, and so on, warrant a shift to eco-friendly pest management methods **(Birthal and Sharma, 2004)**. In 2017, India used 0.31 kg per ha of insecticide, compared to 19.6 kg per ha in Saint Lucia, 16.59 kg in Hong Kong, 13.9 kg in Ecuador, 13.3 kg in Taiwan, and 13.07 kg in China. America has reduced its use to 2.54 kg per hectare **(Roser, 2019).**

Pesticide usage patterns in India vary from those worldwide. In India, pesticides, fungicides, and herbicides are commonly employed. A significant portion of the total is made up of insecticides. In India, pesticides are used in the following order: insecticides > herbicides > fungicides + bactericides > other-pesticides, whereas globally, pesticides are used in the following order: herbicides > fungicides + bactericides > insecticides > other-pesticides. India is currently the world's fourth-major producer of pesticides. According to Research and Markets, the Indian pesticides revenue was worth Rs 214 billion in 2019. The market is predicted to reach Rs. 316 billion by 2024, with an 8.1 percent compound annual growth rate **(Nayak & Solanki, 2021).**

The most commonly used insecticide pesticide is chlorpyriphos. Its usage has increased from 471 metric tonnes (MT) in 2014-15 to 1431 MT in 2019-20. Sulphur is the most commonly used fungicide, with 1548 MT consumed in 2014-15, rising to 3878 Mt in 2019-20. A large concentration of 2, 4-D amine salts is used as a weedicide in India. (herbicide). In 2014-15, it was used 1MT, but in 2019-20, it was used 1067 MT. From 2014 to 2020, the most commonly used rodenticide was zinc phosphide, with consumption varying from 65 to 200 MT **(Nayak & Solanki, 2021).**

Organophosphates are the most commonly used pesticides, followed by neonicotinoids and pyrethroids. According to one research, Cotton is the most pesticide-consuming agri-product (93.27%), followed by vegetables (87.2 %), wheat (66.4%), millet (52.6 %), and mustard (12.6%) **(Maurya and Malik, 2016; Yadav and Dutta, 2019; Nayak et al., 2020).**

2.4. PESTICIDES CONSUMPTION SCENARIO

Pesticide production in India started in 1952 with the establishment of a BHC manufacturing plant in Calcutta, and India is now Asia's second biggest producer of pesticides after China, ranking twelfth globally **(Mathur and Tannan, 1999; FAO, 2018)**. Manufacturing of scientific grade pesticides in India has steadily increased from 5,000 metric tonnes in 1958 to 102,240 metric tonnes in 1998. Pesticide demand was expected to be Rs. 22 billion (USD 0.5 billion) in 1996-97, accounting for approximately 2% of the world market **(Kumar, 2013).**

According to the graph, pesticide use in India has increased hundreds of times over the preceding seven decades, from 154 MT in 1953-54 to 57,000 MT in 2016-17. India used the most insecticides in a single year (80,000 MT) in 1994-1995. The decrease was observed between 2000 and 2010 as a result of a prohibition or limit on the use of

organochlorine pesticides such as HCH (BHC), DDT-aldrin, and others. The adoption of the Stockholm Convention with high levels of application and the creation of integrated pesticide management plans are two reasons for reducing pesticide usage **(Nayak & Solanki, 2021).**

Pesticide application in India is hampered by the use of low-grade pesticides and the lack of pesticide information. According to the Economic Survey 2015-16, pesticide use without adequate restrictions has resulted in an increase in pesticide residue found in food products in India **(Nayak & Solanki, 2021).**

Maharashtra had the greatest total pesticide consumption in 2016-17, followed by Uttar Pradesh, Punjab, and Haryana. While Punjab had the highest per acre pesticide usage (0.74 kg), Haryana (0.62 kg), and Maharashtra (0.62 kg), (0.57 kg). According to the statistics, Maharashtra and Uttar Pradesh consume 41% of all pesticides in India. The top six states in India use more than 70% of crop protection chemicals altogether **(Nayak & Solanki, 2021).**

Many people nowadays favour natural alternatives to synthetic chemicals because they care more about the environment and their own health. Biopesticides are becoming more common due to their environmental safety, target-specificity, efficacy, biodegradability, and applicability in integrated pest management (IPM) programmes. Biopesticides' potential for environmentally safe application is well recognised. Interest has grown because of the greater demand for organic food **(Kumar and Singh, 2015).**

Biopesticides are pesticides drawn from natural resources such as plants, animals, microbes, and certain minerals. Biopesticides include natural pests (Biochemical Pesticides), pesticide control (Microbial Pesticides), microorganisms, and biochemical plant development regulators. Biopesticides have gone a long way since the 17th

century, when more dangerous synthetic pesticides were first used to control agriculture **(Koul, 2011; Villaverde et al., 2016; Samada and Tambunan 2020).**

The graph shows the evolution of chemical and biological insecticide use over the last six years. Bio-pesticide consumption makes for about 9% of total pesticide consumption in India. Bio herbicides are becoming less popular. However, statistics show that biopesticide use has increased in India over the last few decades.

Consumption of neem, one of India's most widely used biopesticides, rose from 83 metric tonnes (MT) in 1994-1995 to 686 MT in 1999-2000, while *Bacillus thuringiensis* (Bt) consumption increased from 40 to 71 MT during the same time period. During the 15th Lok Sabha, the standing committee on chemicals and fertilisers presented a report on pesticide output and availability in India. (2012–2013). That report stated that biopesticide use increased considerably from 123 metric tonnes (MT) in 1994-1995 to 8110 MT in 2011-2012, far exceeding predictions. According to PPQS data, overall biopesticide consumption in India grew by 40% between 2014-2015 and 2018-2019. There are presently 970 biopesticide products registered with the Central Insecticides Board and Registration Committee (CIBRC), India's primary regulatory body for all types of biopesticide use. Bacterial, fungal, viral, and other biopesticides (plant-based, pheromones) make for 29, 66, 4, and 1% of total biopesticide production, respectively **(Nayak & Solanki, 2021).**

Bioinsecticides continue to be in high demand in contrast to other products such as bioherbicides, biofungicides, and bionematicides. Bioinsecticides account for roughly 70% of the market, with makers placing a particular emphasis on this category to provide greater control and food safety **(Market, 2021).**

Only 12 distinct types of biopesticides have been recorded in India under the Insecticide Act of 1968 **(Kandpal, 2014)**. In India, the most common biopesticides are neem-based herbicides, *Bacillus thuringiensis*, NPV, and *Trichoderma* **(Sharma et al., 2018).**

The most commonly used pesticides in 2019-20 are *Tricoderma, Psedomonas*, and NPV-H (nuclear polyhedrosis virus of *Helicoverpa armigera*). Except for those used in agriculture, most biopesticides are used in public health. Pest control in India also includes the use of transgenic plants and beneficial organisms known as bio-agents **(Nayak & Solanki, 2021).**

In India, when chemical pesticides failed to eradicate *Helicoverpa armigera*, *Spodoptera litura*, and other cotton pests, a major technical breakthrough in the field of biocontrol occurred **(Kranthi et al., 2002).** Biocontrol was discovered to be the only technology capable of controlling the widespread resistance of chemical pesticides to pest insects in a safe, cost-effective, and ecologically beneficial way. Biopesticides were later incorporated into IPM, which had previously depended solely on the use of chemical pesticides **(Samada and Tambunan 2020); Mishra et al., 2020).**

CHAPTER IV

MATERIALS AND METHODS

2.5. Materials and methods

2.5.1. Sampling Sites:

Samples were collected from 3 locations (Miramar Beach, Vagator Beach and Anjuna Beach) in January and February 2023. The locations are as follows:

Table 2. Locations of the sampling sites.

SR. NO.	SAMPLING SITES	LATITUDE	LONGITUDE
1.	Miramar Beach	15°28'57.0"N	73°48'24.9"E
2.	Vagator Beach	15°36'10.6"N	73°44'00.8"E
3.	Anjuna Beach	15°34'23.5"N	73°44'27.6"E

2.5.2. Collection

Centrifuge tubes were rinsed with the water sample. The samples were collected in centrifuge tubes from each location by gently dipping the mouth of the tube into the water ensuring that no sand is collected and was immediately kept on ice in an ice box till they were brought to the laboratory for further processing.

2.5.3. Transport and processing of water samples for Viable Count

After collection, the tubes were transported immediately in an ice box to the laboratory to be used within 24 hours for isolation and to perform a viable count.

2.5.4. Analysis of Viable Count

After transportation to the laboratory, the centrifuge tubes containing water samples were surface sterilized using ethanol in the laminar air flow under sterile conditions.

Serial dilutions were carried out till 10^{-3} and the last three dilutions (10^{-1}, 10^{-2}, 10^{-3}) were spread plated on sterile nutrient agar plates. The plates were incubated at room temperature for 24 hours. After 24 hours, the number of colonies was counted to

calculate the viable count. Morphologically distinct colonies were chosen, purified in three cycles, and their colony characteristics were noted.

2.5.5. Maintenance of cultures on slants

Sterile nutrient agar slants were prepared in sterile test tubes, and each culture was streaked on the respected labelled slants with a sterile nichrome loop, allowed to grow and the test tubes were sealed with parafilm, wrapped in aluminium foil and stored in the fridge at 4ºC until further use.

2.6. EFFECT OF DIFFERENT INSECTICIDES ON THE MICROBIAL COMMUNITIES

NOTE: Before starting the main experiment for insecticides, the O.D. of all the cultures (24 hr old) were noted to check whether the cultures are viable.

2.6.1. PROCEDURE:

2.6.1.1. Monocrotophos Insecticide

A. For Day 0 (inoculating cultures in test tubes containing broth)

The sterile nutrient broth was added to sterile labelled test tubes. One loopful of each culture was inoculated in each tube containing broth. The tubes were incubated at room temperature for 24 hours till turbid to be used as inoculum for further experiment. Growth was confirmed by checking OD at 620 nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd.

B. Preparation of Insecticide stock solution

The insecticide was diluted by 1000 times; 50µL of insecticide was added in a centrifuge tube containing 50mL distilled water under sterile conditions.

C. Preparation of nutrient broth containing insecticide

2mL of the above Monocrotophos insecticide stock solution was added to the broth after removing 2mL of nutrient broth from 200mL sterile nutrient broth, to give a final dilution of 10^{-5}.

D. For negative control tube

In 1 test tube, 8mL of nutrient broth and pesticide was added. The tube was incubated at room temperature for 24 hours. This tube was used as the control for autozero at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd.

E. For positive control tubes (without the insecticide)

In all test tubes, 8mL of nutrient broth was added. 150µL of each 24-hour-old culture was inoculated in all the respective tubes. The tubes were incubated at room temperature for 24 hours. After 24 hours the tubes were checked for turbidity. O.D. was noted at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates.

F. For experimental test tubes (with the insecticide)

In all test tubes, 8mL of nutrient broth containing insecticide was added. 150µL of each 24-hour-old culture was inoculated in all the respective tubes. The tubes were incubated at room temperature for 24 hours. After 24 hours the tubes were checked for turbidity. O.D. was noted at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates. 2

2.6.1.2. Monocrotophos Insecticide (Higher Concentration)

A. For Day 0 (inoculating cultures in test tubes containing broth)

The sterile nutrient broth was added to sterile, labelled test tubes. One loopful of each culture was inoculated in each tube containing broth. The tubes were incubated at room temperature for 24 hours till turbid to be used as inoculum for further experiment.

B. Preparation of nutrient broth containing insecticide

0.2mL of the Monocrotophos insecticide was directly added after removing 0.2mL of nutrient broth from 200mL sterile nutrient broth (10^{-3} dilution).

C. For negative control tube

In 1 test tube 8mL of nutrient broth and pesticide was added. The tube was incubated at room temperature for 24 hours, and used as the control for autozero at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates.

D. For positive control tubes (without the insecticide)

In all test tubes, 8mL of nutrient broth was added. 150µL of each 24-hour-old culture was inoculated in all the respective tubes. The tubes were incubated at room temperature for 24 hours. After 24 hours the tubes were checked for turbidity. O.D. was noted at 620m using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates.

E. For experimental test tubes (with the insecticide)

In all test tubes, 8mL of nutrient broth containing insecticide was added. 150µL of each

24-hour-old culture was inoculated in all the respective tubes. The tubes were incubated at room temperature for 24 hours. After 24 hours the tubes were checked for turbidity. O.D. was noted at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt.

Ltd. This was performed in duplicates.

2.6.1.3. Dimethoate Insecticide

A. For Day 0 (inoculating cultures in test tubes containing broth)

The sterile nutrient broth was added to sterile, labelled test tubes. One loopful of each culture was inoculated in each tube containing broth. The tubes were incubated at room temperature for 24 hours till turbid to be used as inoculum for further experiment.

B. Preparation of nutrient broth containing insecticide

0.2mL of the Dimethoate insecticide was directly added after removing 0.2mL of nutrient broth from 200mL sterile nutrient broth (10^{-3} dilution).

C. For negative control tube

In 1 test tube 5mL of nutrient broth and insecticide was added. The tube was incubated at room temperature for 24 hours, and used as the control for autozero at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates.

D. For positive control tubes (without the insecticide)

In all test tubes, 5mL of nutrient broth was added. 150µL of each 24-hour-old culture was inoculated in all the respective tubes. The tubes were incubated at room temperature for 24 hours. After 24 hours the tubes were checked for turbidity. O.D. was

noted at 620m using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates.

E. For experimental test tubes (with the insecticide)

In all test tubes, 5mL of nutrient broth containing insecticide was added. 150µL of each 24-hour-old culture was inoculated in all the respective tubes. The tubes were incubated at room temperature for 24 hours. After 24 hours the tubes were checked for turbidity. O.D. was noted at 620nm using Auto Colorimeter from BR Biochem Life Sciences Pvt. Ltd. This was performed in duplicates.

2.7. CHARACTERIZATION OF ISOLATES

Gram staining and biochemical tests were used to characterize all bacterial isolates.

2.7.1. Gram Staining

A clean grease-free slide was taken and washed thoroughly. A loopful of culture was taken and a smear was prepared on the clean slide. The smear was air-dried and heat fixed. The smear was stained with crystal violet for 1 minute and then washed with a gentle stream of water. Further, the slide was stained with Gram's Iodine for 1 minute. The excess stain was drained and the slide was flooded with decolourizer (95% alcohol) for 1 minute. Smears were washed and flooded with Safranin for 1 minute. Slides were washed, air dried and were observed under oil immersion (100x) objective.

2.7.2. Motility Test

Nutrient soft agar butts were prepared in sterile test tubes and each culture (24-hourold culture grown in Nutrient Broth) was stab inoculated. All the tubes were incubated at room temperature for 24 hours. Tubes were checked to see if the cultures are motile (diffused growth in agar butt) or non-motile (non-diffused growth in agar butt).

2.7.3. Catalase Test

Each culture was streaked on sterile nutrient agar slants. The tubes were incubated at room temperature for 24 hours. A few drops of hydrogen peroxide were added.

Immediate bubbling/ effervescence indicated catalase activity (positive test).

CHAPTER V

RESULTS AND

DISCUSSION

2.8. Results and Discussion

2.8.1. Isolation of Cultures

Seven morphologically distinct colonies were isolated from Nutrient Agar. These cultures were purified in three cycles and were given culture codes **(Table 1)**. **Table 3.** Codes of purified bacterial isolates from water samples

Isolated on N ıtrient Agar		
Miramar	**Vagator**	**Anjuna**
M1	V2	A1
M2		A2
		A3
		A4

2 cultures were isolated from Miramar, 1 from Vagator and 4 from Anjuna **(Table 1)**.

The majority of the cultures were isolated from Anjuna, followed by Miramar and Vagator **(Fig. 1)**.

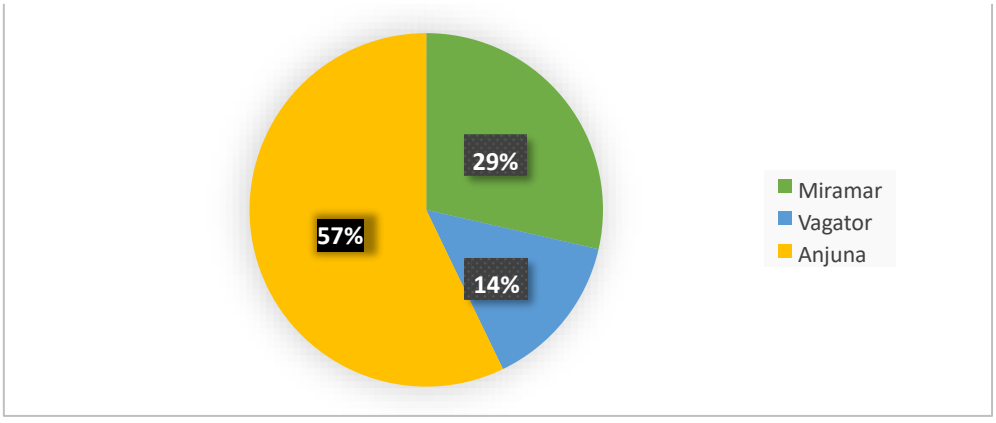

Fig. 2. Percentage of cultures isolated for water samples collected from Miramar, Vagator and Anjuna

2.8.2. Viable count

The viable count could not be calculated since the number of colonies did not fall in the range 30-300 CFU/mL.

2.8.3. Colony Characteristics

Table 4. Colony characteristics of bacterial isolates.

Colony Characteristics	**Cultures**						
	M1	**M2**	**V2**	**A1***	**A2**	**A3**	**A4**
Medium	Nutrient Agar	Nutrient Agar	Nutrient Agar	Nutrient Agar	Nutrient Agar	Nutrient Agar	Nutrient Agar
Time	24 hrs	24 hrs	24 hrs	24 hrs	24 hrs	24 hrs	24 hrs
Temperature	RT	RT	RT	RT	RT	RT	RT
Shape	circular	Circular	circular	circular	circular	circular	circular
Size	small	Small	small	small	small	small	small
Colour	Off-white	Off-white	Off-white	Off-white	Off-white	Off-white	Off-white
Opacity	Opaque	Opaque	translucent	Opaque	Opaque	Opaque	Opaque
Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Consistency	Butyrous	Butyrous	Slimy	Butyrous	Butyrous	Butyrous	Butyrous
Surface Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Gram Character	Gram- Positive Short rods in clusters	Gram- Positive Long rods in chains	Gram- Positive Short rods in clusters	Gram- Positive Short rods in clusters	Gram- Positive Short rods in clusters	Gram- Positive Short rods in clusters	Gram- Positive Short rods in clusters

Motility	Non-Motile	Non-Motile	Non-Motile	Non-Motile	Non-Motile	Non-Motile	Motile

NOTE: A1*- This culture isolated from Anjuna showed agar liquefying properties.

2.8.4. Characterization of bacterial isolates

Table 5. Characterization of bacterial isolates by Gram staining, Morphology, Motility and Catalase activity

Sr. No.	Cultures	Gram Character	Morphology		Motility Test	Catalase Test
			Shape	Arrangement		
1.	M1	Gram-positive	Short rods	in clusters	Non-Motile	Positive
2.	M2	Gram-positive	Long filamentous rods	in chains	Non-Motile	Positive
3.	V2	Gram-positive	Short rods	in clusters	Non-Motile	Positive
4.	A1	Gram-positive	Short rods	in clusters	Non-Motile	Positive
5.	A2	Gram-positive	Short rods	in clusters	Non-Motile	Positive
6.	A3	Gram-positive	Short rods	in clusters	Non-Motile	Positive

7.	A4	Gram-positive	Short rods	in clusters	Motile	Positive

All 7 bacterial isolates were Gram-positive **(Table 4)**. Out of 7 bacterial isolates, only one isolate (A4) diffused through the medium to a considerable distance away from the line of stabbing, indicating that it is highly motile. All 7 bacterial isolates showed immediate bubbling/effervescence after adding hydrogen peroxide, which showed that all the isolates had catalase activity **(Table 4)**

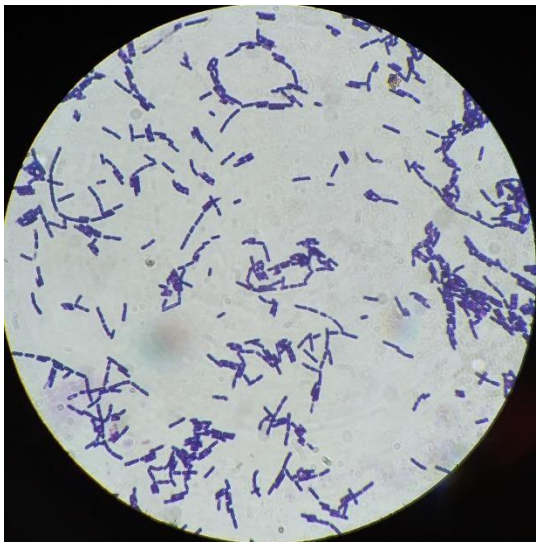

Fig. 3

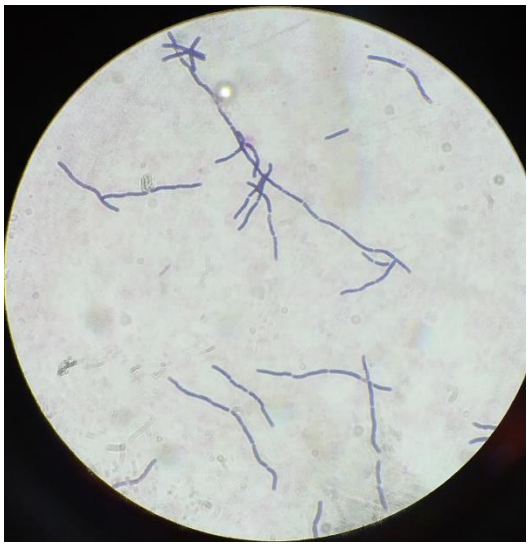

Fig. 4

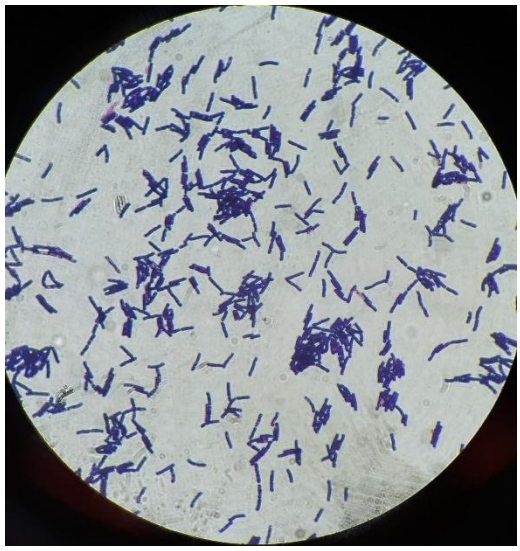

Fig. 5

Fig 3. Isolate M1- Gram-positive short rods in clusters

Fig 4. Isolate M2- Gram-positive long filamentous rods in chains

Fig. 5. Isolate V2- Gram-positive short rods in clusters

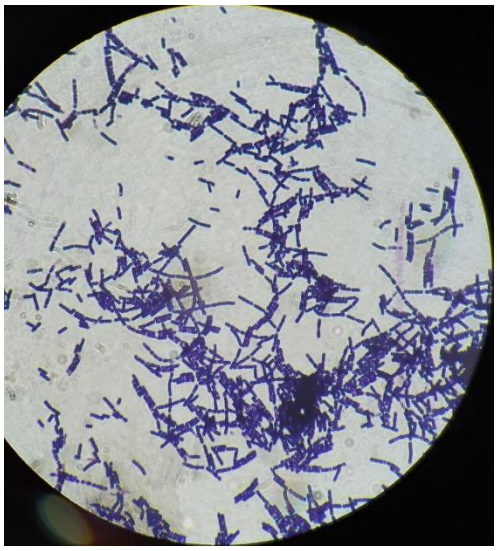

Fig. 6

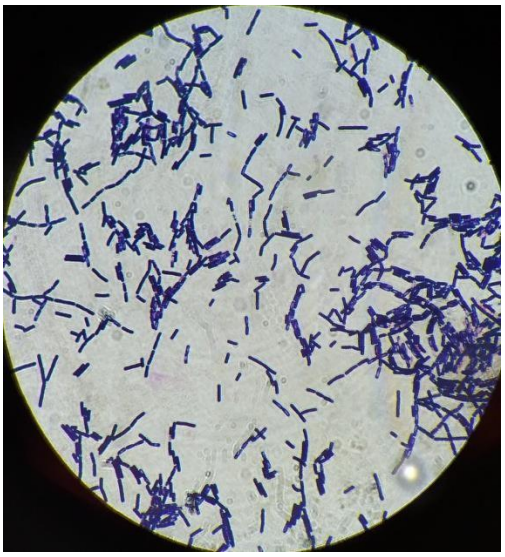

Fig. 7

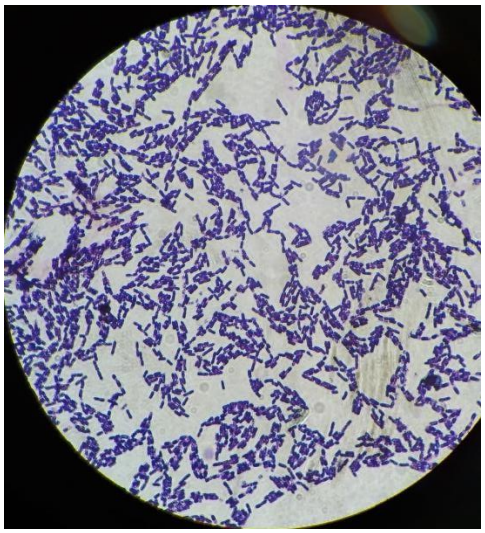

Fig. 8

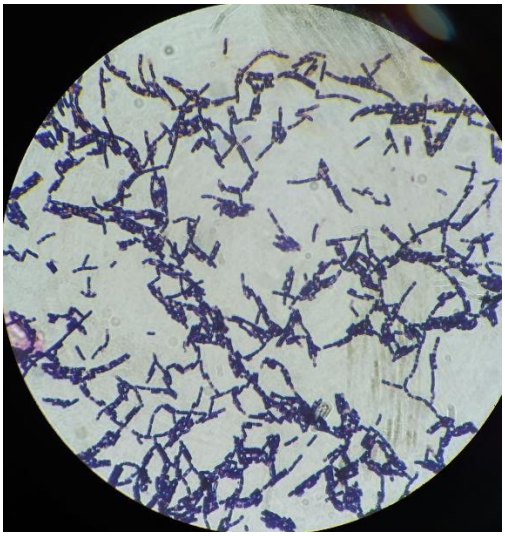

Fig. 9

Fig. 6. Isolate A1- Gram-positive short rods in clusters

Fig. 7. Isolate A2- Gram-positive short rods in clusters

Fig .8. Isolate A3- Gram-positive short rods in clusters

Fig. 9. Isolate A4- Gram-positive short rods in clusters

2.8.5. MOTILITY TEST

Fig. 10.

Out of 7 bacterial isolates, only one isolate (A4) diffused through the medium to a considerable distance away from the line of stabbing, indicating that it is highly motile.

2.8.6. CATALASE TEST

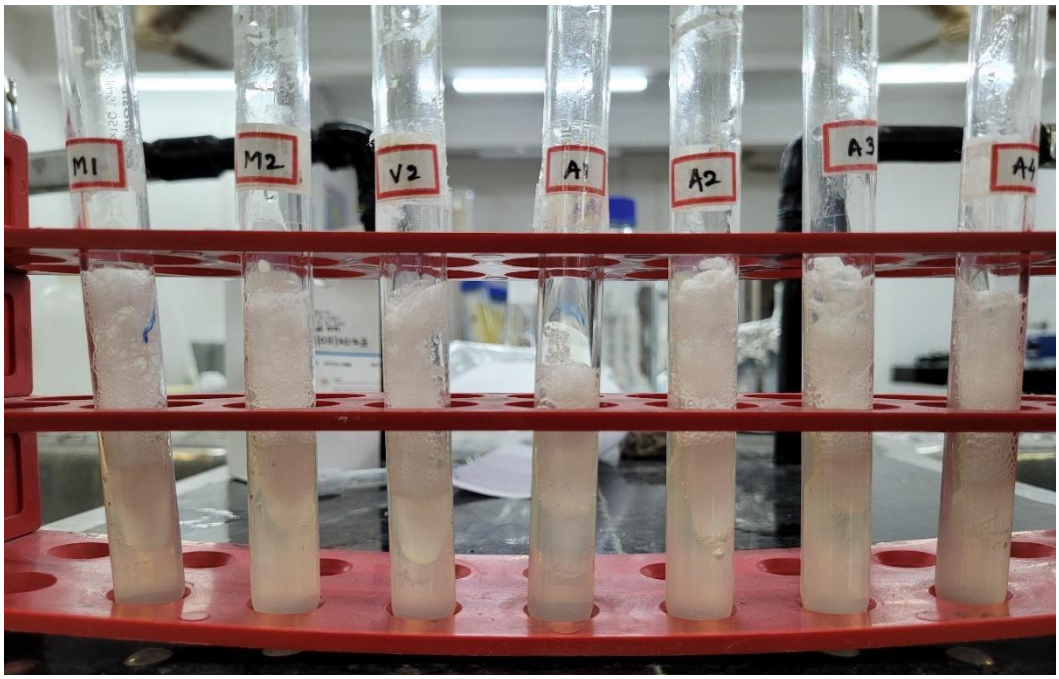

Fig 11. Tubes showing catalase test positive

All 7 bacterial isolates showed immediate bubbling/effervescence after adding hydrogen peroxide, which showed that all the isolates had catalase activity

Table 6. O.D. at 620nm of isolates grown in nutrient broth for 24 hours collected from Miramar, Vagator and Anjuna.

Culture	O.D. at 620nm
M1	0.950
M2	0.930
V2	0.378
A1	0.751
A2	0.719
A3	0.820

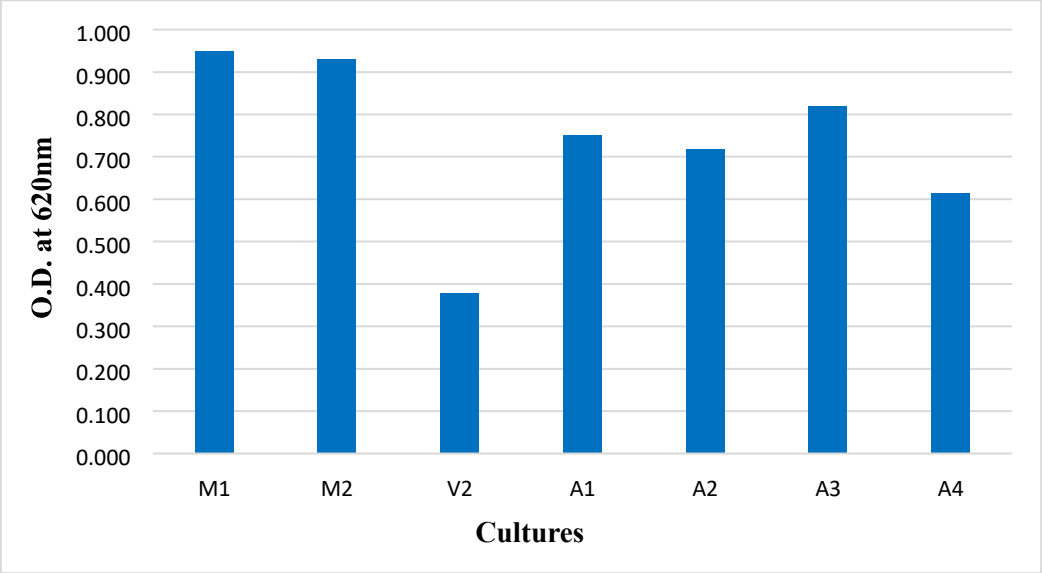

Fig. 12. O.D. at 620 nm of bacterial isolates grown in nutrient broth for 24 hours

O.D. at 620nm of bacterial isolates ranged from 0.378 to 0.950 **(Table 5)**.

2.9. Effect of different insecticides on the bacterial isolates

2.9.1. Monocrotophos Insecticide (10^{-5} dilution)

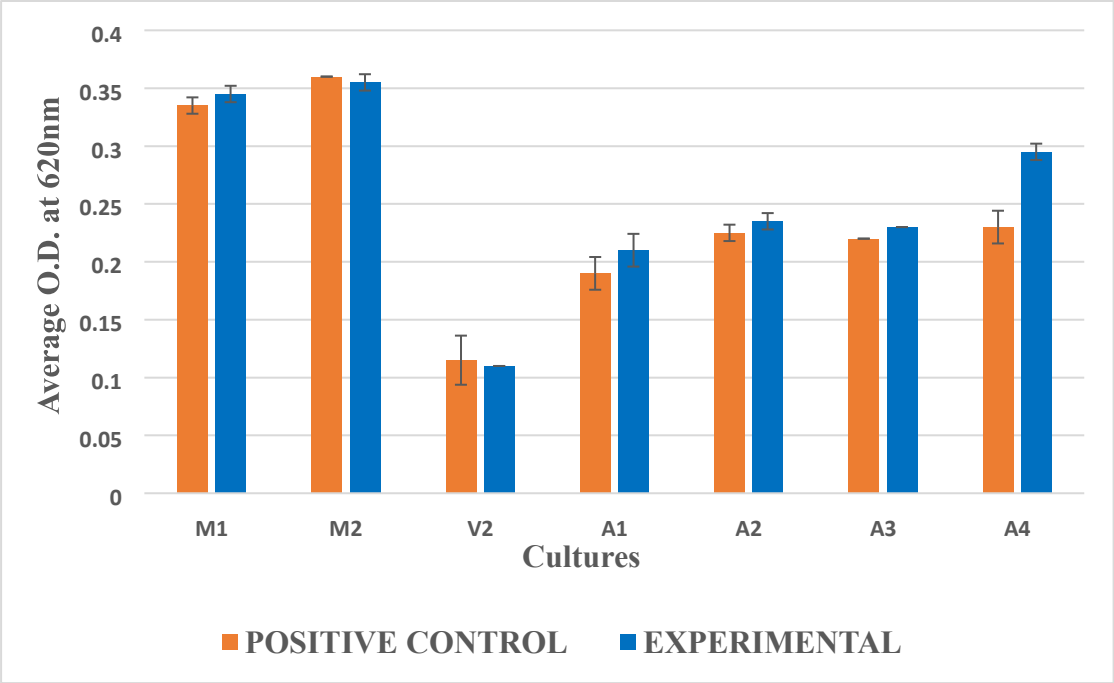

Fig. 13. Growth of bacterial cultures in presence of Monocrotophos compared to control

There was not much reduction in the growth of all the bacterial isolates when treated with Monocrotophos. **(Fig. 13)**. Isolate M2 showed higher growth in the presence of Monocrotophos compared to when grown in its absence. **(Fig. 13)**.

NOTE: Since the cultures were not affected by the above insecticide concentration, the experiment was performed again using higher concentration of insecticide.

2.9.2. Monocrotophos Insecticide (10^{-3} dilution)

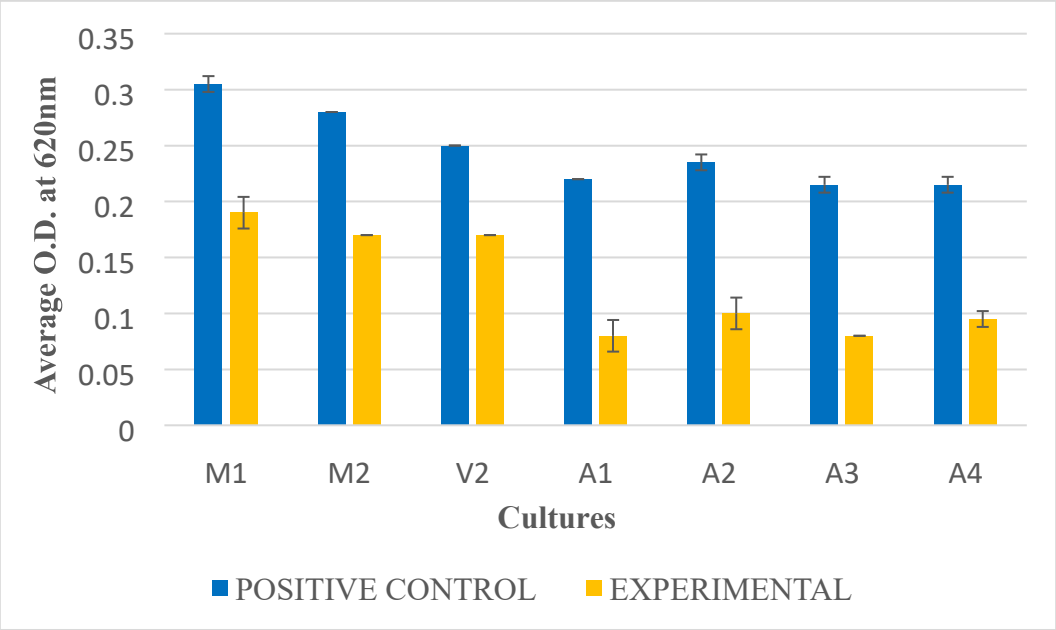

Fig. 14. Growth of bacterial cultures in presence of a higher concentration of Monocrotophos compared to control

All the cultures showed a reduction in growth when subjected to a higher concentration of insecticide **(Fig. 14)**. Isolate A1 showed the maximum extent of reduction compared to control in Monocrotophos in experimental treatments. **(Fig. 14)**.

2.9.3. Dimethoate Insecticide (10^{-3} dilutions)

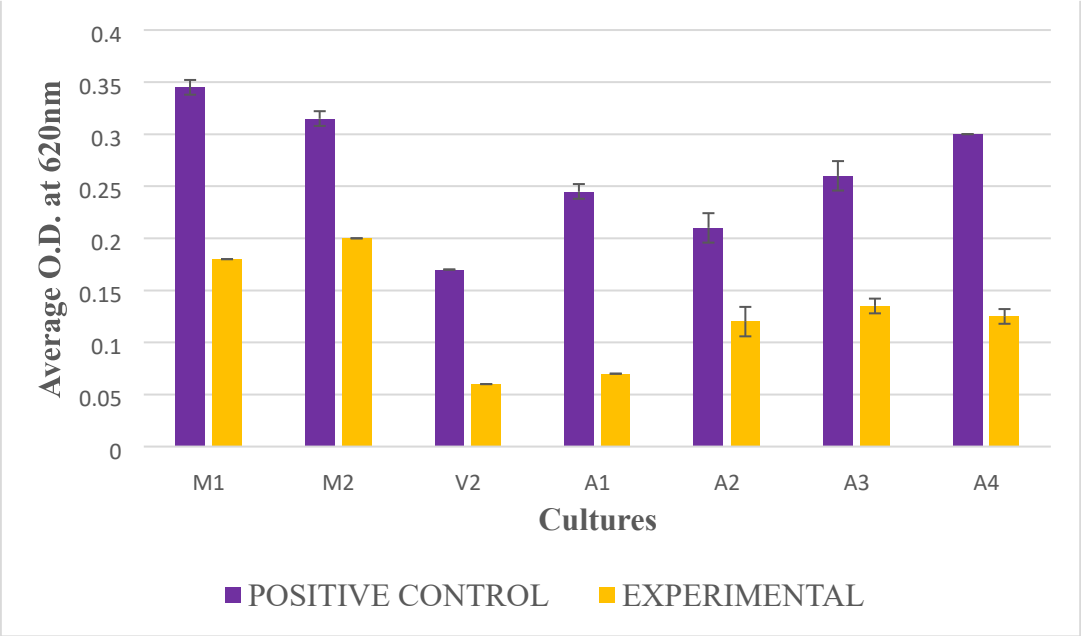

Fig. 15. Growth of bacterial cultures in presence of Dimethoate compared to control

All the cultures tested showed a reduction in growth when subjected to insecticide **(Fig. 15)**. Isolate A1 showed the maximum extent of reduction compared to control in Dimethoate in experimental treatments **(Fig. 15)**.

3. DISCUSSION

Concentrations of insecticides (Monocrotophos and Dimethoate) used for the experiment (10^{-5} and 10^{-3} dilution) were environmentally relevant concentrations. Use of lower dilution of insecticide showed rediuction in growth of all the bacterial isolates. Monocrotophos was an easily tolerable pesticide for all the bacterial isolates at lower concentrations (10^{-5} dilution). The effect of both pesticides on all the bacterial isolates at 10^{-3} dilution differed from one another. Two bacterial cultures M1, M2 (from Miramar) showed different sensitivity patterns to both Monocrotophos and Dimethoate. M1 was found to be more sensitive to Dimethoate than Monocrotophos compared to control. Culture V2 (from Vagator) was more sensitive against Dimethoate than Monocrotophos compared to control. All 4 cultures A1, A2, A3 and A4 (from Anjuna) were highly sensitive to Monocrotophos as compared to Dimethoate. Culture A1 and A3 showed a similar sensitivity pattern and were highly sensitive against Monocrotophos.

Mohamed (2017) investigated the consumption of Oxamyl by *Micrococcus luteus* OX obtained from Egyptian soil. He discovered that *Micrococcus luteus* consumed 87.8% of oxamyl in 48 hours making use of MSM supplemented with the nematicide as the sole carbon source under shaking conditions. The finding is comparable to that of **Rousidou et al. (2016)**. They degraded oxamyl into oxamyl oxime using four different freshly identified *Pseudomonas* bacteria. Three of these strains finished the oxamyl degradation in 96 hours. Longer incubation times beyond 2 days resulted in no significant decrease in his study. Furthermore, **Osborn et al. (2010)** observed oxamyl utilisation by *Aminobacter* spp. and *Mesorhizobium* sp. Carbaryl, like oxamyl, belongs to the carbamate family of insecticides, and a bacteria isolated from garden soil may

use carbaryl as its only carbon source **(Doddamani & Ninnekar 2001)**. He also found that *Micrococcus luteus* strain OX is not only strongly recommended for oxamyl remediation, but may also be useful for bioremediation of other pesticides, particularly carbamates **(Mohamed, 2017)**.

Shefali et al. (2021) reviewed the effects of increase in human population on the aquatic ecosystem, which can be viewed as climate change, nutrient enrichment of aquatic bodies, and pollution from various types of toxic substances, including pesticides, on a regional and global scale. These man-made environmental disruptions are responsible for negatively impacting the normal functioning of living species, including developmental defects ranging from invertebrates to larger organisms such as mammals. Pesticide use has increased in recent years, and it has been observed that it impacts non-target creatures at many biological scales. **(Shefali et al., 2021)**.

Muturi et al. (2017) studied the effects of pesticides on microbial communities in container aquatic habitats. They employed a microcosm technique to investigate the impacts of two herbicides (atrazine and glyphosate) and three insecticides (malathion, carbaryl, and permethrin) on the microbial communities of container aquatic habitats. MiSeq sequencing of the V4 region of both bacterial and archaeal 16S rRNA genes was used to characterise the microbial communities of indoor microcosms exposed to a single pesticide, a combination of herbicides, a combination of insecticides, or a combination of all five insecticides. Individual insecticides (but not herbicides) reduced microbial diversity and richness, and two insecticides, carbaryl and permethrin, also changed the structure of the microbial community. A herbicide mixture had no effect on microbial diversity or structure, whereas a pesticide mixture or all five pesticides

reduced microbial diversity and altered community structure. These findings indicate that individual pesticides or pesticide mixes can disturb aquatic microbial populations **(Muturi et al., 2017).**

The key issue and focus in the field is the consequences of the bacterial community having pesticide-degrading capabilities. The molecular mechanism and broad opinion of pesticide breakdown by microbes distinct from bacterial communities will surely improve our understanding and antiquity, indirectly lowering the danger to farmers' health, soil health, and agricultural field production in the future **(Kumar et al., 2020)**.

Shefali et al. (2021) reviewed the effects of increase in human population on the aquatic ecosystem, which can be viewed as climate change, nutrient enrichment of aquatic bodies, and pollution from various types of toxic substances, including pesticides, on a regional and global scale. These man-made environmental disruptions are responsible for negatively impacting the normal functioning of living species, including developmental defects ranging from invertebrates to larger organisms such as mammals. Pesticide use has increased in recent years, and it has been observed that it impacts non-target creatures at many biological scales. **(Shefali et al., 2021)**.

This study shows that microorganisms are sensitive to pesticides even when the pesticides are present at environmentally relevant concentrations. Pesticides are used to control boring, chewing, and sucking insects (such as aphids, Helicoverpaspp, caterpillars, moths, mites, jassids, budworms, scale and stem borer, and locusts) for a wide variety of crops **(Sayed, 2014)**, red spider mites, pea midges, thrips, wheat bulby, sawy, suckers, and woolly aphids **(Deshpande et al., 2004; Pappas & Kyriakidis 2003)**. Though pesticides are synthesized to specifically destroy pests and other disease carriers, they also cause a reduction in the growth of bacteria, indicating an inhibitory effect on non-target organisms.

CHAPTER VI

SUMMARY

Pesticides are substances used in agronomic practices to manage pests, weeds, and diseases in plants **(Dhaliwal et al., 2015)**. Pesticides enter the aquatic ecosystem through surface runoff, spillage, industrial effluent, vapourization, agricultural returns, groundwater intrusions, pesticide-treated soils, adsorption, or plant uptake **(Pataik & Patra, 2006; Boran et al., 2007; Jabali & El-Hoz, 2020); Ansara et al., 2012; Uddin et al., 2013; Pico et al., 2020)**. Most pesticides used in urban and agricultural environments harm aquatic organisms such as birds, fish, and zooplankton **(Ware, 1980)**. Pesticides mainly affect aquatic organisms through the skin, breathing or orally **(Shefali et al., 2021)**. Different classes of insecticides include Organochlorines, Organophosphates, Carbamates and Synthetic pyrethroids **(Shefali et al., 2021)**. Sampling was done in 3 areas (Miramar, Vagator, and Anjuna). 7 cultures were isolated after three purification cycles using nutrient agar and were characterized by Gram staining, Motility and Catalase activity. The results indicated that isolates were Grampositive. Out of the 7 cultures tested, only one isolate (M2) was long filamentous rods rest were short rods. All the isolates were catalase positive and only one of the isolate (A) was highly motile.

In the next experiment, sensitivity of the isolates was tested to 2 different insecticides i.e., Monocrotophos (10^{-5} and 10^{-3} dilution) and Dimethoate (10^{-3} dilution) which are environmentally relevant concentrations. In the presence of insecticide, cultures isolated showed different sensitivity profiles. All the isolates could tolerate Monocrotophos at 10^{-5} dilution i.e., there was hardly any inhibition. Whereas all the isolates were sensitive to both insecticides (Monocrotophos and Dimethoate) at 10^{-3} dilution.

The effect of both the pesticides on all the bacterial isolates at 10^{-3} dilution differed from one another. Two bacterial cultures isolated from Miramar (M1, M2) showed different sensitivity patterns to both Monocrotophos and Dimethoate. M1 was found to be more sensitive to Dimethoate than Monocrotophos. Culture V2 isolated from Vagator was found to be more sensitive against Dimethoate than Monocrotophos. All the 4 cultures (A1, A2, A3 and A4) isolated from Anjuna were highly sensitive to Monocrotophos as compared to Dimethoate. Culture A1 and A3 showed a similar sensitivity pattern and were highly sensitive against Monocrotophos.

ANNEXURES

Annexure I

A) Agar

1. Nutrient Agar 1.5%

Ingredients	Grams/Litre
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	1.50
Sodium chloride	8.00
Agar	15.00

Final pH (at 25ºC)	7.3 ± 0.2

2. Soft (Nutrient) Agar

Ingredients	Grams/Litre
Peptone	5.00
Sodium chloride	5.00
Meat extract	1.50
Yeast extract	1.50
Agar	2.00
Final pH (at 25ºC)	7 ± 0.2

Annexure II

A) Broth

1. Nutrient Broth

Ingredients	Grams/Litre
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Meat extract	1.50
Yeast extract	1.50

Distilled water	1000mL
Final pH (at 25ºC)	7 ± 0.2

Annexure III

A) Stains

1. Gram Staining Reagents

- **Crystal Voilet**

a) Crystal Violet: Dissolve 20g crystal violet in 200mL methylated spirit.

b) Ammonium oxalate: Dissolve 8g ammonium oxalate in 800mL of distilled water. Mix together to make 1000mL stain solution.

- **Gram's Iodine**

Ingredients	Grams/Litre
Iodine	10.0
Potassium iodide	20.0

- **95% Alcohol**

Mix 950mL absolute alcohol with 50mL distilled water.

- Safranin

Dissolve 0.25g of safranin in 10mL ethanol and make up the volume 100mL with distilled water.

Annexure IV

A) Preparation of stocks

1. Stock solution of Monocrotophos (10^{-5} dilution)

Dissolve 50µL of the Monocrotophos to 50mL distilled water. Add 2mL of this stock to 200mL nutrient broth.

2. Stock solution of Monocrotophos (10^{-3} dilution)

Add directly 2mL of this solution from the bottle to 200mL nutrient broth.

3. **Stock solution of Dimthoate (10^{-3} dilution)**

Add directly 2mL of this solution from the bottle to 200mL nutrient broth.

Table 2. Viable count for 3 locations

Sr. No.	Location	Dilution	Number Of Colonies
1.	Miramar Beach	10^{-1}	5
		10^{-2}	3
		10^{-3}	2
2.		10^{-1}	4

	Vagator Beach	10^{-2}	2
		10^{-3}	1
3.	Anjuna Beach	10^{-1}	48
		10^{-2}	41
		10^{-3}	36

PLAGIARISM REPORT

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