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RECENT ADVANCES IN THE FIELD OF LIFE SCIENCES-VOL 2

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PREFACE

We are delighted to publish our book entitled “Recent Advances in the Field of Life Sciences - Vol 2". This book is the compilation of esteemed chapter of acknowledged experts in the fields of Life Science. This book is published in the hopes of sharing the excitement found in the study of Life Science. We developed this digital book with the goal of helping people achieve that feeling of accomplishment. The chapters in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

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RECENT ADVANCES
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CHAPTER-1
VERSATILITY OF GRASS PEA (LATHYRUS SATIVUS L.,) AS AN EXCELLENT CYTOGENETIC MODEL TO EVALUATE GENOTOXIC STRESS USING ROOT TIP ANA-TELOPHASE CYTOLOGY

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Abstract

With more than 150 species worldwide, Lathyrus sativus L. is frequently known by several names, including grass pea, dogtooth pea, grass pea vine, Indian pea, riga pea, and wedge pea vine. This crop has long been utilized as a grain for human consumption as well as for animal feed. It dates back thousands of years. This crop is best suited for arid and semi-arid environments due to its resilience to drought, adaptation to a variety of soil types, and durability. This genus, which has a bimodal karyotype of 2n=14, is a great model to assess the gentoxicity testing of various contaminants, such as heavy metals, salinity, plant-derived alleolochemicals, prooxidants, and anticancer medications. The potential and adaptability of grass pea is covered in length in this chapter. This chapter in detail encompasses the possibility and versatility of grass pea chromosomal aberration system CAs and numerial aberration tests NAs over the traditional models as a ready, reliable reproducible result yielding option to opt for all plant biologists.

Introduction:

Known by several names, including grass pea or khesari dal, Lathyrussativus is an important crop in agricultural history because of its rich nutritional profile and ability to withstand harsh environments. This legume, which has its origins in the Mediterranean, has spread around the world and is especially well-known on the Indian subcontinent. Lathyrus sativus has been grown for generations in India, where it has influenced both traditional customs and daily life. The current agricultural status of grass pea in India has fluctuated despite its nutritional value and flexibility due to a variety of variables, including socioeconomic dynamics and agronomic techniques.

Historical Roots and Origin:

Archaeological evidence indicates that *Lathyrus sativus* was cultivated thousands of years ago by ancient civilizations throughout the Mediterranean region. Its adaptability to a variety of climates and resistance to pests and illnesses made this bean an essential staple for populations in the past. Grass peas traveled across trade channels and arrived in the Indian subcontinent, where they were incorporated into diets and agricultural systems.

Modern Agricultural Status of *Lathyrus sativus* in India:

Lathyrus sativus is still an important crop in modern India, especially in areas with harsh weather conditions like drought or poor soil. Because of its resilience and capacity to fix nitrogen, smallholder farmers find it to be a desirable alternative that improves livelihoods and food security in rural areas. Furthermore, grass peas have nutritional value as they are an excellent source of fiber, protein, and important minerals. This helps to address the issue of malnutrition, particularly in regions with limited resources. Lathyrus sativus has advantages, however there are drawbacks to

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its agricultural status in India. Regulations have been restricted and consumers have been wary due to concerns of its neurotoxic characteristics, especially when ingested extensively over an extended period of time. Its widespread adoption has also been hampered by changes in market demand and pricing as well as a lack of research and development efforts.

Botanical Description of Grass Pea (*Lathyrus sativus*):

The grass pea (2n = 14) is an Angiosperm crop belonging to the kingdom Plantae, family Fabaceae, sub-family Faboideae, tribe Vicieae, order Fabales, and genus Lathyrus. About 150 species make up the genus; among them, L. sativus is significant for food, feed, and fodder; winged vetchling L. Ochrus and red vetchling L. cicera are significant for feed and fodder as well. There are 187 species and subspecies in the broad genus Lathyrus (Allkin et al. 1983). Four species of these are present in India: L. sativus, L. odoratus, L. ochrus, and L. aphaca (Allkin et al. 1983). While grass pea grain is high in protein, its use is severely limited because of b-Noxalyldiaminopropionicacid (b-ODAP) [also known as b-oxalylaminoalanine (BOAA)], a water-soluble non-protein amino acid that functions as a neurotoxin and paralyzes lower limbs. Large-scale extended consumption can result in neurolathyrism, a disease that affects up to 6% of the population and causes paralysis of the lower limbs in humans (Sharma et al. 2000). As a result, the crop is no longer included in initiatives to improve agriculture. In fact, several nations, like India, have outright prohibited the selling of lathyrus. These commercial grass pea cultivars are essential to maintaining agricultural livelihoods, particularly in areas vulnerable to environmental stressors like soil deterioration and drought. Acknowledging their agronomic advantages notwithstanding, it is imperative to confront the possible health hazards linked to the ingestion of grass peas and advocate for safe use and dietary variety. Furthermore, in order to ensure food security and human health, continuous research and breeding efforts are concentrated on creating new grass pea cultivars with increased nutritional content and decreased toxin levels.

At present 25 grass pea genotype varieties are available throughout India (ICAR 2009)

1. Mahateora (high yielding low ODAP content).
2. Ratan (high yielding low ODAP content).
3. Nirmal Mutant. (high yielding low ODAP content).
4. Pusa 24’.
5. Berhampur Local.
6. Bankura Local.
7. Kaikhali Local.
8. BK-10.
9. BK-3-6.
10. BK-10-2-1.
11. BK-11-3.
12. BK-2.
13. BK-27-1.
14. BK-37-2.
15. BK-28-1.
16. BK-7-1.
17. BK-20-5.

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18. WBK-5.
19. BK-35-1-1.
20. BK-2-2.
21. BK-13-1.
22. BK 2-46.
23. BK-14-1.
24. BK-14-8-3.
25. BK-26-5.

Its cultivation in India is restricted to Chhattisgarh, Madhya Pradesh, West Bengal, parts of Bihar and Maharashtra.

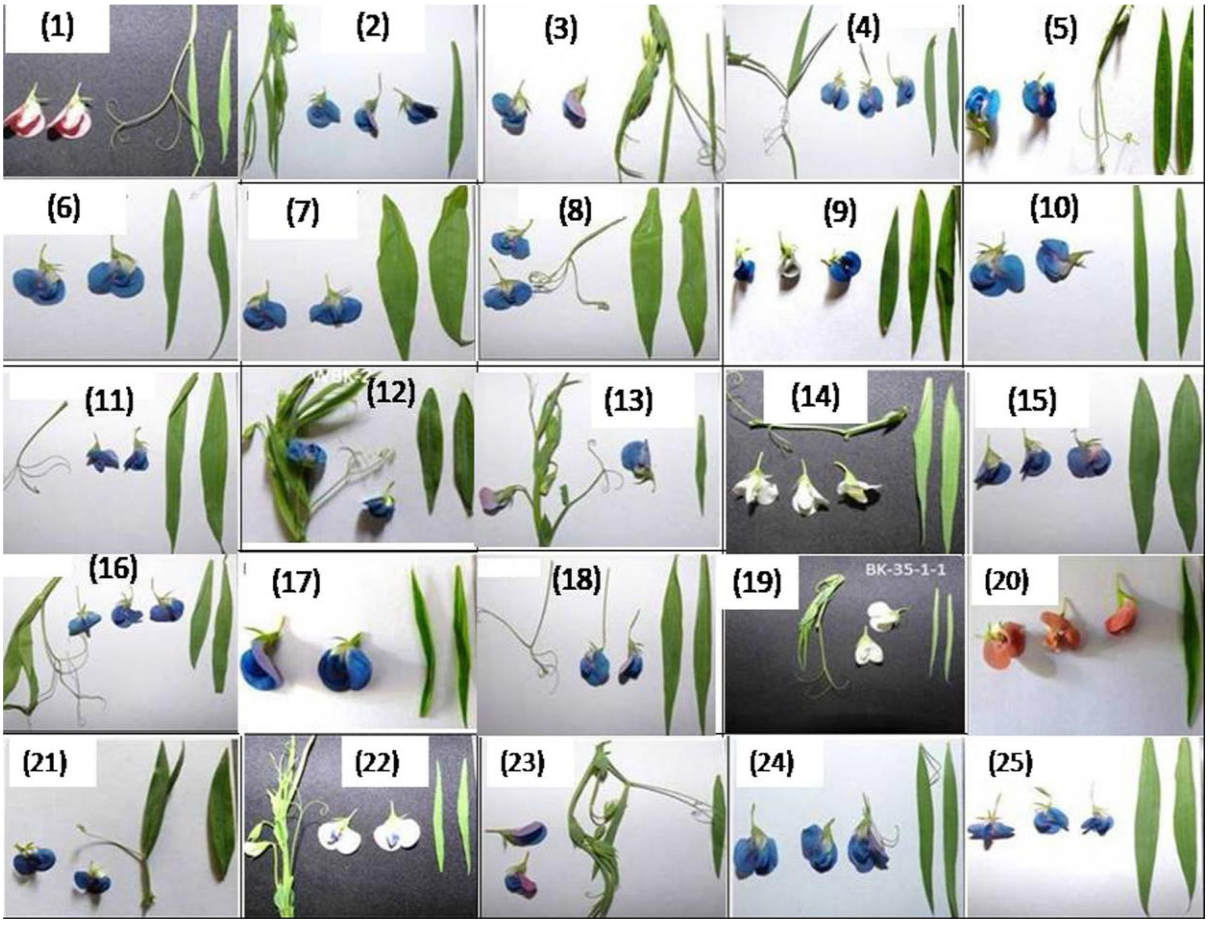

Fig 1: Flower colour variation and variability of leaf and tendril structure of all the 25 genotypes (after Chatterjee et al., 2019).

Grass pea (Lathyrus sativus), also known as khesari dal or chickling pea, is an annual legume belonging to the Fabaceae family. It is cultivated primarily for its edible seeds and forage. Below is a concise botanical description of the plant:

1. Morphology:

i) Stem: Grass pea features a slender, erect stem with branching, typically reaching a height of 30-90 cm.Leaves:The leaves are compound, consisting of two leaflets, each ranging from ovate to lanceolate in shape, with pointed tips.Flowers: The plant bears showy, papilionaceous flowers, typically in shades of blue, pink, or white, arranged in clusters at the end of slender stalks.Inflorescence: The inflorescence is a raceme, with several flowers arranged along the stalk.Fruit: Grass pea produces pods that are linear-oblong in shape, containing 2-5 seeds. The pods

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are typically 2-4 cm long and covered in fine hairs.Seeds: The seeds are rounded to oval, varying in color from white, cream, yellow, to brown, depending on the variety. They are approximately 0.5-1.5 cm in diameter.

ii) Root System: Grass pea develops a taproot system, penetrating the soil deeply to access nutrients and water.

2. Habitat and Distribution: Grass pea is adaptable to a wide range of climates and soil types, although it thrives in well-drained, loamy soils.It is primarily cultivated in temperate and subtropical regions across Asia, Africa, and parts of Europe.

3. Growth Habit: It exhibits determinate growth, with a relatively short growing season of about 90-150 days. Grass pea is known for its resilience to drought and poor soil conditions, making it suitable for cultivation in marginal lands.

4. Uses:

i) Food Source: The seeds of grass pea are edible and rich in protein, carbohydrates, and essential minerals. They are often used in soups, stews, curries, and as flour for making bread and snacks.

ii) Forage: The plant is also utilized as a forage crop for livestock, providing nutritious fodder.

5. Cultivation:

i) Grass pea is propagated from seeds, sown directly into the soil after the last frost date.

ii) It requires moderate watering and sunlight for optimal growth.

iii) Crop rotation is recommended to prevent soil depletion and disease buildups.

6. Health Considerations: While grass pea seeds are nutritious, they contain a neurotoxin called ODAP (β-N-oxalyl-L-α,β-diaminopropionic acid) in varying concentrations. Prolonged consumption of grass pea seeds as a staple food can lead to a neurological disorder known as lathyrism. In conclusion, Lathyrus sativus L., or grass pea is a versatile legume with edible seeds and forage potential, adapted to diverse environmental conditions. However, caution must be exercised regarding its consumption due to the presence of neurotoxins.

7. **Grass pea somatic cytology:**Grass pea, scientifically known as Lathyrus sativus L., is a leguminous plant that exhibits distinct cytological features and genomic attributes. Here's a cytological description along with its genomic characteristics:

Cytological Description:

Since the nucleus, mitochondria, and chloroplasts are membrane-bound organelles, grass pea cells are classified as eukaryotic. These organelles are essential to several biological functions, including photosynthesis, energy synthesis, and metabolism. With a mean chromosome length (TL) of 6.03 µm (4.7-6.7 µm) and a total chromosome volume mean (TCV) of 24.2 µm3 (18.1-35.1 µm3), all genotypes examined were diploid (2n = 2x = 14), according to cytological investigations. The majority of species are diploid, with polyploids being the rare exceptions (Yamamoto*et al*. 1984; Broich 1989; Battistin and Fernandez 1994; Klamt and schifino-Wittmann 2000; Seijo and Fernandez 2001). Large differences in chromosome size have contributed significantly to the development of Lathyrus species, which are linked to a four-fold variation in the amount of 2C nuclear DNA, despite the stability of chromosomal number (Narayan and Ress, 1976). Numerous karyotypic analyses of Lathyrus old world members have been conducted(Lvania and Sharma 1980; Yamamoto*et al*. 1984; Sahin*et al*. 2000; Verma, 1979; Biswas and Biswas, 2004).

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2. Genomic Attributes:

Genome Size: Lathyrus sativus is thought to have a genome with about a particular number of base pairs (the precise amount may differ based on the cultivar and the genome sequencing techniques employed). It has a collection of genes in charge of several characteristics like development, growth, and reaction to external cues. All genotypes had an average 2C DNA content of 14.7 pg, with a range of 13.8 pg to 15.6 pg. (Karimzadeh et al., 2011).

Genetic Diversity: Grass pea exhibits genetic diversity within its species, which is essential for adaptation to different environmental conditions and for breeding programs aimed at improving traits such as yield, disease resistance, and nutritional content.

Genetic Markers: Molecular techniques have been employed to identify and characterize genetic markers in grass pea, facilitating genetic mapping, marker-assisted selection, and genetic diversity studies.

Domestication: Studies of the grass pea genome provide insights into its domestication history and genetic changes that have occurred during cultivation.

Functional Genomics: Functional genomics approaches, such as transcriptomics and proteomics, have been utilized to understand gene expression patterns and regulatory mechanisms underlying various biological processes in grass pea.

So, grass pea (Lathyrus sativus) exhibits specific cytological features such as a diploid chromosome count, eukaryotic cellular structure, and genomic attributes including genome size, genetic diversity, and functional genomics insights, which collectively contribute to its biological characteristics and agricultural importance.

Importance of Higher Plant based bioassays to evaluate the cytotogenotoxic effects of different xenobiotics: Lathyrus sativus L., as an excellent alterative to Allium cepa, Allium sativum, Vicia Faba, etc. To prepare Bioassay-based dosimeters for ecotoxicity monitoring:

A number of studies (Islam et al., 2015; Ullah et al., 2013; Younas et al., 2015) have reported that rapid advancement of industrialization poses a severe threat to both the ecological balance and the survival of living things. Concerning effects produced by chemical agents to exposed species include mutagenic and genotoxic effects, which can cause numerous health problems and also affect future generations due to inheritable modifications in genetic material (Leme and MarinMorales, 2009). Genetic toxicology is the study of agents that damage DNA with the goal of understanding the molecular mechanisms and potential biological effects of genetic material. A biological assessment of pollutants present in environmental samples obtained through the use of the in vivo method is necessary for the reliable measurement of the extent of pollution load prior to effluent discharge from industries being mixed with watersheds and adjacent territories (de Souza Pohren et al., 2013; Hemachandra and Pathiratne, 2015; Kannangara and Pathiratne, 2015b;). Biological testing, especially short-term bioassays on bacteria or higher plants, is the most efficient way to determine and estimate the pollutant load in any matrix methodology (Mesi and Kopliku, 2012). Many plant species can be used as indicators of the cytogenetic and mutagenic effects of physical and chemical environmental contaminants (Çavuşoğlu et al., 2011). Plant cytogenetic tests are a rapid, sensitive method of environmental monitoring that is also affordable, easy to use, and has a solid track record of integration with other bio-testing systems (Fiskesjo, 1988). Worldwide, plants like *Hordeumvulgare*, *Pisumsativum*, *Zea mays*, *Tradescantia*, *Nicotianatabacum*, *Vicia faba*, and *Allium cepa*have been used (Akintonwa et al., 2009; Goncharuk et al., 2011; Iqbal et al.,

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2015a; Iqbal and Nisar, 2015; Srivastava and Mishra, 2009; Srivastava et al., 2005). However, it is extremely uncommon to employ *Lathyrus sativus* L. as a viable substitute for cytogentoxicity assay models in bioassay-based research (Adhikari et al., 2024).

Advantages of Lathyrus sativus L. over other popular models such as A. cepa test versus other biological assays are manifold:

i) *Lathyrus sativus* L., is a harsh environment resilient crop.

ii) Availability and maintenance of Lathyrus sativus L., are throughout the year making it almost a no-costly system across all laboratories.

iii) Almost availability of nearly 25 well documented varieties throughout West Bengal, researchers can access any germplasmthrought the year for experimental purposes.

iv) Very easy germination makes Lathyrus sativus L., makes one of the excellent test material for nucleolar, chromosomal, morphological, anatomical and enzymatic bioassays throughout the year. Allium cepa and Allium sativum chiefly being a winter crop put across several difficulties in rooting and seed germination during high summer months in India.

v) Lathyrussativus's traits make it a great genetic model for evaluating environmental contaminants and can be applied to the observation of both chemical and physical hazardous substances. Similar to Allium cepa and Allium sativum (Leme and Marin-Morales, 2009; Paz et al., 2006) *Lathyrus sativus* can be used because it is sensitive to mutagens and can be used to evaluate a variety of genetic endpoints, from point mutations to chromosome aberrations (CA) in meristematic cells and the F1 generation.

vi) The Lathyrus sativus test is a good example of an in vivo biological test because it allows seeds to be primed prior to germination in direct contact with the substance of interest, detecting potential DNA damage, and yields results that can be used as a model for a variety of animal and plant biodiversity. Furthermore, direct visualization is possible for the structural aberration and numerical chromosomal changes. Lathyrus sativus also stands out for being more efficient than other bioassays, having a high connection with other test systems, being easy to handle, being inexpensive, and having the right amount of chromosomes (2n = 14; bimodal karyotype).

vii) Lathyrus sativus can be used for the indication of toxic compounds (mutatoxic, cytotoxic and genotoxic etc; Adhikari et al, 2021, Adhikari 2023, Adhikari, 2019, Ghosh et al, 2020) and due to its similar karyokinetic characteristics of proliferation as compared to *Allium cepa*, Allium sativum, *Viciafaba, Zea maysTradescantia, Nicotianatabacum, Crepis capillaries, Pisumsativum* and *Hordeum vulgare*, is more suitable for chromosomes and nuclear study (Leme et al., 2008; Leme and Marin-Morales, 2009; Magdaleno et al., 2008; Smaka-Kincl et al., 1996; Souza et al., 2009). In addition, cytogenetic tests are thought to be one of the most accurate and sensitive ways to measure the harmful effects of any agent of interest when compared to other physical, chemical, physiological, radiological, and other methods on a genetic level using a variety of plant-based bioassays. These tests are appropriate for identifying the harmful effects of specific known substances in various concentrations over different exposure times.

viii) While physical and chemical analyses only determine the presence and concentrations of various pollutants, biological tests are essential for assessing how living organisms respond to complex environmental pollution and for indicating potential synergistic effects of various pollutants. Additionally, they take a lot of time and effort (Bianchi et al., 2011; Chandra et al.,

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2004; Leme and Marin-Morales, 2008, 2009). Similar to *Allium cepa* L., *Lathyrus sativus*L., assay can be considered an effective and dependable test system for the quick screening of compounds for mutagenic and clastogenic effect evaluation (Rank and Nielsen, 1997b). This test often gives an approximation of the overall toxic effect that comes from treating root tip cells with a combination of wastes, such as the Allium system(Fiskesjo, 1993).

ix) A quick way to check for genotoxic effects of chemicals found in the environment is to use the micronucleus (MN) and chromosomal aberration (CA) tests. Furthermore, Lathyrus sativus may be utilized frequently because of its length of cell cycle, its response to a variety of recognized mutagenic substances, and its ability to assess the action mechanism of toxic agents (Cisplatin, Etoposide, Vinblastine, *Piper betle*Leaf Extract, pro-oxidant (H_2O_2), antioxidant i.e., ascorbic acid; and an anticancerous drug methotrexate; MTX etc.) on genetic material based on their clastogenic and aneugenic effects. (Adhikari et al, 2021, Adhikari 2023, Adhikari, 2019, Ghosh et al, 2020; Samanta et al, 2015; Samanta et al, 2023).

x) Compared to other biological tests, such as *A. cepa*, *Lathyrus sativus* application in situ studies is simpler (Kovalchuk et al., 1998). The chromosomal characteristics facilitate the execution of the CA's test for both determining the test agent's action mechanisms and assessing hazardous effects. (Fiskejo, 1985; Rank and Nielsen, 1997b).

xi) *Lathyrus sativus*, in addition to chromosome damage and disruptions in the cell division cycle, makes it easier to assess the hazards of aneuploidy using models such as the Allium model (Leme and Marin-Morales, 2008). There is established evidence of a strong correlation between the *Lathyrus sativus* test findings and data derived from both prokaryotic and eukaryotic systems (Adhikari et al, 2021, Adhikari 2023, Adhikari, 2019, Ghosh et al, 2020; Samanta et al, 2015; Samanta et al, 2023).

Origin of CAs and NAs in Lathyrus sativus root system out of genotoxic exposure:

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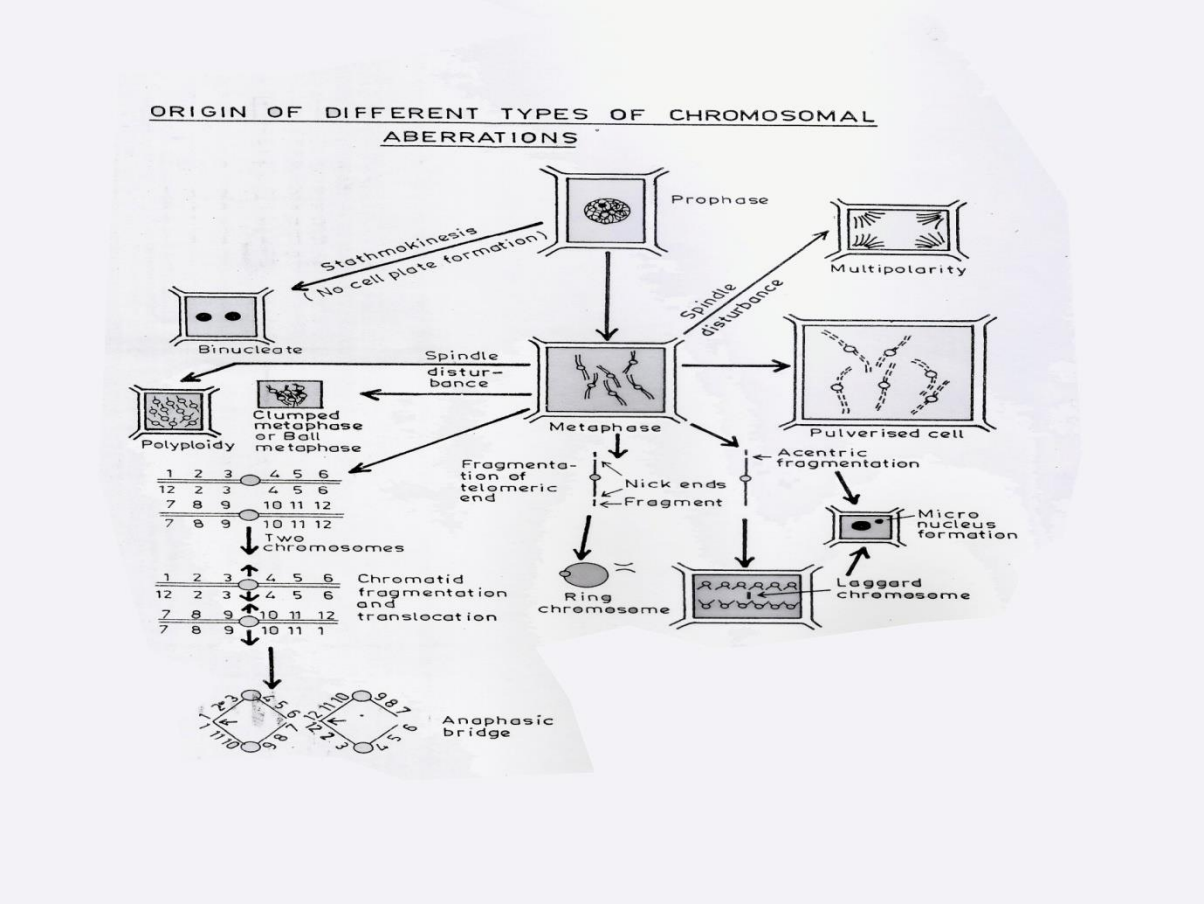
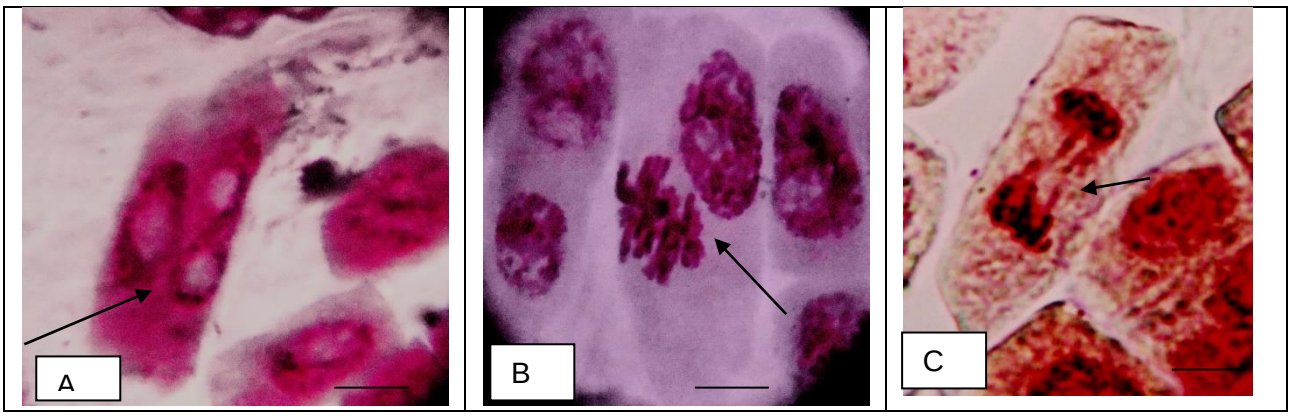


Fig 2: Origin of different types of chromosomal aberrations in Lathyrus sativus L.,

Lathyrus sativus Ana-telophase chromosomal aberration (CAs) and abnormal mitotic index (MIs) studies have been explored (Adhikari, 2019, Adhikari; Adhikari et al, 2020, Adhikari 2021) to evaluate different test substances, like heavy metals,(Pb, Cd, Cr, and Al), phytoextractsviz, Cascabellatheveita seed powder extract) CTSAE pretreatments (24h) and pomegranate peel powder aqueous extract and increasing salt concentrations to evaluate their cytogeotoxic potentials and mechanism of action fruitfully (Adhikari et al, 2024). The different types of cellular aberrations and possible outcomes out of toxic effects of these heavy metals and phytodecoctionsin pictorial forms are described below with suitable naming.

Cytological assessments of different types of Chromosomal aberrations in *Lathyrus sativus* L.,



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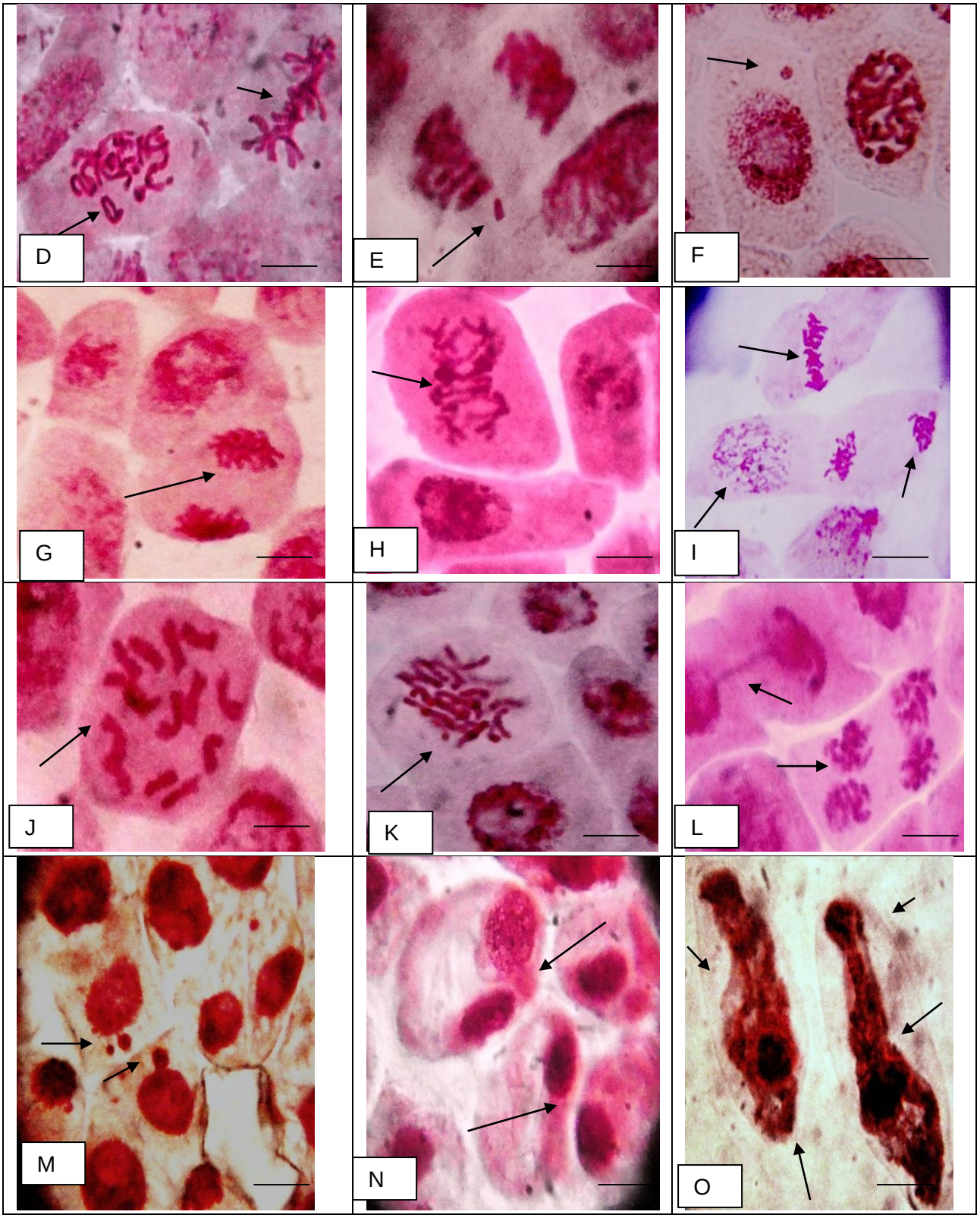

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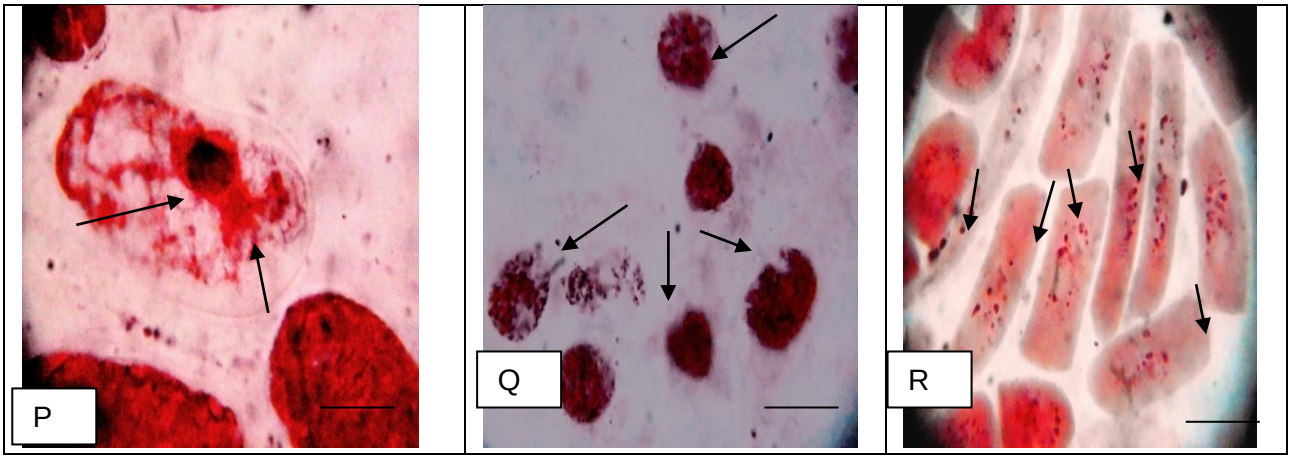

Figure 3 (A-R): showing the cytogenotoxic effects of CTSAE pretreatments (24h) *Lathyrus sativus* L. germinating root tip cells with increasing concentrations (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively). A= Binucleate cells with vacuolation (5mg/ml), B= Megadikaryon with prophase and metaphase after 5 mg/ml pretreatment, C= Anaphasic Sticky Bridge after 5mg/ml pretreatment, D= Early ball metaphase with ring formation, pole to pole metaphase after 10 mg/ml pretreatment, E= Disturbed anaphase with arm breakage after 10 mg/ml pretreatment, F= Micronuclei at interphase and pulverized chromatin after 20 mg/ml pretreatment, G= micro and macrcell formation with stellate anaphase after 20 mg/ml pretreatment, H= equatorial anaphasic separation after 20 mg/ml pretreatment, I= Giant cells with metaphasichypercondensation; chromosomal erosions and stellate anaphase after 20 mg/ml pretreatment, J= c-Mitosis after 20 mg/ml pretreatment, K= Polyploidy after 20 mg/ml pretreatment, L= Interphasic nuclear bridge formation and "Somatic Tetrad" formation after 30 mg/ml pretreatment, M= nuclear budding at interphase nucleus after 30 mg/ml pretreatment, N= Giant cells with early symptoms of necrosis, nuclei migrated to corners with cytoplasmic disappearance after 40 mg/ml pretreatment, O= Giant cells with cytoplasmic shrinkage away from cell walls leading to apoptosis after 40 mg/ml pretreatment. P= Apoptotic cells completely disintegrated protoplasm with disappearing nucleus after 40 mg/ml pretreatment. Q= Interphase nuclei showing chromatinal erosion, karyorrhexis at onset of apoptosis after 40 mg/ml pretreatment. R= Complete karyolysis in all the cells after 40 mg/ml pretreatment. Bar (_____) = 10µ.

Assessment of cytotoxicity Based on the number of dividing cells in the cell cycle, the NA and CAare crucial endpoints for the evaluation of toxicity and is primarily used by researchers as a cytotoxicity indicator (Abdel Migid et al., 2007; Leme and Marin-Morales, 2008; Porras Torres et al., 2013). A cell division anomaly is indicated by a MI that is less than the control, which is required for proper cell division. A greater MI value is also a sign of aberrant growth, such as cell proliferation and uncontrollable growth relative to negative control. Thus, both the decline and the increase in MI are significant markers for the evaluation of cytoxicity of diffent ecological contaminants (Leme and Marin-Morales, 2009).

The impact of ambient chemicals on the biological system's DNA/protein production may be the cause of the inhibition of MI (Andrade et al., 2008; Chandra et al., 2005). According to Abdul Migid et al. (2007), a drop in MI below 22% compared to control has deadly effects on test organisms, although a drop below 50% (the cytotoxic limit value) typically has sublethal

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consequences. As a result, a reduction in MI indicates that root development inhibition is actually a measure of cell division inhibition (Marcano et al., 2004).

The decrease in MI due to the exposure of wastewater/any other physical or chemical agent indicates the presence of cytotoxic agent and can be used for the estimation of pollution level in the sample (Smaka-Kincl et al., 1996). Consequently, because Lathyrus sativus L. root is more sensitive to the harmful effects of physical or chemical agents different researchers have used the MI as a cytoxicity indicator in Lathyrus(Adhikari et al, 2021, Adhikari 2023, Adhikari, 2019, Ghosh et al, 2020; Samanta et al, 2015; Samanta et al, 2023).

A change in chromosomal number or structure is a characteristic of CA (Fernandes et al., 2007b). Prophase, anaphase, metaphase, and telophase are examples of cell stages that have been thoroughly researched in order to assess the structural anomalies caused by toxic agents (Fiskejo, 1985; Rank, 2003; Rank and Nielsen, 1993). According to Albertini et al. (2000), contact with physical and chemical polluting agents results in DNA breakage, suppression of DNA synthesis, and altered DNA replication, all of which contribute to the development of CA. Chromosome adherence, loss, breakage, bridge, irregular distribution, aberrant separation, stickiness, vagabond, rings, late separation, and unorientation are among the CA. Furthermore, chromatin degeneration, anaphase with multiple bridges, late anaphase stage with double bridge, disturbed telophase, bridge sticky metaphase, sticky prophase, polar slip, drifting away from the metaphase plate, disturbed telophase, bridge at telophase, and chromatin degeneration are among the other types of abnormalities associated with CA. (Abu and Ezeugwu, 2008; Gupta and Ahmad, 2012a). According to reports, metaphase containing sticky chromosomes appears abnormal, with a sticky "surface" that leads to chromosome aggregation. Such aberrations are indicative of chromatin toxicity, which typically results in irreversible cell death. All these CAs are well documented in *Lathyrus sativus* L., root tip bioassays with different reports (Adhikari et al, 2021, Adhikari 2023, Adhikari, 2019, Ghosh et al, 2020, Adhikari et al, 2024).

According to El-Ghamery et al. (2003), the development of hazardous agent complexes with phosphate groups in DNA, DNA condensation, or the creation of inter- and intra-chromatid crosslinks are the causes of chromosomal stickiness. Furthermore, the effect on microtubule assembly is suggested by the late segregation of chromosomes, C-metaphases, and multipolar anaphases. According to Jordan and Wilson (1998), microtubules play a crucial role in chromosome migration, cell shape, and the creation of the cell wall during the growth and mitosis cycle. Spindle poisoning, also known as changes and disruptions in microtubule dynamics, is the cause of late segregation and multipolar anaphases in the C-metaphase number of chromosomes (Andrade et al., 2012).

Since the C-metaphases completely deactivate the cell's mitotic spindle, they are indicative of aneugenic agents (Fiskejo, 1985). When there is no ordered equatorial plate, the spindles become inactive;it blocks the centromere division (Leme et al., 2008). While the induction of MN is the most common outcome, multinuclear cells can arise from the presence of C-metaphases, according to Fernandes et al., 2007b and Kirch-Volders et al., 2002. According to some authors, MN can also be caused by chromosomal breaks, losses, and excess materials that are stimulated by DNA replication. These materials can be removed from the cell by creating mini cells, which are tiny cytoplasmic sections with a lower percentage of nuclear material (Fernandes et al., 2007b; Leme et al., 2008) and as a result, nuclear anomalies including nuclear buds, MN, tiny cells, lobated nuclei, and polinucleated cells generate CA types (Fernandes et al., 2007b).

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According to reports, the creation of DNA–DNA and DNA–protein cross-links causes aberrations in the CA, such as breaks and fragments. Heavy metals are thought to be a major contribution in this regard (Chandra et al., 2005). Both aneugenic and clastogenic processes are involved in the pathophysiology of CA. Aneugenic action involves the inactivation of a cell structure, such as the mitotic spindle, resulting in chromosomal losses, whereas clastogenic action is defined by the triggering of chromosomal breakage during cell division (Fenech, 2002; Leme & Marin-Morales, 2009). Chromosome adherence is another typical indicator of harmful effects on the genetic material. It can result in chromosomal breaks and chromosomal bridges, which can have an irreversible effect on the cell and cause the cell death process (Fiskesjo, 1993; Turkoglu, 2007). According to Leme and Marin-Morales (2008), adhesion can proliferate and last until the telophase stage, which is when chromosomal bridges are formed.

Additionally, chromosomal adhesion contributes to the development of chromosomal bridges, which in turn cause chromosomal breakdowns (Marcano et al., 2004). A malfunction of the mitotic spindle causes an uneven distribution of chromosomes, directing them toward more than two poles, in contrast to the normal division cycle, which is the cause of multipolar anaphases (Rank and Nielsen, 1998). It is not an easy task to evaluate the CA using Lathyrus sativus L.; this is because, similar to the Allium system, it requires accurate, appropriate, and precise knowledge of cell division stages and their potential anomalies (Leme & Marin-Morales, 2009).

Genotoixicty and Mutagenicity (MN) evaluation in Lathyrus sativus L., :

Beginning at the start of the current decade, the toxicity evaluation was based on the numerical aberrations (NA) endpoint, which is defined by morphological abnormalities in nuclei during cell division. Bi-nucleated, multi-nucleated, lobulated, nuclei carrying nuclear buds, mini-cells, vacuolated nuclei, nuclei with lesions in the nuclear wall, and deformed nuclei are among the NA. The NA assessment is a sensitive technique that can identify any mutagen's harmful effects. (Abdel Migid et al., 2007; Abu and Ezeugwu, 2008; Abu and Mba, 2011; Akinsemolu et al., 2015; Leme and Marin- Morales, 2009). The presence of multipolarity during nuclear division does not appear to prevent the reorganization of the nuclear envelope and the membrane would follow the uneven distribution of the genetic material within the cell, resulting in the lobated nuclei and polynuclear cell. Multipolar anaphases with chromosomal bridges can cause the lobated nuclei. (Leme et al., 2008).

The triggering of the cell death process occurs when NA is present (Leme et al., 2008; Leme and Marin-Morales, 2008). Another type of NA that is regarded as one of the most common abnormalities is the nuclear bud. One well-known genotoxic modification is the nuclear bud, whose creation is linked to the beginning of the nuclear envelope formation before the chromosomes fully migrate to the opposite poles and are subsequently incorporated into the nuclei. When clastogenic substances prevent the correct reconstruction of chromatin in the nucleus, chromosomal breaks, bridges, and rearrangements may ensue, giving rise to the nuclear bud. According to Mazzeo et al. (2011a) and Shimizu et al. (2000), cellular processes that encourage the removal of the amplified genetic material may also be the cause of the nuclear buds. For the purpose of evaluating the genotoxicity of contaminants, some researchers have employed this end point (Asita and Makhalemele, 2009; Chaparro and Pires, 2015; Olorunfemi et al., 2014a; Phugare et al., 2010) in plant bioassays to elucidate the mechanism of actions of environmental pollutants.

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In contrast to the NA and CA endpoints, which are the products of damaged and improperly healed cells, the MN endpoint in the *Lathyrus sativus* L. test is straightforward and easy to assess. The descendant cell's nucleus is smaller than the parent cell's due to the MN defect. Given that *Lathyrus sativus* L., has asymmetric bimodal karyotype with big and few chromosomes that is homogenous in regard to chromosomal size, the MN size can be a useful metric to evaluate the clastogenic and aneugenic effects in this species. Large MN, therefore, would suggest an aneugenic effect as a result of chromosomal loss, whereas tiny MN may suggest a clastogenic action as a result of chromosome break.

To improve the analysis's accuracy and dependability, additional cytogenetic methods like chromosomal banding and in situ hybridization have to be used (Leme and Marin-Morales, 2008, 2009). Acentric fragments (clastogenic action), lagging chromosomes, spindle malfunctions, or malformations of the mitotic fuse (aneugenic action) are frequently the cause of MN abnormalities. These can lead to cell death due to primary gene deletion during telophase of mitotic cells. These findings have been reported by Andrade et al. (2008) and Sudhakar et al. (2001). The development of chromosomal fragments and MN cells is also linked to chromosome breakage (Fiskesjo, 1993). Moreover, spindle poisoning, an unusual condition, can also cause MN to occur which may also result from spindle poisoning which is an anomalous disjunction of chromosomes at anaphase stage (Chandra et al., 2005; Grover and Kaur, 1999) or by eliminating the amplified genetic material (Mazzeo et al., 2011a).

MN is thought to be an indicator of a real mutation effect (Abdel Migid et al., 2007). Generally speaking, the induction of MN in root meristems is the manifestation of chromosome breakage and disruption of the mitotic process due to spindle abnormalities (Grover and Kaur, 1999). According to Abdul Migid et al. (2007), the failure of cell plate formation or a disruption in cytokinesis may lead to the production of multinucleated cells. Due to multipolar anaphases, which may or may not be connected to chromosomal adhesion, lobate nuclei and polynucleated cells are the end product of CA, rendering the cells nonviable (Fernandes et al., 2007b). Nuclear buds are another source of micronucleated cells. These buds actively remove nuclear material from the nucleus during the during the S phase (synthesis phase- stage of cell cycle in which DNA is replicated) of the cell cycle (Shimizu et al., 1998).

On the basis of MN, the clastogenic/aneugenic effects of hazardous substances can be carefully assessed. According to reports, the MN obtained from clastogenic agents is smaller than the MN derived from toxic compounds' aneugenic effect (Leme and Marin-Morales, 2008). The nuclear buds can also be associated with the production of MN, which may then be removed from the cytoplasm as a micro cell. The nuclear buds are suggestive of a preliminary step of releasing the excess nuclear material (Fernandes et al., 2007b).

According to Mazzeo et al. (2011a), MN is caused by the induction of chromosomal breaks during cell division as well as the expulsion of excess genetic material by nuclear buds. This induction is correlated with the inhibitory action of topoisomerase II, an enzyme that reassembles DNA fragments during replication, leading to the clastogenic effect of toxic agents (Mondrala and Eastmond, 2010; Whysner et al., 2004). Furthermore, it is confirmed by Alberts as al., 2008 that topoisomerase II suppression causes the daughter chromosomes to become tangled and prevent them from separating after replication.

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Because additional material is created by amplification and removed from the nucleus as an MN, replication of damaged DNA may result in genetic material amplification if such damage is not repaired (Fernandes et al., 2007b; Mazzeo et al., 2011a).

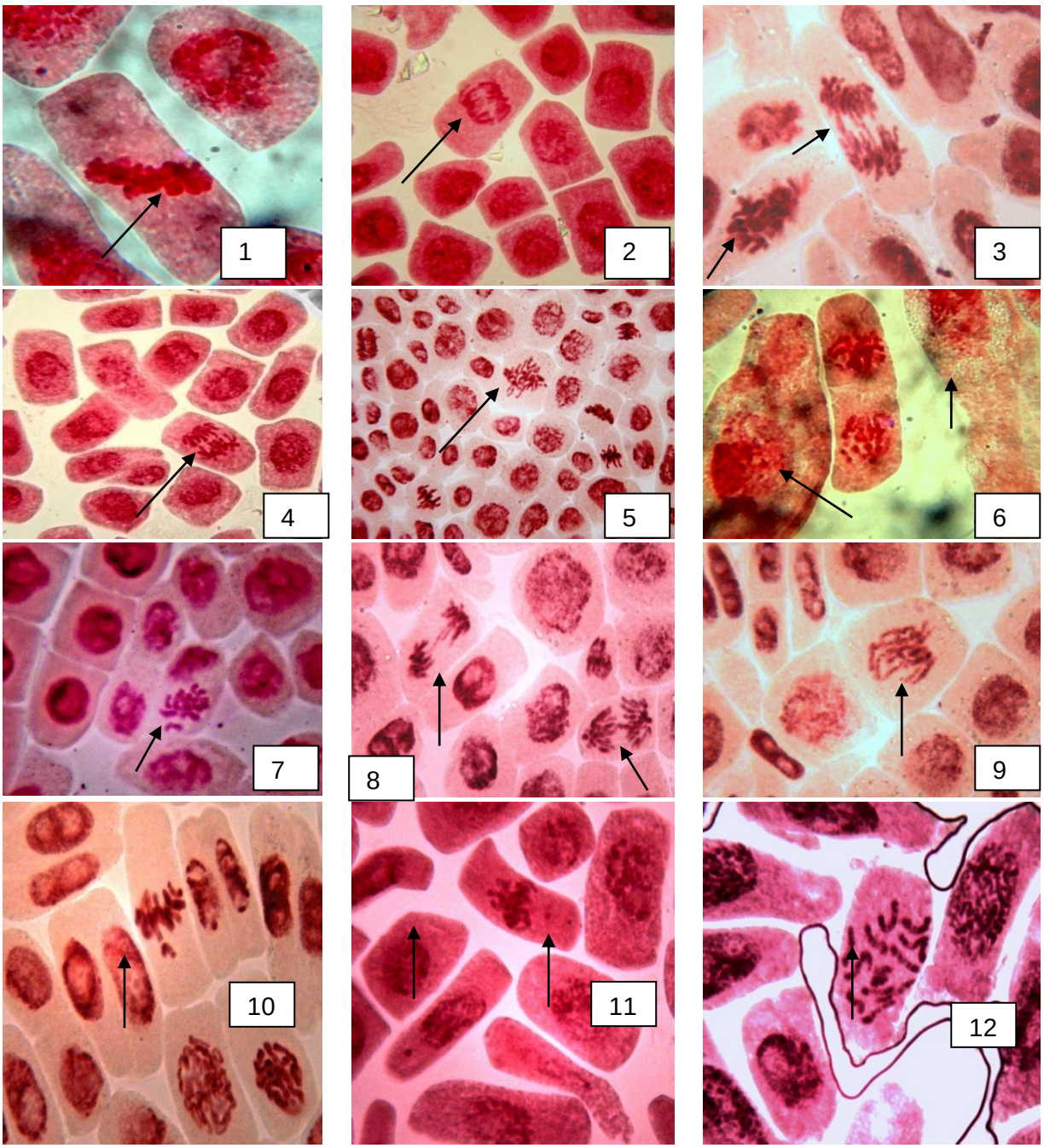

Fig 4: Effect of Lead on Lathyrus sativus L., chromosomes: fig 1: Metaphasic clumping and vacuolated nucleus, fig 2: Metaphasic Sticky bridge, fig 3: Anaphasic double bridge,fig 4: puffy anaphase , fig 5: Multipolarity with early fragmentationfig 6: Giant ghost cells with degrading chromosomes and disappearing nuclear membrane leading to apoptosis,Fig 7: stathmokinesis leading to polyploidzation fig 8: Telophasicsticky bridge fig 9: Pulverized chromosomes showing hetropycnosis, Fig 10: Early separation leading to laggard,Fig 11: Micronuceli,Fig 12: C-mitosis showing hetropycnotic chromosomes

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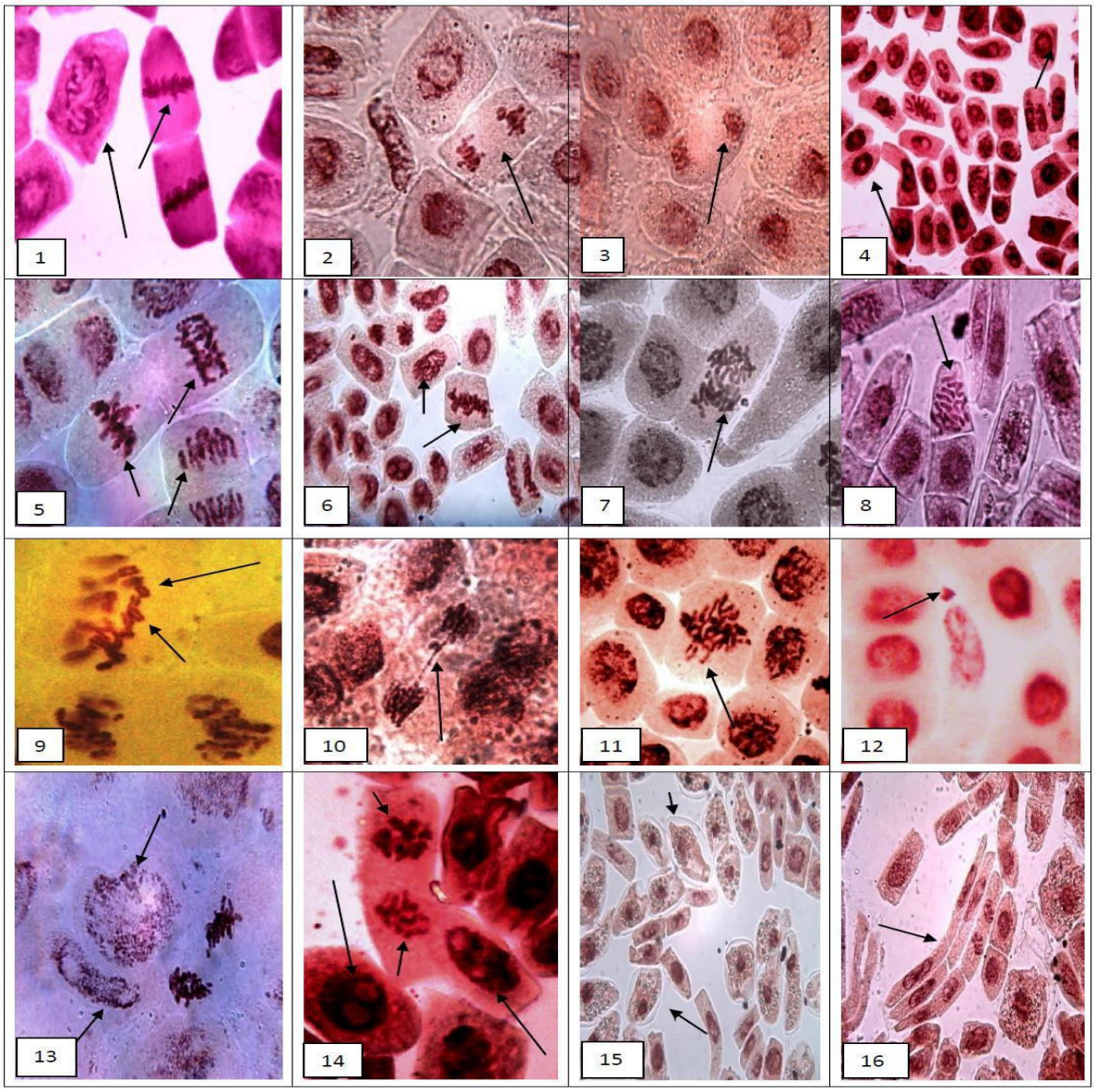

Fig. 5 Abnormal Cytology Pictures after treatment with different concentrations of copper salt at germinating root tips of *L. sativus* L after 72 hrs

1= Control (Prophase and Metaphase), 2= Control (Anaphase), 3= Control (Telophase), 4= nuclear blebbing and Nuclear lesion at 4 ppm, 5= Metaphasic clumping, acentric fragmentation and trophokinesis at 4 ppm, 6=Nuclear lesions, metaphasic stickiness, trophokinesis, and early condensation at 4 ppm treatments, 7= C-Mitosis and Polyploidization at 4 ppm treatment, 8= C-Mitosis at 6 ppm treatment, 9= Heteropycnosis and chromatid swelling (puffiness) at metaphase forming centric fission showing chromosome gaps at 6 ppm treatment, 10= Telophasic stickiness with early fragmentation at 6 ppm treatment, 11= Multipolarity and early separation at metaphase after 6 ppm treatment, 12= Micronuclei formation at 8 ppm treatment, 13= Pulverized nucleus with chromatin erosion at 8 ppm treatment in early apoptotic cells, 14= Binucleate condition with nuclear erosions and disrupted telophase without formation of nuclear membrane at 8 ppm treatment. 15= Necrotic cells with autophagy and degrading nucleus, cells are highly vacuolated with degrading protoplams and nuclear fragmentations at 8 and 10 ppm, 16= Giant Dead cells with nuclear lesion and erosion at 10 ppm treatment.

Higher plants, like *Lathyrus sativus* L.'s chromosomal characteristics (relatively big chromosomes, fewer in number with stable karyotype, 2n=14) would make it simple and repeatable to identify aberrant mitotic phases and chromosomal aberrations following genotoxic sensitization (Adhikari

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2019; Adhikari et al., 2020, Adhikari et al, 2024). In addition to ready frequency of MN as an easy cellular marker to assess ecotoxic actions of different classes of xenobiotics, cytogenotoxic end points would result in an increase or decrease in MI compared to control. These end points could be visualized by numerical scoring and visual microscopic accounting of structural and numerical changes in chromosome identified as CA. *Lathyrus sativus* L.'s chromosomal characteristics and biochemical machinery fulfil all the necessary crieterion that can effectively put forth thecytogenotoxicassoications and finer nuances of evaluation thus making it one of the most easy to handle, rugged and easy to produce reproducible results must make it fit in the accepted brand among cell biologists across globe.

Conclusions:

Based on available scientific reports, it can be concluded that various natural and artificial compounds have the potential to cause severe effects on the genetic material of *Lathyrus sativus* L. Somatic cells. These effects range from DNA damage to root inhibition, with chromosomal aberrations being the most commonly observed feature in the somatic cells located at the tips of the roots. It was also discovered that the Lathyrus sativus L test is sensitive and effective at identifying the harmful substances that are present in contaminated bodies and industrial effluents. As a result, *Lathyrus sativus* L. can also be used to assess the toxicity of industrial wastes and other nearby areas, with the results serving as a warning to biological and ecological systems. Additionally, the *Lathyrus sativus* L test needs to be standardized because its sensitivity may be impacted by variables such solvent extraction, sampling season, pH, temperature, and the exposure of *Lathyrus sativus* L seeds to background contaminants. Since all aspects of life are contaminated, it may be beneficial to establish global stock seed centres to produce and supply unexposed seed for experimentation. The results obtained in this way can be used as biomarkers to authenticate the toxicological status of industrial wastewater plus all forms of bio stimulantsvis-a-vis retardants affecting plant systems vs eukaryotic cellular toxicity. More and more researchers must employ this plant germination test in presence of pollutants to explore the morphological, cellular, enzymatic and molecular biomarkers to evaluate the mechanism of actions of these test chemicals in terms of minimal lethal dose vs cytotoxicity. Although Allium cepa and Allium sativum enjoy the most trusted plant model for genotoixicity study but Lathyrus sativus L., with its finer cellular attributes and positive response towards different allelochemicals and anthropogenic pollutants soon might get enlisted among the list of superior and sought after plant models in near future.

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CHAPTER-2
ROLE OF GUT MICROBIOTA AND PREBIOTICS

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Abstract

The search for novel substances with potential for technical uses and health benefits is currently gaining popularity. Prebiotics are beneficial substances that can be found naturally in several plant and animal food. Because of their beneficial effects on health, the food and pharmaceutical sectors have paid close attention to prebiotics. To meet the demands of the consumer market, strategies aimed at boosting prebiotic production must be implemented in response to the growing demand.The role of bacteria in the gut in health has been receiving more attention in recent times. Thus, therapies that have the ability to control the microbiota and its interactions with the host are becoming more and more popular. Food components with prebiotic properties include non-digestible carbohydrates like polysaccharides (resistant starch, pectin, and dextrin) and oligosaccharides (fructooligosaccharides, galactooligosaccharides, xylooligosacharides, isomaltooligosaccharides, mannanooligosaccharides, raffinose oligosaccharides, arabinoxylan-oligosaccharides, AXOS), lactulose, and others).The gut microbiota ferments these substances to create short chain fatty acids (SCFA), which include acetate, propionate, and butyrate. These substances can be used to produce a wide range of goods, such meat products, dairy products, sugary treats, baby formulae, whole wheat bread, cereal bars, and chocolate. Certain prebiotics are found in plants and animal products like honey in their natural state. Microorganisms and enzymes can be employed to synthesize prebiotic chemicals in addition to natural sources.

Keywords: Human gut microbe, Prebiotics, Dietary fibres,Galactocidase,Bifidobacteria

Introduction:

Prebiotics are short-chain carbohydrates that specifically increase the activity of specific types of good bacteria while remaining indigestible to human digestive enzymes. Beneficial bacteria in the gut digest prebiotics to create short-chain fatty acids. In the large intestine, prebiotics provide numerous additional health advantages, including a lower risk of cancer and improved absorption of calcium and magnesium. Prebiotics are regarded functional food ingredients that offer substantial technological benefits. They can be found in a variety of fruits and vegetables.When added to a wide range of food applications, including bread and dairy products, they increase mouth feel, flavour, and texture as well as the stability of foams and emulsions. This contribution examines prebiotic bioactive found in food sources. Furthermore covered are the uses of bioactive prebiotics in food, promoting probiotic viability, health advantages, epidemiological research, and prebiotic safety issues. [1]

According to Quigley, Hudson, and Englyst (1999), prebiotics are short-chain carbohydrates (SCCs) that are resistant to digestion by human digestive enzymes. These liquid polymers are known as non-digestible oligosaccharides (NDOs) when they dissolve in 80% ethanol. A prebiotic is a non-active food ingredient that travels to the colon where it undergoes targeted fermentation.

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The selective stimulation of the growth and/or activity of one or a small number of bacteria mediates the advantage to the host .Prebiotics and dietary fiber are defined similarly, with the exception of prebiotics' selection for different genus or types of native bacteria. Prebiotics travel from the small intestine to the lower gut, where probiotic bacteria can access them without the other intestinal bacteria being able to use them. Inulin and its hydrolysates, resistant starch, lutein, galactooligosaccharides, fructooligosaccharides, and maltooligosaccharides are examples of prebiotics that are typically found in nutrition. Short-chain fatty acids—acetic, propionic, and butyric acid in particular—are crucial end products of the metabolism of carbohydrates because they provide the host organism with energy. [1]

Additionally, they are present in a variety of foods, including tomatoes, bananas, artichokes, asparagus, onions, garlic, and many other plants. Generally speaking, oligosaccharides are mixes of sugars with varying levels of polymerization .Three alternative processes can be used to produce prebiotic oligosaccharides: enzymatic breakdown of polysaccharides, microbiological generation or enzymatic synthesis, and isolation from plant resource. Prebiotic oligosaccharides are primarily produced and sold in markets. Prebiotic oligosaccharide patents have been submitted in great numbers, and this number is still rising. [1]

Human gut microbiota:

There isa dynamic mutualistic relationships between the microbial species and the host, the human gut microbiota (HGM) is composed of microbial complexes that perform metabolic, immunologic, and protective functions, making it a highly active metabolic organ .Aspects of human health may therefore be impacted by changes in the composition of HGM. [2]

Internal and external factors, including genetics, host physiology (age, diseases, stress situations), and environmental factors (lifestyle, usage of drugs, pesticides, pollutants, nutrition, and pollution), can all affect the composition of HGM. According to Carlson, Erickson, Lloyd, and Slavin (2018), diet has been extensively researched and determined because the positive health impacts rely on how HGM metabolizes dietary components. Research on these food components has shown that prebiotic ingestion may have a favourable impact on the composition of HGM and metabolic processes in the colon and small intestine. [2]

What exactly these prebiotics are?

Prebiotics are described as "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2010. Improvements in gastrointestinal function and barrier homeostasis, increased mineral absorption, modification of energy metabolism and satiety, and decreased risk of intestinal infections are only a few of the health advantages of prebiotics metabolism by HGM.[2]

On the other hand, in addition to poor diet quality, the lack of dietary fibre and prebiotics in Western diets causes HGM diversity to be lost, which in turn causes a rise in chronic non-communicable diseases like obesity, heart disease, type 2 diabetes, and colon cancer. In the meantime, the consumption of diets rich in prebiotics increases the diversity of heterogeneous gut microbiota (HGM), which influences both microbial metabolic activities and the formation of

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determined fermentative end products. Examples of these include short-chain fatty acids (SCFAs), branched-chain fatty acids, organic acids, peptides, ammonia, amines, phenolic compounds, and gases. Certain metabolites produced by bacteria. As the most beneficial genera in human genetic modification (HGM), bifidobacteria and lactobacilli are classified as probiotics. As such, they have been employed as indicators of the impact of prebiotic supplementation studies. [2]

Difference between prebiotics and dietary fibres:

Prebiotics

Although they are not recognized, some writers classify dietary fibers as prebiotics. The distinction between the definitions of prebiotics and dietary fiber lies in the fact that the former are metabolized by particular genus or types of native microorganisms, while the latter are metabolized by most colonic microorganisms. Therefore, the terms should not be used interchangeably.[2]

Dietary fibers:-

The majority of nations have accepted the Codex Alimentarius' definition of dietary fiber, which comprises those polymers of edible carbohydrates that have three or more monomeric units and are resistant to the body's natural digestive enzymes while still being neither hydrolysed nor absorbed in the small intestine.By this definition, edible carbohydrate polymers from natural sources such fruits, vegetables, and cereals, as well as edible carbohydrate.[2]

Production of prebiotics:-

Prebiotics are essential to human health. Asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichokes, wheat, honey, bananas, barley, tomatoes, rye, soybeans, human and cow's milk, peas, beans, and, more recently, seaweeds and microalgae are among the dietary food products that naturally contain them. Owing to their low food concentration, they are produced on an industrial basis. Lactose, sucrose, and starch are used as basic materials to make several prebiotics. Numerous pertinent studies on the manufacture of prebiotics are available, as the majority of them are categorized as GOS and FOS on an industrial scale.[3-5]

Fructo-oligosaccharides(FOS):-

Approximately 36,000 plants contain fructose , however, these sources do not contain sufficient amounts of FOS to have prebiotic benefits. FOS should therefore be synthesized. Numerous publications have provided explanations of the various FOS production techniques .Glycosidase and glycosyl-transferase are enzymes that can be used to chemically produce fosfat. The concentration of the final product (FOS) is relatively low, and the chemicals used in these reactions are expensive and dangerous. As such, industrial production of it is not feasible. An essential enzyme in the synthesis of fructosyl-transferase (FOS) is FTase. One to three fructose molecules are transferred by FTase from sucrose to create FOS. Many microbes, including Arthrobacter sp., Fusarium sp., Aspergillus sp., Aureobasidium sp., Penicillium sp., and Aspergillus sp. [6-10]

The entire cell of a microorganism or a free enzyme can be utilized to produce FOS .Numerous variables can influence the amount of FOS that is generated. The starting sucrose content (theoretically between 55 and 60%) determines the maximum quantity of fructose that FTases can create. One of the fermentation byproducts, glucose, prevents trans-glycosylation .Thus, one of the most important steps in increasing FOS fermentation yields is eliminating glucose and sucrose

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residues. A few researchers reported using β-fructofuranosidase and glucose oxidase to increase the amount of FOS that was produced .FOS can be produced from sucrose by the enzyme β-fructofuranosidase. Glyconic acid is created when glucose oxidase breaks down the glucose generated during FOS fermentation. Ion-exchange resins or other methods can extract gluconic acid, unlike glucose. [7-19]

By breaking down tiny saccharides into carbon dioxide and ethanol, S. cerevisiae and Zymomonasmobilis are able to remove saccharides like glucose, fructose, and sucrose. Oligosaccharides containing four or more monosaccharide units are indigestible by S. cerevisiae. Z. mobilis ferments sucrose, producing minor amounts of sorbitol and FOS as well [20- 23].

Galactooligosaccharides(GOS):-

Originally created chemically through nucleophilic and electrophilic displacement, GOSs are now thought to be unfeasible to produce on an industrial scale . The two essential enzymes for the production of GOS are galactosidase and galactosyl-transferase. High concentrations of GOS can be produced by the stereoselective enzyme galactosyl-transferase . Nevertheless, because nucleotide sugars are required for this reaction as a donor, the bio-catalysis of GOS by galactosyl-transferase is extremely expensive. Using human milk oligosaccharides or producing globotriose are two methods to lower the cost of this process.[24-28]

Compared to galactosyl-transferases, galactosidase is far less expensive in the formation of GOS. Galactosidase, on the other hand, is less stereospecific than galactosyl-transferase and generates GOS in smaller amounts. Galactosidase can produce more GOS in a number of ways, including: (i) raising the concentration of donors and acceptors in the reaction; (ii) lowering the reaction's water activity; (iii) shifting the reaction equilibrium to the direction of the end product by eliminating the product from the medium; and (iv) changing the synthesis conditions [24,29]

The entire cell or simply the free form of β-galactosidase can be employed for GOS bio-catalysis. There is also a recombinant version of this enzyme available. When the β-galactosidase isolation technique is not cost-effective, the entire cell is utilized. Because β-galactosidase employs metal ions as co-factors, using the entire cell is also considerably less expensive because of co-factors found naturally in the cell and cell membrane. However, this is not very important for GOS production.[30-32]

Certain by-products, such glucose and galactose, have no prebiotic properties and may even reduce the yield of GOS production. These by-products can be eliminated by other metabolic activities when the entire cell is utilized. For example, when cultivated on lactose medium for GOS production, Sirobasidium magnum, S. elviae, and Rasoponeminutause glucose as a carbon source. Another example is that glucose is used as a carbon source in yeast cells, while galactose can stimulate the production of β-galactosidase. However, using living whole cells can result in the synthesis of various metabolic end products, such as ethanol, lactic acid, and acetic acid, which can influence the generation of GOS. [33-38]

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Thus, in order to eliminate these metabolic products, alternative techniques are needed. When employing the entire cell, temperature is another adverse factor in addition to metabolic end products interfering with the creation of GOS. The yield of GOS production is frequently increased by temperature, which is undesirable and can even be lethal for non-thermophilic cells. Non-viable and resting cells are taken advantage of in some research. The GOS production yields of these cells are significantly larger and they lack the disadvantages of live cells [21, 39].

Recombinant β-galactosidases have more advantages than native β-galactosidases, such as high production yield, easy purification, and improved enzyme stability, as well as an activity through molecular approach. *Escherichia coli* and *Bacillus subtilis* are mostly used for producing recombinant β-galactosidases. *E. coli* has some disadvantages, such as endotoxins production, difficulty in disulfide bonds expression, and acetate formation, which has toxic effects. In contrast, the engineered *B. subtilis* does not produce any endo- or exo-toxins. But this bacterium has also some disadvantages, including producing proteases in high quantities (which are able to degrade proteins) and plasmid instability [40-43].
Recombinant versions of β-galactosidase have been produced by some yeasts, including Pichiapastoris and S. cerevisiae. Compared to bacteria, yeast has a few benefits, such as (i) a wider productivity range, (ii) the ability to form disulfide bonds, and (iii) superior protein folding.[42-45]

HEALTH IMPACTS OF PREBIOTICS:-

1. Influence on the Hindgut bacteria:-

These bacterial species have numerous health-promoting characteristics, which make them popular candidates for dietary stimulation and widely used indicators of the health of the microbiota. GI tract mucosal inflammation has been demonstrated to be down regulated by lactobacilli. In addition to helping those who are lactose intolerant digest lactose, lactobacilli can potentially prevent traveler'sdiarrhea, ease constipation, and improve symptoms of irritable bowel syndrome (IBS). Due to their great inclination to ferment specific oligosaccharides, bifidobacteria are found naturally in the GI tracts of healthy adult humans and are frequently used as a diagnostic tool for prebiotic capability. Saccharolytic bacteria, which are frequently utilized as markers for good bacteria, are similar to Lactobacillus [46- 48]

Additionally, no known carcinogenic chemicals are produced in vivo by bifidobacteria. Obesity and weight increase have been found to be inversely correlated with Bifidobacteria concentrations [49-51].Since not all Bifidobacteria species have the same level of impact, certain species may be crucial to this connection .Lower bacterial diversity and Bifidobacteria levels have been linked to increased inflammation and IBS.Although the exact mechanisms underlying illness states and Bifidobacteria and Lactobacilli are unknown, a sufficient body of research indicates that these bacteria are strongly linked to health.[52-55]

2.Metabolite production:

There are numerous health advantages for humans associated with the formation of primary and secondary metabolites that result from the direct or indirect fermentation of specific substances. Because of the fermentation of nutrients such as amino acids, carbohydrates, and other unabsorbed substances in the proximal small intestine, the gut microbiota produces short-chain fatty acids (6

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C). Of all the SCFAs produced in the colon, 90–95% are composed of butyrate, propionate, and acetate. More than half of the SCFAs found in human feces are similar to acetate and muscles prefer to metabolize acetate as a source of energy. Butyrate and propionate have been found to have a negative correlation with ulcerative colitis and other GI illnesses caused by inflammatory response pathways. Responses vary widely, despite the fact that SCFA production has several favorable effects. [56-58]

Clinical studies have demonstrated an increase in urine hippurate concentrations following the fermentation of inulin-type fructans. Hippurate is a microbial co-metabolite of mammals that has been detected in lower concentrations in fat than lean people and in diabetics versus non-diabetics .Because inulin ferments, higher quantities of hippurate in the urine are thought to be a good thing .[59-62].

3.Effect on mineral absorption:-

Reducing the risk of osteoporosis and bone fractures is a major concern globally. In the US, over 28 million people have low bone mass or osteoporosis, and 1 in 8 people over 50 break their spine annually in the EU. A crucial goal for maintaining healthy bone structure in adolescent and elderly populations is to increase the bioavailability and absorption of calcium through the consumption of prebiotics. One of the main places where calcium is absorbed is the distal intestine, and the chemical modifications and increased acid fermentation of the prebiotic dietary fibers by different bacteria promote absorption. [63, 64]

Mixed results have been reported from clinical investigations assessing mineral absorption in different groups. Four investigations have demonstrated that when participants took 1–17 g/d of inulin, oligofructose, galactooligosaccharides (GOSs), and short-chain FOSs, there was no significant effect on calcium absorption [65-68]. Significant increases in calcium absorption were observed in six clinical investigations using identical dosages (8–40 g/d) and the same therapies in addition to a lactulose treatment. [69- 74]

The age and physiology of study participants could affect the results, as individuals going through puberty and after menopause may have a higher affinity and desire for calcium uptake. It may also depend on the microbiota variety and development of the participants. Numerous evaluations of research on humans and animals and how they affect bone structure have been done [75].

4.Changes in populations of harmful bacteria:-

Inhibiting pathogens like E. coli, Salmonella spp., Campylobacter, and other harmful bacteria from entering the GI tract is largely dependent on the gut mucosa and microbiota. There are five possible mechanisms that could be involved: the production of inhibitory peptides by lactic acid bacteria, competitive effects resulting from a shortage of colonization sites, lowering the colonic pH below thresholds for pathogenic bacteria, competition for scarce nutrients, and immune system stimulation .[76,77]

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5. Influence on the defense of the immune system:-

The host's gastrointestinal tract contains a wide variety of cells that are essential for immune system response and communication. Prebiotics and the fermented metabolites they produce have an impact on natural killer cells, effector T cells, B cells, and TREG cells .It's unclear exactly how prebiotic fermentation produces metabolites like SCFAs, but one possibility is that these are what affect the immune system. Macrophages, T cells, and dendritic cells have all been demonstrated to be impacted by butyrate [78]

Conclusion:-

Prebiotics are well known for being able to support a healthy gastrointestinal system. Prebiotics offer everything from pathogen inhibition to intestinal native bacterial multiplication. New uses for prebiotics are being developed daily as a result of the growing body of research on their benefits, improving the options available to consumers for leading healthy lives. Prebiotics are a novel approach to substitute fat and carbohydrates in a variety of food industry products, while also improving mouth feel by improving tongue lubrication. Sometimes a product's greater heat stability comes with additional sensory, textural, and physiological advantages.

Prebiotic consumption is essential for regulating the gut microbiota, producing more short-chain fatty acids (SCFA), and lowering the risk of developing a number of illnesses, including type 2 diabetes mellitus, obesity, irritable bowel syndrome, and colon cancer. By using these substances, food's sensory, physical-chemical, and nutritional qualities can all be enhanced. Enzymes and microorganisms can synthesize non-digestible carbohydrates, which is one way to expand the range and accessibility of these components in the market.

Prebiotics are among the most commonly utilized drugs to preserve a well-balanced micro biome or to restore it when bacterial homeostasis is compromised.

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CHAPTER - 3
IMPACTOFHEAVY METALS ACCUMULATIONDEPOSITSON GREEN VEGETATION – A REVIEW

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Abstract

Heavy metals are one of the major pollutant wastes that poses a major challenges to green vegetation as well on animals also. They metals are highly toxic to plants and living life. Heavy metals play a vital role in nature as they are essential for the plant's normal growth. These heavy metals are also involved in redox reactions, transferring electrons, basic functions in nucleic acid metabolisms, and being an integral part of several enzymes as a direct participant. The availability at a certain concentration of these essential metals in growing medium is very important, but their excess concentration results in several toxic effects. Therefore, the present book chapter comprised the information for better understanding of heavy metal toxicity and their accumulation mechanism by the plants.

Keywords: Heavy metals, Toxic effects, Stress tolerance, redox reactions etc

1. Introduction

“Heavy metals” are natural elements characterized by their rather high atomic mass and their high density. Although typically occurring in rather low concentration, they can be found all through the crust of our planet. Commonly, a density of at least 5 g cm−3 is used to define a heavy metal and to differentiate it from other, “light” metals. Other, broader definitions for “heavy metals” require an atomic mass higher than 23 or an atomic number exceeding 20; these definitions are highly error prone and confusing. Both alternative definitions cause the inclusion even of nonmetals; resorting to the atomic mass criterion, the maximum number of elements classified as “heavy metals” rockets high to 99 out of the in total 118 building blocks of our universe. Looking at the periodic table of elements, we learn that heavy metals sensustricto (according to the density criterion) occupy the lion’s share, namely, columns 3–16, of the periods 4 to 6, encompassing the transition metals, post-transition metals, and lanthanides [1].

Some heavy metals like copper, selenium, or zinc are essential trace elements, with functions indispensible for various biological processes also driving the entire human metabolism [2]. The heavy metal cobalt, acting as the central atom in the vitamin B12 complex, is a key player in the reductive branch of the propionic acid fermentation pathway [3]; without this special heavy metal compound, the gourmet would have to do without the unique flavour of Emmentaler cheese. Many heavy metals are of outstanding technological significance, e.g., iron, zinc, tin, lead, copper, tungsten, etc. Recently, different heavy metals act as the central atom of artificially designed “bioinorganic” catalysts for special chemical transformations [4]. Moreover, among them we find precious noble elements like gold, silver, iridium, rhodium, or platinum [5]. On the other hand, many of them, e.g., mercury, cadmium, arsenic, chromium, thallium, lead, and others, classically represent the “dark side of chemistry”; they exert toxic effects already at low concentration [6]. In

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this context, some heavy metals have gained dubious popularity by being the materials major crimes can be made of [7].

Heavy metals were literally heaven's sent by originating from asteroid impacts. Typically, heavy metals occur in the earth's crust in rather low concentrations between the low ppb ranges (noble metals) and up to 5% (iron); here, heavy metals are mainly found chemically bound in carbonate, sulfate, oxide, or silicate rocks or also occur in their metallic, elemental form. Weathering and erosion resulted in their leaching and mitigation into soil, rivers, and groundwater. About 4–5 billions of years ago, when Earth's mantle was still liquid, heavy metals sank to Earth's center and formed Earth's core, which today predominately consists of the heavy metals iron and nickel [1].

2. Heavy metals and the eco- and biosphere

It is important to emphasize that there are some trace elements among the heavy metal family, which are essential for many biological processes; they are predominantly found in period 4 of the periodic table of elements. For strict aerobic beings as we are, it would not be possible to survive without having cytochromes, which make aerobic life forms breath since the very beginning [8]. Iron also plays a major role in our respiration system as central, oxygen-affine, atom of the blood pigment heme. Copper plays a similar role in the transport of electrons and oxygen, especially as central atom in hemocyanin in mollusks and arthropods [9]. Zinc in turn is pivotal as constituent of zinc finger enzymes [10]. Selenium is described as an antioxidant; further, it is involved in hormone biosynthesis [11]. Cobalt was found to be significant in biosynthesis of complex compounds and different steps in cellular metabolism, especially as central atom in vitamin B12, which is needed for cell division, blood formation, the nervous system, and in propionic acid biosynthesis (vide supra) [12]. Moreover, vanadium and manganese are important for regulation and functioning of several enzymes [13], whereas some metabolic functions are also assigned to the typically known-as-toxic elements chromium, arsenic, and nickel. Regarding arsenic, this element was only recently revealed as a natural constituent in herring caviar, where it was shown to substitute phosphorus in phosphatidylcholine-like lipids, the so-called arsenolipids [14]. Further, we should mention the role of molybdenum in some redox reactions [15], in addition to the function of cadmium in the metabolism of some microalgae from the Diatomophyceae class [16]. Moreover, the role of the heavy metal tungsten in the metabolism of prokaryotes is scientifically confirmed [17].

To give an impression on the quantities of heavy metals present in our human body, hence, the "heavy metal load" we are steadily carrying along with us, we can estimate that only about 0.01% of our mass originates from the presence of heavy metals, with iron (about 5 g in a person weighing 70 kg), zinc (2 g), lead, and copper (0.1–0.2 g each) being the top four heavy metals in our body; the rest, from the mass-related perspective, can be considered negligible [18].

Besides the abovementioned leaching and mitigation of heavy metals by erosion and weathering, these elements are mainly mobilized of by the action of humans during their physical (extraction, smelting) or chemical (reductive) release from ores and the subsequent processing for diverse applications. Other processes releasing heavy metals into the ecosphere involve their (agro)industrial, domestic, automotive, medical, electrical, and other technological use, resulting in their extensive distribution in both aquatic and terrestrial environments.

Currently, we witness increasing global worries regarding their possible adverse health effect and their negative enduring impacts on biosystems. Some heavy metals are reported or at least

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suspected to be carcinogenic (hexavalent chromium, arsenic, cobalt, nickel, antimony, vanadium, mercury), mutagenic (arsenic, vanadium), teratogenic (arsenic), allergenic (nickel), or endocrine-disrupting (silver, copper, zinc, selenium). Others (e.g., thallium) lead to neurological and behavioral alterations, particularly in the case of children, central nervous system damage (mercury, lead, thallium, manganese, and tin), bone marrow damage, and osteoporosis (cadmium); are hepatotoxic and/or nephrotoxic (cadmium, cadmium, mercury, heptavalent manganese); cause heart rhythm disturbances (thallium); or negatively affect the immune system (lead) [19].

3. Determining heavy metals

Classically, quantification of heavy metals involves well-established techniques, such as wet chemical methods (gravimetric, titrimetric, colorimetric, etc.), coupled plasma/atomic emission spectrometry (ICP/AES), inductively coupled plasma with mass spectrometric detection (ICP/MS), or atomic absorption spectroscopy (AAS) [20, 21, 22]. Moreover, diverse ion selective electrodes are frequently reported for heavy metal determination [22, 23]. Currently, new, robust, sensitive, selective, inexpensive, and fast optical [24, 25, 26], chemical [27, 28], and biological [29, 30, 31, 32, 33] sensory systems are currently in status of development. Such advances in analytical chemistry are currently tightly connected to nanotechnology [26, 28, 34]. Moreover, so-called lab-on-paper sensors were also developed for heavy metal determination, as demonstrated for quantification of mercury, silver, copper, cadmium, lead, chromium, and nickel [29]. This sensor, operated by an immobilized enzyme, is an example for so-called biosensors, which synergistically combine the scientific fields of biotechnology and microelectronics; such “biosensors” consist of an immobilized biological component in combination with a transducer [30]. As a very recent technology, so-called genetically encoded fluorescent sensors can be used for monitoring heavy metals inside biological cells and were already assessed for determination of heavy metals like zinc, copper, lead, cadmium, mercury, or arsenic [35].

Some of the major heavy metals absorbed by different plants initiated the different roles in plant metabolism. These are as described under the following headings.

Aluminum (Al) :

Aluminum is the third richest element in the crust of the Earth, occurring at around 8%. It has some useful features that allow us to use it in various industries such as electrical, metallurgical, transportation, packaging, and chemical manufacturing. The various aluminum residences are often used in the manufacture of paper, sugar refining, water purification, wood preservation, leather tanning and textiles for water resistance (Kumar and Aery, 2016). As insoluble aluminosilicates and oxides, Al is present in the soil. In the initial phases of plant growth, it is easily accumulated by plant roots and translocated within the plant, but then drops sharply with advancing maturity. The concentration of other elements (P and Ca) in the rhizosphere affects the accumulation of Al.

Aluminum in the soil can inhibit the growth of plants at a level as low as 1 mg/Kg (Rana and Aery, 2000). The root tip, which turns brown, shows the earliest symptoms of Al toxicity. The damage is limited to the root tip's active growth of tissues. In particular, the distal part of the root apex transition zone is highly sensitive to Al toxicity (Kumar and Aery, 2016). This extensive damage to the root structure leads to the reduced and damaged root system and the absorption of limited water and mineral elements.

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Cadmium (Cd) :

The usual Cd content is 0.1 and 0.41 mg/Kg, respectively, in the Earth's crust. Most cadmium is used in the production of batteries (Ni-Cd and Ag-Cd). It is also used in relatively large quantities as pigments (yellow), coatings and stabilizers.Excess Cd in growing soil may cause leaf chlorosis, but it may be due to iron deficiency and toxic metal interaction. Due to direct or indirect interaction with Fe in leaves, chlorosis may appear.

Cobalt (Co) :

Cobalt has cobaltite, smaltite, and erythritis in the minerals. Like many other metals, Co-contaminated soil pollution is largely due to mining and smelting, sewage sludge dispersal, and fertilizer use, which can pose an environmental risk (Bakkaus*et al*., 2005).

Some studies found that when given in high doses, Co is relatively toxic to plants. Cobalt uptake and distribution in plants is dependent on species and controlled by various mechanisms. Root absorption of Co2+ involves active transportation across cell membranes, even though the molecular mechanisms remain unknown. Although low mobility of Co2+ in plants restricts its transportation from roots to shoots, its distribution may involve organic complexes (Lock *et al.*, 2007).

Chromium (Cr) :

Chromium (Cr) is Earth's 7th most abundant component and 21st in the crust, with an average concentration of 100 mg/Kk.Plants use inactive mechanisms to absorb chromium in its trivalent form, i.e. Cr(III), while Cr(VI) is inhibited by SO42− and Ca2+ (Vikram*et al*., 2011). Due to their high oxidation power, hexavalent ions, i.e. Cr(VI), damage the root membranes. Cr enters plant roots through root exudates reduction and/or complexation, enhancing solubility and mobility through root xylem (Shanker*et al*., 2005). However, Cr's accumulation and mobilization within the storage tissue depends on its ionic state, it accumulates mainly in roots and is poorly translocate to shoots.

Iron (Fe) :

Iron plays an important role in animals, plants and as well as microbes. Plants mostly get Fe from the plant's rhizospheric zone.Iron plays an important role in many plant forms of physiology and biochemistry. It assists as a component of many vital enzymes such as the electron transport chain's cytochromes and is therefore essential for a wide range of biological activity. Iron is involved in chlorophyll synthesis in plants and is vital for maintaining the construction and function of chloroplast (Rout and Sahoo, 2015). Fe is accumulated primarily by plants, solubilizing Fe3+ and then reducing it to Fe2+ for absorption or root transport. Fe is transferred as ferric citrate or iron(III) chelate form from roots to shoots and transported to active growing shooting regions. Iron is a crucial mineral for plants that is essential for the biological redox system, and it is also a vital component of numerous enzymes that play significant roles in plant physiology and biochemistry. It acts as a cofactor of key enzymes involved in plant hormone synthesis and is involved in many reactions to electron transfer (Jeong*et al.*, 2008).

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Manganese (Mn) :

Mn is a common metal in the crust of the earth and its occurrence in soils is primarily the result of the parent material. In recent times, however, the severe anthropogenic has focused on increasing manganese content and obtainability in many soils.

Mn is a vigorous plant component that interferes in several metabolic activities, mostly in photosynthesis and as an antioxidant-cofactor enzyme. Several studies on the toxicity of manganese and translocation of Mn from soil to plant tissue in the form of Mn2+ have confirmed their significance under low pH and redox soil conditions. Mechanisms that can tolerate this toxicity are also recorded when Mn metal is inside the plant cell, making it vital to compartmentalize this metal in different plant tissue (leaves, roots, shoots, and leaf plant cells) (Millaleo*et al*., 2010).

Molybdenum (Mo):

Mo is present in the lithosphere at an average concentration of up to 23 mg/Kg but may increase concentration (300 mg/Kg) in shales containing important organic matter. Mo is present as numerous different complexes in agricultural soils depending on the soil section's chemical speciation (Kaiser et al., 2005). The strong relationship between Mo and Fe metabolisms is presumed because I the absorption mechanisms for Mo and Fe affect each other, (ii) the majority of molybdoenzymes also require the-containing organic reductions or organic oxidation groups such as the-sulfur groups or heme, (iii) Mo metabolism has enrolled mechanisms typical of iron-sulfur cluster synthesis, and (iv) both Mo cofactor synthesis and extra synthesis. Tomatsu et al. (2007); Bittner et al. (2014) the studied that Mo present in the soil are many forms in the soil such as molybdenite (MoS2) or ferrimolybdite [Fe2(MoO4) 3], and its dissolved and plant-available formmolybdate (MoO4 2 −).

4. Mitigating heavy metals :

To elevate the negative impacts of heavy metals, remediation techniques are increasingly improved in order to address the growing public pressure to reduce prevailing environmental hazards and to bequeath the subsequent generations a future worth living.

Traditional physical, thermal, chelating, and other chemical techniques often display serious shortcomings such as too high cost, excessive expenditure of work, and invasive change of soil properties and microflora [36]. Traditionally, remediation of soils contaminated by heavy metals resorts to simply digging the contaminated soil and subsequently disposing it at landfills. Of course, this disposal strategy merely postpones the eco-problem by shifting it from one location to the next and, moreover, generates hazards connected with transportation of precarious soil and leaching of heavy metals at the ultimate disposal site. In the case of water polluted by heavy metals, alkaline lime precipitation is known as a better advanced and maybe the most efficient traditional technique for treating heavily polluted effluents. Lime precipitation can effectively be used to treat wastewater with metal loads exceeding 1 g L−1. However, the remaining heavy metal-alkali-sludge stills need ultimate disposal [37].

Modern physical and chemical approaches for remediation of heavy metal pollution involve the use of adsorption on new adsorbents such as nano-carriers, ion exchange techniques, removal via advanced membrane filtration techniques, electrodialysis, or photocatalysis. Among these novel physicochemical techniques, new adsorption- and membrane filtration-based methods are most thoroughly investigated and are most commonly applied to treat contaminated wastewater [37, 38].

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Among new absorbents, both inorganic (kaolinite, montmorillonite) [39] and organic materials (e.g., agricultural waste or bio-char) [40, 41, 42, 43] were studied for heavy metal recovery. In this context, the application of carbon-, metal-, or metal oxide-based nanoparticles as adsorbents benefits from high surfaces susceptible toward metal adsorption and expedient reactivity. Here, the mechanisms of interactions of nanomaterials with, on the one hand, heavy metals and, on the other hand, heavy metal with additional wastewater constituents with metal-binding groups need to be understood in order to optimize the recovery processes [44].

Photocatalysis uses photons from the UV-near vis region of light's electromagnetic spectrum and, when operated in a smart way, is able to degrade toxic organic pollutants in parallel to metal recovery in just one single process step. This technique resorts to photocatalytic semiconductors, e.g., TiO2, which, when illuminated with UV light, generate highly reductive electrons that in turn reduce heavy metal ions in contaminated wastewater. As an example, photocatalysis was successfully implemented for reduction of the dramatically precarious hexavalent chromium to its about 500 times less toxic trivalent form. In the case of precious noble metals like gold, this process does not only mitigate an environmental pollutant but also mines value-added materials for further use ("photorecovery") [45].

Phytoremediation is an emerging technology to overcome shortcomings of above-discussed methods. During phytoremediation, plants act synergistically with diverse soil microbes, which convert the heavy metals in a form bioavailable for the plants, finally decreasing the concentrations of contaminants in affected environments. Phytoremediation resorts to the ability of many plants for specific and efficient uptake, translocation, and storage of hazardous elements with chemical properties mimicking those of elements essential for plant growth [46]. Being a relatively recent technology, phytoremediation is supposed to be efficient, cost-effective, and ecologically benign; is driven by sunlight as the sole energy source; and enjoys an excellent public acceptance [47]. In the context of heavy metals, new powerful "heavy metal hyperaccumulator" plants are currently assessed for both phytoremediation (getting rid of the unwanted heavy metal) and phytomining (accumulating the precious heavy metals for further use). Such "hyperaccumulators" are characterized by their capacity to take up toxic metal ions at levels of thousands of ppm. In the optimum scenario, the toxic metals are transported from the plant's rhizosphere up to the shoots in the plant's periphery; now, the shoots, enriched with the target contaminants, can easily be harvested and burned for energy generation and, if economically reasonable, recycling the metal from remaining ash [48]. Using aquatic plants, phytoremediation can also be used to cure polluted water bodies. Various plant species have successfully performed in absorbing heavy metals such as arsenic, cadmium, chromium, lead, and even radionuclides from contaminated soil. Among the different phytoremediation categories, phytoextraction can be used to mitigate heavy metals from soil by profiting from its ability to uptake and accumulate those heavy metals, which constitute elements essential for plant development, such as iron, copper, manganese, molybdenum, or nickel. In addition to these essential elements, some chemically similar heavy metals with unknown or not yet confirmed biological function can also be phytoextracted, such as cadmium, chromium, silver, lead, cobalt, selenium, or mercury [49]. Excellent results for phytoremediation of arsenic were reported for the fern Pterisvittata L. species [50]; here, the uptake capacity of the plant yielded more than 4 g heavy metal per kg plant material [51]. In the case of lead, several plants, most of all different mustards (Brassica ssp.), are described to be able to accumulate between 50 and 100 mg of this heavy metal per gram plant dry mass [46].

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CHAPTER- 4
L-ASPARAGINASE- A BOON TO PHARMACEUTICALS

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ABSTRACT
L- Asparaginasehave attracted a lot of attention in the pharmaceutical market. The health benefits and medicinal application have been known since past. Its applications were limited due to accompanied negative impacts exerted on the patients after administration. The novel sources of the enzyme, genetic modification and research focused on limiting the side effect of the enzyme have again created a market for its production. The chapter deals with history, microbial sources, production, characteristics, advantages and limitations of Asparaginase.

Keywords:L-Asparaginase, tumor, Penicillin, *Pseudomonas*, *Rhodotorula*.

Introduction:
The discovery of Asparaginase and its effect in cancer was based on the observation when lymphomas in rat receded when treated with serum of guinea pig. Further research revealed that the actual factor which lead to regression of the tumor was not serum as a whole but a single component i.e. L-Asparginase. Since, this discovery in 1953, a lot of research was carried out decipher its structure, mechanism of action and scope of applicability (Zuo*et. al.*,2015; Faizan*et al.*, 2022)

Sources of Asparginase:
There are many sources of Asparginase in nature. The most popularly used source of Asparginasease*E.coli* and *Erwiniachrysanthemi* due to its high efficiency in the treatment and better results. The added advantages of the bacterial sources are they can produce enzyme in he large quantity which is industrially desirable and favourable (Shukla*et al.*, 2014)

Structure of L-Asparginase:
The enzyme is a tetramer protein. It contains a pair of dimers. Each dimer carries two active centers. There are four reaction centers and studies reveal that four molecules of L-Aspartate are bound to the enzyme. (Rani *et al.*, 2012) The structural studies reveal that the active centers essentially have 2 Threonine residues T-12 and T-89. Which are present in all members of this enzyme family and are proved to be indispensable for activity. The positions of these Threonine residues are in close approximately to the bound substrate. The mechanisms of action reveal that they act as a primary nucleophile reaction (Alam*et al.*, 2019)

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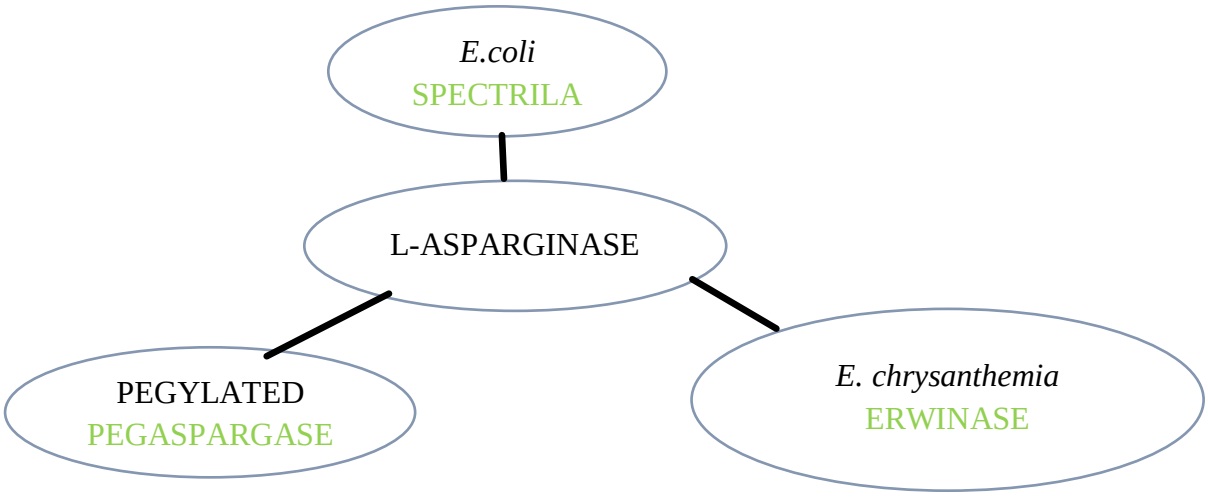

Figure-1: Classes of L-Asparginase on basis of sources

There are multiple microbial sources of L-Asparginase which have variation in properties and effectiveness. Table-1 elucidates the various organism studied for Asparginase production.

Table-1:Mcrobial Sources of L-Asparginase

CATEGORY	MICROBE	REFERENCES
MOLDS	1. Aspergillus	Mishra (2012); Qeshmi*etal*.(2018)
	2. Penecillium	Patro and Gupta (2012)
	3. Fusarium	Meghavarnam and Janakirman (2016)
YEASTS	1. S. Cerevisiae	Pradhan*et al*. (2013)
	2. Rhodotorula	Borah *et al*. (2012)
	3. Issatchenkia	Mu Borah *et al*. (2012)
	4. C.utilis	Muslin (2014)
BACTERIA	1. E.coli	Borah *et al*. (2012)
	2. Erwinia	Deokar*etal*. (2010)
	3. Pseudomonas	Husain *et al*. (2016)
	4. Serratia	Borah *et al*. (2012)
ACTINOMYCETES	1. Actinomycesmeyeri	Muslin (2014)
	2. Actinomycesisraelii	Muslin (2014)

Mechanism of l-asparginase as anticancer agent:

The studies on L-Asparginase from *E.coli* reveal that the enzyme acts upon the Aspargine in the cancerous cells by converting into L-Aspartic acid and ammonia. This degradation limits the DNA, RNA and protein synthesis which results in arrest of cell growth and finally diverts the cell to Apoptosis. Figure-2 gives on insight to the mechanism of action of L-Asparginase as anticancer agent (Benchamin*et al*., 2019)

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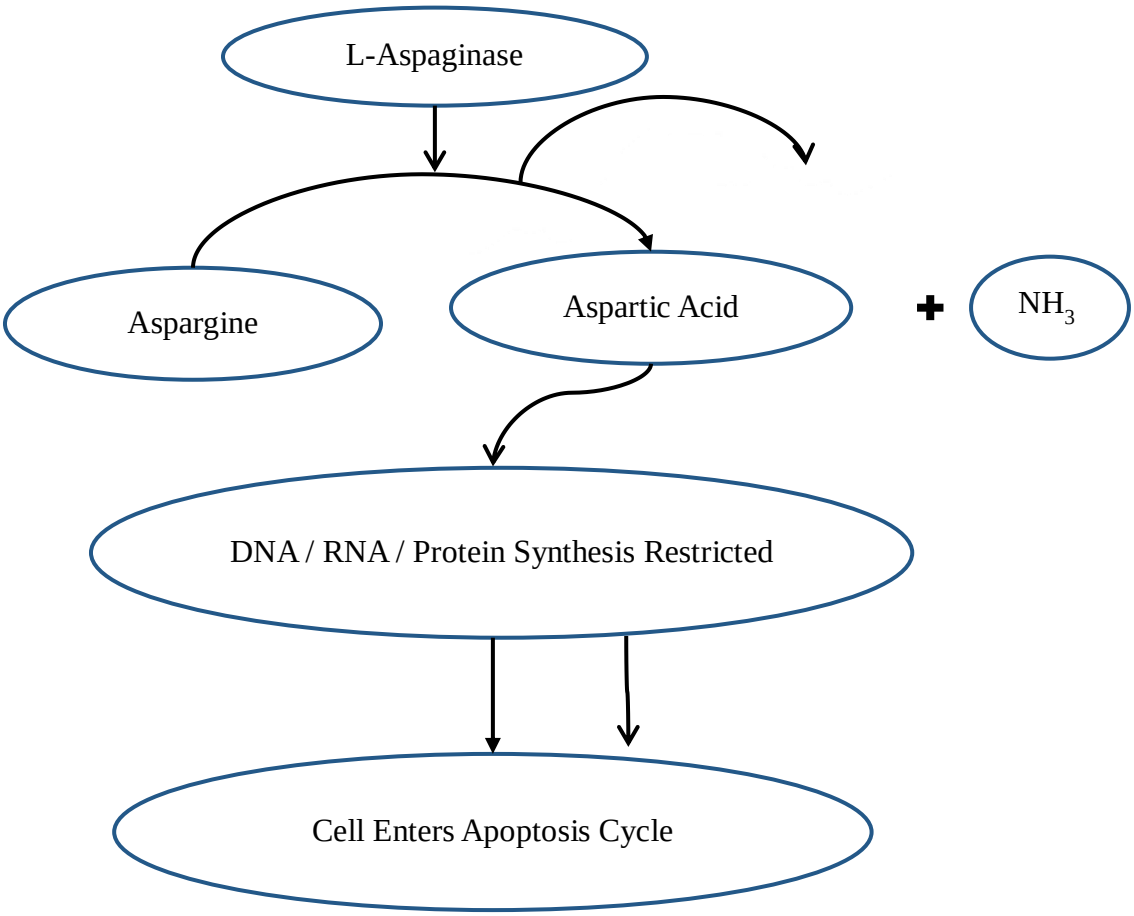

Figure-2: Mechanism of the action of L-Asparginase as anticancer agent

Microbial production of l-asparginase:

The L-Asparginase producing organism is selected and grown under aerobic condition on a roller shaker in 250 ml Erlenmeyer flasks containing culture medium. The medium used is Mineral Salt Medium deficient of citrate. (Baskar*et al*., 2011; Cachumba*et al*., 2016) The Flow-sheet of L-Asparginase production is given in Fig:3.

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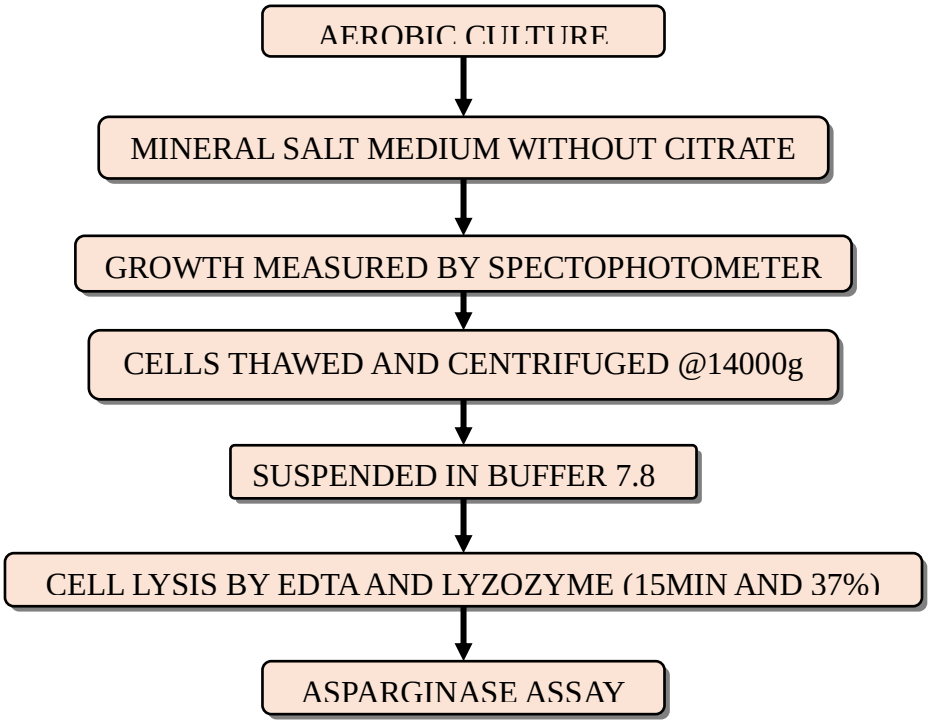

Recovery and purification of l-asparginase:

The industrial production of L-Asparginase has two main objectives to attain (1) High yield (2) Low production cost. Industrial production of L-Asparginase generally employs E.coli, E. chrysanthemi as bacterial source and A.oryzae as fungal source. The common nitrogen sources used are glutamine, proline which has proved to be best inducer of L-Asparginase. Enzyme purification is one of the most vital slip of the industrial production (Luhana*et al*., 2013; Dange*et al*., 2015; Emmanuel *et al*., 2015; El-Naggar*et al*., 2016). The important steps of recovery anf enzyme purification are provided in Figure-4.

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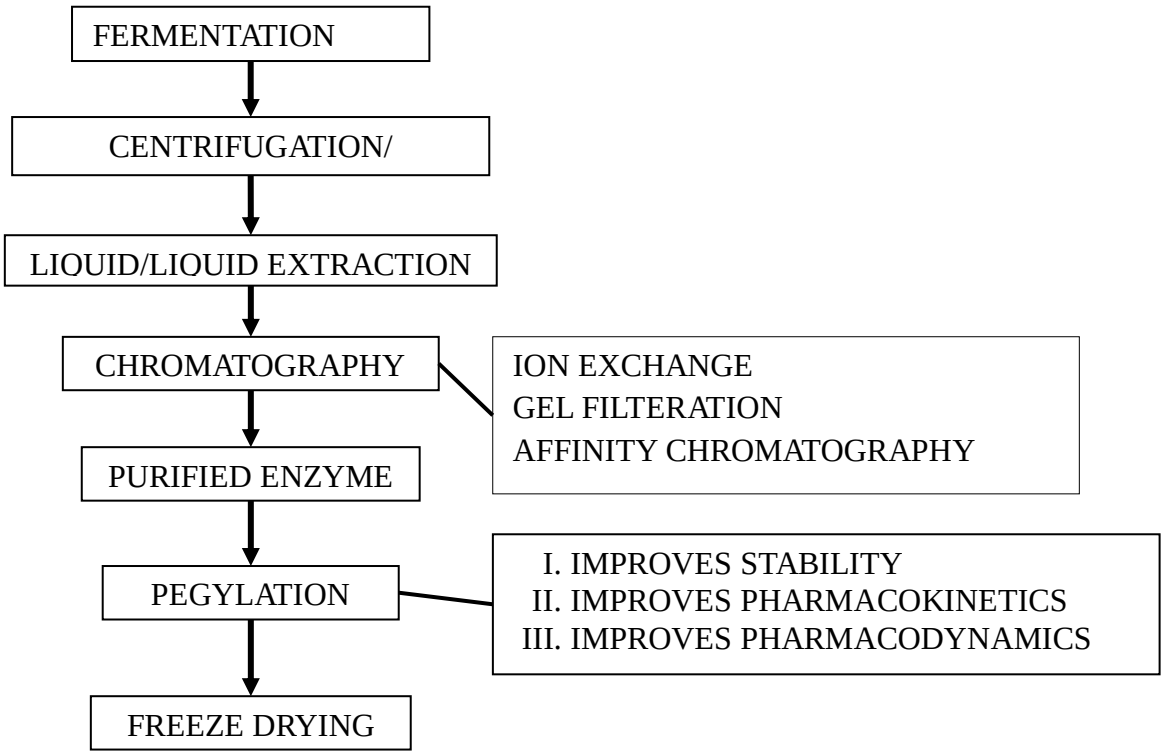

Figure-4: Recovery and purification of L-Asparginase

Applications of l-asparginase:

Asparginases find a wider application in field of pharmaceutical and food industries. It is now acknowledged as an anticancer agent. In food industry it is used to reduce levels of acrylamide when food items baked above 100℃ have high starch content. It finds wider application in Biosensor fabrication (Baskar*et al*., 2013; Batool*et al*., 2016; Alam*et al*., 2019; Baskar*et al*., 2019)

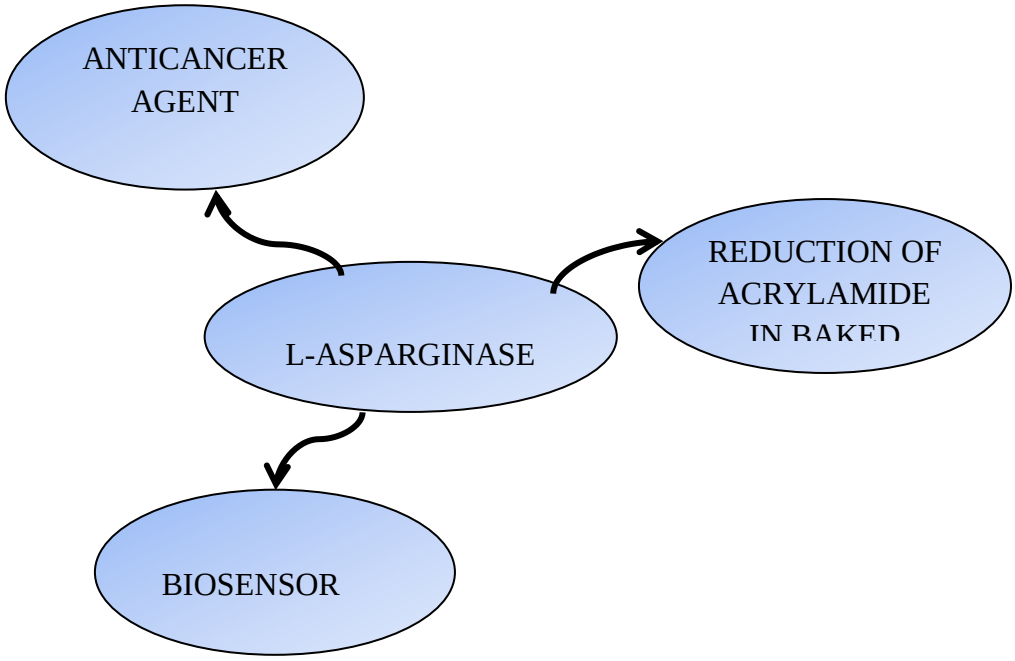

Figure-5:Major Application of L-Asparginase

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Limitations of asparginase use:

Asparginase administration is accompanied with a numerous medical side effects. It can trigger allergies, anaphylaxis, fever, damage to kidney, pancreas and liver. Therefore, its usage is under speculation due to intense side effects in some patients.

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CHAPTER - 5

INTRODUCTION TO SALINITY STRESS IN VEGETABLE CROPS

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Abstract

Among the major Abiotic stresses, Salinity stress poses a significant threat to agricultural productivity, particularly impacting vegetable crops. The problem of salinity stress is caused by a combination of natural factors and human activities, making it a complex challenge. Mechanisms of salinity stress in vegetable crops, such as water and nutrient absorption, physiological responses, and molecular mechanisms, are discussed. The harmful effects of salinity stress on vegetable crops include reductions in seed germination, seedling establishment, vegetative growth, biomass accumulation, and reproductive development, highlighting the multifaceted impacts of salinity stress on crop productivity. Strategies for managing salinity stress in vegetable crops include cultural practices, soil amendments, water management techniques, and breeding/genetic approaches. These strategies help in mitigating the detrimental effects of salinity stress, thereby enhancing the resilience and sustainability of vegetable crop production in saline conditions.

Keywords: Abiotic Stress, Salinity, Soil, Vegetable, Growth.

Introduction:

Salinity stress arises when the concentration of soluble salts, primarily sodium chloride (NaCl), in the soil or irrigation water exceeds the levels conducive to optimal plant growth. The excessive salt concentration around the root zone has an immediate effect on plant growth due to osmotic stress and a somewhat slower effect due to ionic toxicity in the older plant tissues. Such effects disrupt water uptake by plant roots and reduce shoot growth, hindering essential physiological processes. Salinity problems occur under both natural and human-induced activities such as mineral weathering (soil parent material), shallow saline groundwaters, irrigation under poor drainage, and chemical additions to soils in the form of fertilizers and marginal waters. Salinity has been degrading major agricultural productive regions throughout the world, the understanding of salinity stress in agricultural crops is important for various reasons including vegetables. To begin, salinity is the major contributor to reduced yields in vegetable crops. Elevated salt levels impede nutrient uptake and metabolic processes, ultimately leading to diminished productivity. Also, vegetables play a crucial role in balanced nutrition, making it essential to manage salinity stress to ensure consistent supply and food security, especially in regions where vegetables are dietary staples. Moreover, salinity stress degrades soil quality and disrupts ecosystem balance, highlighting the necessity of sustainable management practices to prevent soil salinization and maintain environmental integrity as acknowledged in the Special Report on Climate and Land by IPCC.

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Economic burdens on the agricultural sector due to decreased yields and increased production costs is another salinity impact in vegetable production.

Impact of salinity stress on vegetable crop production is multifaceted. Firstly, it hampers germination and seedling establishment, as excessive salt levels hinder seed germination and seedling emergence, resulting in poor stand establishment. Additionally, salinity stress disrupts physiological processes such as photosynthesis and nutrient uptake, leading to stunted growth and leaf chlorosis. This disruption extends to reproductive development, causing reduced flower formation, fruit set, and yield. Furthermore, salinity stress alters the nutritional composition and sensory qualities of harvested vegetables, impacting their marketability. Salinity-stressed plants are also more vulnerable to pest infestations and diseases due to weakened defense mechanisms, exacerbating production challenges. Continual exposure to salinity stress degrades soil quality and fertility over time, compromising long-term agricultural sustainability.

Factors contributing to Soil Salinity:

Soil salinity arises from both natural phenomena and human activities as we discussed earlier. Naturally, rocks and minerals weather over time, releasing various minerals, including salts including chlorides of sodium, calcium, magnesium, carbonates, and sulfates, into the soil. This process is fundamental to soil formation and contributes to the background levels of salts found in soils and irrigation water (lakes, streams, and groundwater) worldwide. Additionally, erosion plays a crucial role in transporting soluble salts from geological formations to nearby soils, primarily through water and wind erosion. Coastal regions are particularly vulnerable to soil salinity due to their proximity to seawater, which infiltrates coastal soils through tidal movements and storm surges. This phenomenon is commonly known as seawater intrusion. Human-induced activities exacerbate soil salinity issues when the land management is not handled properly in salt-prone areas. Poor irrigation practices, such as under irrigation, over irrigation or the use of saline water for irrigation without proper drainage contribute to salt accumulation in the soil. As water evaporates from the soil surface, salts are left behind in the soil profile, gradually increasing their concentration over time - a process known as secondary salinization. Capillary movement of shallow saline groundwater also contributes to the salt accumulation in the similar manner. Excessive use of fertilizers containing soluble salts further elevates soil salinity levels. Inadequate drainage worsens the salinity problem, which restricts the salt leaching below the root zone concentrating salts in the root zone. Land clearing and deforestation disrupt natural ecosystems, increasing soil erosion rates and exposing bare soil surfaces to salt transport.

Climate change adds complexity to soil salinity dynamics. Altered precipitation patterns, including increased drought frequency and reduced rainfall, lead to soil desiccation and salt buildup. Higher temperatures increase evaporative demand, concentrating salts in the root zone and exacerbating salinity issues, especially in arid and semiarid regions. Reduced irrigation water availability is another factor limiting the salinity management through leaching. Rising sea levels and storm surges associated with climate change intrude salt water into coastal soils, further elevating salinity levels. Indirectly, changes in vegetation patterns and land use practices driven by climate change influence soil salinity by altering soil moisture levels and nutrient cycling processes.

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Mechanisms of Salinity Stress in Vegetable Crops:

Absorption and transport of water and nutrients in plants:- Salinity stress disrupts the absorption and transport of water and nutrients in vegetable crops due to osmotic stress, fundamentally affecting their physiological processes. Excessive salt concentrations in the root zone induce an osmotic imbalance hindering the ability of plant roots to absorb water, resulting in water stress due to reduced water potential in leaves. Water absorption by the plant root is restricted by the water potential gradient as the soil water potential becomes lower than that of the plant roots during salt stress. Osmotic stress further inhibits cell expansion, decreases cell turgor pressure, reduces shoot growth and ultimately impairs overall plant growth. Additionally, the presence of high salt levels in the soil solution interferes with the uptake of essential nutrients by plant roots. This interference occurs because salt ions, particularly sodium (Na^+) and chloride (Cl^-), compete with nutrient ions for uptake sites on the roots creating nutrient imbalance. As a consequence, this leads to nutrient deficiencies and restricts various enzyme activities in vegetable crops, which further compromises their growth and development.

Physiological responses of vegetable crops to salinity stress:-

Vegetable crops demonstrate a variety of physiological responses to salinity stress as part of their adaptation mechanisms to adverse conditions. One key response is osmotic adjustment, wherein plants accumulate compatible solutes, such as sugars, amino acids, and organic acids, within their cells. These compatible solutes aid in maintaining cellular osmotic balance and water uptake under saline conditions. By stabilizing cell membranes and maintaining cell turgor pressure, these solutes help protect cellular structures from dehydration and damage. Moreover, in response to salinity stress, plants modulate their metabolism and hormone signaling pathways. This adjustment involves reallocating resources towards stress-responsive pathways, such as the synthesis of stress-related proteins, antioxidants, and osmoprotectants. These compounds play crucial roles in scavenging reactive oxygen species (ROS), stabilizing proteins, and maintaining cellular integrity. Additionally, salinity stress triggers adjustments in leaf physiology, including stomatal conductance, leaf water potential, and photosynthetic rates. These adaptations allow plants to optimize water use efficiency and carbon assimilation under saline conditions, albeit at the expense of reduced growth and yield potential.

Molecular mechanisms underlying salinity stress tolerance in vegetable crops:

At the molecular level, vegetable crops deploy complex mechanisms to tolerate salinity stress and maintain cellular homeostasis. A central mechanism involves the regulation of ion transport and compartmentalization to minimize the adverse effects of excess salt ions on cellular metabolism. Plants actively regulate the uptake, translocation, and sequestration of ions, such as sodium (Na^+) and chloride (Cl^-), to prevent their accumulation in the cytoplasm and other vital organelles. Key components in ion homeostasis include ion transporters and channels located in the plasma membrane and tonoplast, which control the flux of ions across cellular membranes. By modulating ion movement, plants sustain favorable ion concentrations in the cytoplasm and vacuole, thereby preventing salt-induced toxicity and osmotic stress. Additionally, plants activate various stress-responsive genes and signaling pathways to coordinate their responses to salinity stress. These genes encode proteins involved in stress perception, signal transduction, transcriptional regulation, and metabolic adjustments. For instance, transcription factors, such as members of the AP2/ERF,

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MYB, and NAC families, govern the expression of stress-responsive genes implicated in osmotic adjustment, ion homeostasis, and antioxidant defense. Furthermore, plants deploy antioxidant defense systems to counteract the detrimental effects of reactive oxygen species (ROS) generated under salinity stress. Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidases, and glutathione peroxidase (GPX), scavenge ROS and safeguard cellular structures and macromolecules from oxidative damage.

Effects of Salinity Stress on Vegetable Crop Growth and Yield:

Reduction in seed germination and seedling establishment:- Salinity stress presents hurdles for seed germination and seedling establishment in vegetable crops. Excessive salt levels in the soil or irrigation water disrupt the osmotic balance, hindering water absorption by seeds. This limitation in water availability delays or prevents germination by impeding essential enzymatic processes. Additionally, salt ions directly interfere with metabolic activities within seeds, such as respiration and enzyme function, further hampering germination. Seed germination rates decline, and seedlings may exhibit reduced vigor and survival rates due to compromised root and shoot development.

Impacts on vegetative growth and biomass accumulation:- Salinity stress affected the vegetative growth and biomass accumulation of vegetable crops. The presence of excessive salts in the soil disrupts water uptake by plant roots, causing water stress and inhibiting cell expansion. As a result, processes like stem elongation, leaf expansion, and branching are stunted. Moreover, salinity stress disrupts the uptake and translocation of essential nutrients within plants, leading to nutrient deficiencies and decreased photosynthetic efficiency. Consequently, plants allocate fewer resources to above-ground growth, resulting in diminished biomass accumulation. This reduction in nutrient availability and photosynthetic capacity leads to stunted growth and reduced overall vigor in vegetable crops.

Influence on reproductive development and yield:- Salinity stress impacts the reproductive development and yield of vegetable crops, affecting both quantity and quality. High salt concentrations during the reproductive phase disrupt critical processes such as flower development, pollen viability, pollination, and fruit set. Salinity stress may lead to reduced flower production, poor pollen germination, and impaired pollen tube growth, ultimately resulting in decreased fruit set and seed formation. Additionally, fruits grown under saline conditions may exhibit altered quality parameters such as size, color, flavor, and nutritional composition. This leads to reduced market value and consumer acceptance of the produce. Vegetable crops exposed to salinity stress often yield lower harvests, impacting the economic viability of farming operations.

Strategies for Managing Salinity Stress in Vegetable Crops:

A. Cultural practice for mitigating salinity stress:-

(1) Crop rotation: Beyond merely alternating between salt-sensitive and salt-tolerant crops, strategic crop rotation involves careful consideration of the specific crops' water and nutrient requirements, growth habits, and salt tolerance levels. By diversifying the crop rotation cycle and incorporating leguminous cover crops or green manures, growers can further enhance soil fertility, suppress weeds, and promote beneficial microbial activity, thereby contributing to overall soil health and resilience to salinity stress.

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(2) Proper irrigation scheduling: Precision irrigation management extends beyond monitoring soil moisture levels and evapotranspiration rates. It entails tailoring irrigation schedules to the specific growth stages of each crop and adjusting water application rates based on real-time weather conditions and soil moisture data. Implementing innovative irrigation technologies such as soil moisture sensors, automated weather stations, and computerized irrigation controllers enables growers to fine-tune their irrigation practices and optimize water use efficiency, minimizing the risk of soil salinization while maximizing crop yield potential.

(3) Mulching: While mulching with organic materials like compost or straw effectively conserves soil moisture and reduces evaporation, it also serves as a critical component of soil conservation and erosion control. By maintaining a protective layer of mulch on the soil surface, growers mitigate the impact of raindrops and surface runoff, prevent soil compaction, and enhance soil water infiltration rates. Organic mulches decompose over time, they contribute valuable organic matter to the soil, enriching soil fertility, improving soil structure, and enhancing microbial activity, all of which are essential for combating salinity stress.

(4) Planting density and spacing: Optimal planting density and spacing not only influence resource utilization and microclimatic conditions within the crop canopy but also impact weed competition, disease incidence, and pest pressure. By strategically adjusting planting densities and row spacings, growers can modulate light interception, air circulation, and soil coverage, thereby promoting healthier plant growth and reducing the susceptibility of individual plants to salinity stress. Proper spacing allows for more efficient nutrient uptake and root exploration, enabling plants to better access water and nutrients in saline soils.

(5) Soil management: Beyond conventional soil management practices, such as cover cropping, green manuring, leaching, and minimum tillage, growers can explore innovative soil health management strategies to combat salinity stress. Techniques such as biological soil amendments (e.g., microbial inoculants, biofertilizers), soil biostimulants (e.g., humic substances, seaweed extracts), and soil remineralization (e.g., rock dust applications) can enhance soil microbial diversity, improve nutrient cycling efficiency, and enhance soil structure, thereby bolstering soil resilience to salinity stress and promoting sustainable vegetable production practices. Additionally, adopting regenerative agriculture principles, such as agroforestry, agroecology, and holistic land management, helps restore degraded soils, sequester carbon, and mitigate climate change impacts, further contributing to long-term soil health and ecosystem resilience.

B. Soil amendments and water management techniques:

(1) Gypsum application: Gypsum, composed of calcium sulfate, serves as a pivotal tool in managing soil salinity. Gypsum application replaces sodium ions from soil exchange sites with calcium, improving soil structure, and promoting the aggregation of soil particles. This results in improved soil permeability, facilitating better water infiltration and reducing the risk of waterlogging. Additionally, gypsum enhances calcium availability, vital for maintaining proper cell membrane function in plants. By facilitating the leaching of excess salts from the root zone through the flocculation of clay particles, gypsum helps mitigate soil salinity, thereby alleviating the adverse effects of salinity stress on crop growth and productivity.

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(2) Organic matter incorporation: Incorporating organic matter into the soil is fundamental for enhancing soil health and resilience to salinity stress. Organic matter acts as a nutrient source for soil microorganisms and aids in the formation of stable soil aggregates, thereby improving soil structure and water retention capacity. Moreover, organic matter stimulates microbial activity in the soil, fostering nutrient cycling and the decomposition of organic materials into plant-available forms. By increasing soil organic matter content, growers can bolster soil fertility, water holding capacity, and cation exchange capacity, thus mitigating the impacts of salinity stress on crop growth and ensuring sustainable agricultural practices.

(3) Use of saline-tolerant amendments: Certain soil amendments are tailored to enhance soil salinity tolerance and mitigate the adverse effects of salinity stress on crop growth. For instance, specific types of biochar or soil conditioners are formulated to improve soil water retention and nutrient availability, making them suitable for use in saline soils. These amendments may also contain beneficial microorganisms or plant growth-promoting substances, further enhancing plant resilience to salinity stress. Incorporating saline-tolerant amendments into the soil, growers can enhance soil physical and chemical properties, optimize plant nutrient uptake, and improve crop productivity under saline conditions.

(4) Saline water management: Effectively managing saline water resources is critical for minimizing soil salinization and preserving soil health in saline-prone environments. Utilizing efficient irrigation technologies such as drip irrigation or micro-sprinklers helps minimize soil surface wetting and reduce evaporation losses, conserving water and diminishing the risk of soil salinization. Additionally, periodical leaching of salts below the root zone with fresh water during the highest salinity stress reduces the soil profile salt levels for the optimum growth of plants. Long-term risk of marginal waters in regards to their effect of soil hydro-ecological functioning should be considered when utilized. Implementing saline water management strategies, growers can enhance water use efficiency, mitigate soil salinization, and ensure sustainable agricultural practices in saline-prone regions.

C. Breeding and genetic approaches for developing salt-tolerant vegetable crop varieties:

(1) Phenotypic Selection: This traditional breeding approach involves observing and selecting plants based on their physical characteristics or phenotypes. For salt-tolerant vegetable crops, breeders would observe characteristics such as leaf size, shape, and yield performance under saline conditions. By screening diverse germplasm collections, which represent the genetic diversity within a species, breeders can identify individuals with promising traits. These selected plants serve as the basis for further breeding efforts, aiming to develop varieties that thrive in salt-affected environments.

(2) Molecular Breeding Techniques: Molecular breeding techniques rely on advanced genetic tools to expedite the breeding process. High-throughput genotyping platforms enable breeders to analyze the genetic composition of plants rapidly and accurately. By identifying genetic markers associated with salt tolerance, breeders select plants with desired traits more efficiently. Marker-assisted breeding strategies facilitate the incorporation of salt-tolerance genes into elite breeding lines, combining traditional selection with genetic analysis. This approach accelerates variety development and increases the likelihood of success in breeding for salt tolerance.

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(3) Genetic Engineering: Genetic engineering allows for precise manipulation of specific genes associated with salt tolerance. Technologies like CRISPR/Cas9 enable targeted modifications, such as gene knockout or gene insertion, to enhance a plant's ability to withstand salt stress. By introducing or enhancing the expression of genes involved in stress response pathways, researchers engineer crops to exhibit improved salt tolerance without compromising other desirable traits. Genetic engineering offers flexibility in targeting specific genes or pathways related to salt tolerance, potentially leading to the development of highly resilient vegetable varieties.

Conclusion: Continued research and innovation are vital for addressing salinity stress in vegetable crops, especially with escalating challenges like climate change and irrigation water shortage due to urban and environmental competition. Investing in research is crucial to refine breeding techniques, develop new genetic resources, and discover innovative approaches for enhancing salt tolerance in crops. Additionally, ongoing innovation in agricultural practices and technologies is essential for mitigating the impacts of salinity stress on food production and ensuring food security for future generations. In conclusion, sustainable agriculture is indispensable for mitigating the impacts of salinity stress on vegetable crop production. Practices promoting soil health, water conservation, and biodiversity fortify resilience against salinity and other environmental stresses. Embracing agroecological principles and fostering partnerships facilitate the development and adoption of sustainable solutions.

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CHAPTER - 6
MICROBIAL BIOTECHNOLOGY IN AGRICULTURE: APPLICATIONS FOR SUSTAINABLE CROP PRODUCTION AND SOIL HEALTH

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Abstract

An effective device is rising to gain sustainable agricultural manufacturing and soil health: microbial life. This bankruptcy discusses diverse programs of useful microbes in agricultural practices. We are speaking approximately the various network of organisms that stay in and round plant roots, called the plant microbiome, and their importance. Plant increase is facilitated via way of means of loads of useful microorganisms, together with mycorrhizal fungi and growth-selling bacteria (PGPB). Through techniques regarding phosphate solubility and nitrogen fixation, PGPB will increase nutrient availability. Mycorrhiza will increase the supply of vitamins and water via way of means of setting up symbiotic relationships with plant roots.This bankruptcy additionally seems at how microbial biocontrol may be used to manipulate plant illnesses and decrease pesticide use. We will also explore the use of microbes for bioremediation of contaminated soils and for improving soil structure and fertility. Finally, the chapter will address the challenges and future directions of microbial biotechnology in sustainable agriculture, emphasizing the need for continued research and development to harness the full potential of this approach for a more environmentally friendly and productive agricultural future.

Keywords- Microbiome, PGPB, Mycorrhizae, Bioremediation

Introduction:

In agriculture, microbial biotechnology is critical for managing soil health and producing crops in a sustainable manner. This discipline seeks novel ways to increase agricultural productivity with the least amount of negative environmental impact by using the power of microorganisms such as bacteria, fungi, and algae. Scientists and agriculturalists alike are finding innovative ways to combat major issues facing modern agriculture, such as soil degradation, pest control, and nutrient management, through employing the complex interactions between bacteria and plants. In simple terms, the goal of microbial biotechnology in agriculture is to maximize soil health and promote plant growth by taking advantage of microorganisms' inherent ability to thrive. These microbes mediate nutrient cycling, disease suppression and plant tolerance to environmental stresses. Biotechnological intervention in microbial communities can alter soil function and crop performance through improved soil fertility, increased crop productivity, and reduced reliance on chemical inputs related to fertiliser and pesticide use. Microbial biotechnology has promising applications to agriculture, such as the development of biofertilisers and biopesticides through beneficial microbes (nitrification-fixing bacteria, mycorrhizal fungi, etc), which enhance plant nutrition and suppress pathogens and pests while decreasing environmental impacts of agro-

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chemical fertilisers. Microbial inoculation can also serve as a more sustainable and longer-lasting alternative to conventional fertilisers.

Additionally, microbial life has the potential to sustainably increase agricultural productivity, allowing farmers to produce more food on their soil without compromising the integrity of the environment. Using artificial microbes and modern bioremediation techniques, we can repair damaged farmland, adapt to climate change, and develop ecological networks on the farm. Sustainable agriculture is of great importance in today's world population growth and climate change conditions. Microbial biotechnology can bring a sustainable and environment-friendly agriculture, which uses the complex interactions between the microbes, plants and soil to fulfil the human food demands without sacrificing the health of our planet. As the research in this area booms, by introducing biological microbes into conventional farming, we can usher in an era of sustainable and safe food.

Importance of soil microbiome:

The majority of life on Earth is made up of microbes, both in quantity and overall biomass. Since they were the first living things on Earth, they have undergone significant evolution and now display a range of functional, evolutionary, and metabolic variety that much beyond that of any other species in the tree of life. The habitats of certain microbes determine the boundaries of the biosphere and the biosphere-geosphere boundary because they may live in harsh conditions that are inhospitable to most other forms of life.

“Microbes” are essential drivers for the biogeochemical cycles and objectives like sustainability and safety. This vital microbial bulk (soil microbiome) is substantially inspired via way of means of agricultural/farming practices. Therefore, with the assist of microbiome engineering technology like meta-transcriptomics, meta-proteomics, metabolomics, and novel gene-changing techniques, we will without difficulty display screen out the quite numerous and balanced microbial populace withinside the bulk of soil, improving the soil’s fitness and productivity. Importantly, we want to extrade our cultivation techniques to obtain such sustainability.

Symbiotic microbes, an important component of soil microbes, perform many important functions such as:

- Nutrient cycles include all types of biogeochemical cycles, such as sulfur, nitrogen, phosphorus, and carbon cycles.
- To contribute to reducing threats such as soil erosion by preserving soil structure and leading to an increase in organic matter, which is an important factor in improving soil fertility, health, resilience and water potential.
- Rhizosphere-associated microbes are involved in the production of specific plant defense mechanisms that cause plant diseases.
- Increasing the tolerance of plants to different environmental conditions such as temperature and humidity fluctuations.Initiation and initiation of processes such as root growth and nutrient uptake.

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Applications of microbial biotechnology

1. BIOFERTILIZERS

Biofertilizers are microbial mixtures made up of naturally occurring rhizobacteria that promote plant growth (PGPR). PGPRs can either directly or indirectly stimulate plant growth by solubilizing nutrients in the soil, producing hormones that stimulate plant growth, and producing siderophores, which are metabolites that sequester iron. Given how synthetic fertilizers contribute to climate change, microbial products, or biofertilizers, have great potential as instruments for global sustainable agriculture. Successful uses and research indicate that they are effective in growing a variety of crops.

A variety of live microbial inoculants that replenish plant nutrients and enhance soil health are referred to as biofertilizers. By utilizing their innate capacity to convert and release nutrients for plant absorption, these advantageous microorganisms provide a viable substitute for synthetic fertilizers. In this article, we examine three major categories of biofertilizers that have different but complimentary uses:

- Bacteria that Fix Nitrogen: These bacteria form symbiotic partnerships with leguminous plants, chiefly with species of Rhizobium. Through the nitrogenase enzyme complex, they settle in root nodules and transform atmospheric nitrogen (N2) into ammonia (NH3). By making this fixed nitrogen easily accessible for plant growth, it encourages the growth of legumes and lessens reliance on artificial nitrogen fertilizers.
- Microbes that Solubilize Phosphate (PSM): PSM, a broad category that includes fungus and bacteria (such as Pseudomonas and Bacillus), improve plant uptake of phosphorus (P), an essential mineral that is frequently deficient in soils. These bacteria dissolve insoluble phosphate sources, such as rock phosphate, and transform them into forms that are easily absorbed by plant roots through a variety of processes, such as the synthesis of organic acids and phosphatases.
- Mycorrhizae: By growing a massive network of filaments (hyphae) that greatly expand the root surface area for nutrition and water uptake, these symbiotic fungi establish a mutually advantageous interaction with plant roots. In addition to improving phosphorus and water uptake from the soil, mycorrhizae may also help plants absorb other vital nutrients. They also help to increase the stability and structure of the soil.

2. BIOREMEDIATION

Microorganisms play a part in maintaining biological equilibrium by contributing to food chains. The technique of bioremediation involves removing contaminated materials from the environment by means of bacteria, algae, fungus, and yeast. Microbes can develop at temperatures as low as -196, and as high as 1200 degrees Fahrenheit, in the presence of hazardous substances or any waste stream. The biological systems and adaptability of microorganisms make them the perfect option for remediation. The most crucial nutrient for bacteria is carbon. Bioremediation was carried out using microbes from diverse settings. Microbes include, among others, Nitrosomonas,

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Xanthobacter, Arthrobacter, Pseudomonas, Bacillus, Mycobacterium, Corynebacterium, Flavobacterium, and other microorganisms.

3. PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

In general, there are far more bacteria near plant roots than there are in the overall soil. Thus, a variety of soil microbes that have been identified as Rhizobacteria for Plant Growth Promotion are members of the genera that promote plant growth, such as Bacillus, Pseudomonas, Azospirilium, Agrobacterium, Azotobacter, Arthrobacter, Alcaligenes, Serratia, Rhizobium, Klebsiella, Clostridium, etc. Numerous plant species have their rhizosphere colonized by plant growth promoting rhizobacteria (PGPR), which have positive impacts on the plants' growth and resistance to diseases brought on by plant pathogenic bacteria, viruses, nematodes, and fungi. PGPR can be divided into two groups based on their interactions with plants: free-living rhizobacteria that reside outside of plant cells and symbiotic bacteria that live inside plants and exchange metabolites with them directly.

Recent advancements:

Alterations made to the genetic structure of microorganisms to produce new proteins or other food ingredients, to improve/enhance the production of existing proteins/ingredients, or to modify the properties of existing proteins to suit new applications. Several techniques are used to perform gene editing in microorganisms, and the term genetically engineered microorganisms (GEM) refers specifically to microorganisms (i.e.bacteria or fungi, including yeast) that humans have transformed using in vitro molecular biology techniques (also known as modern biotechnology) to perform a specific function.

The extraordinary advances achieved in the fields of biochemistry and molecular biology over the past decades have led to the widespread use of GEMs in the production of medical and food substances, especially as these processes become increasingly popular increasingly recognized as environmentally friendly, animal friendly and cost friendly, effective production methods. For example, insulin is now produced by bacteria instead of sacrificing pigs to harvest the pancreas, the original source of insulin. Similarly, trypsin and chymosin produced by bacteria areavailable as alternatives to harvesting trypsin or rennet from animal sources such as pigs and cows. The benefits of GEM production are not limited to replacing animal production methods.

In the coming decades, the focus will be on creating a clean, green environment using beneficial microbial communities associated with soil and plants. Plant-microbe interactions include the association of bacteria with the plant system: epiphytic, intracellular and rhizospherics. Bacteria associated with plant ecosystems play an important role in the growth, development and health of the soil. In addition, soil and plant microbiota help promote plant growth, directly or indirectly through plant growth-promoting mechanisms, e.g., release of plant growth regulators; dissolves phosphorus, potassium and zinc; biological nitrogen fixation; or by producing siderophores, ammonia, HCN and other secondary metabolites. These beneficial microbial communities represent a new and promising solution for agricultural environmental sustainability by providing biofertilizers, bioprotectants and biostimulants, in addition to in addition to alleviating various types of abiotic stresses in plants.

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Conclusion:

The role of the microbiome in sustainable agriculture and human health. In sustainable agriculture, soil health is largely determined by the presence and diversity of bacteria present in the soil rhizosphere. The diversity and abundance of soil and rhizosphere microorganisms influence the composition, yield and sustainability of crops. Deploying bacteria to improve agricultural productivity is an extremely attractive, non-transgenic approach that can be broadly considered as expanded plant genomics. Because these same bacteria can help restore soil health and productivity, they have a bright future in sustainable, low-input agriculture. Better assessment of soil health indicators is needed to better understand how production strategies and environmental factors influence the physical, biological and chemical stability and dynamics of soils, soil-rhizome-plant systems and their impact on short- and long-term sustainability.

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CHAPTER- 7

BEHAVIOUR ECOLOGY OF PHEROMONE MEDIATED COMMUNICATION IN MOTHS AND ITS IMPORTANCEIN PHEROMONE APPLICATION

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Abstract

Insect pheromones are specific natural compounds that meet modern pest control requirements, i.e., species-specificity, lack of toxicity to mammals, environmentally benign, and a component for the Integrated Pest Management of agricultural pests. Therefore, the practical application of insect pheromones, particularly sex pheromones, have had a tremendous success in controlling low density pest populations, and long-term reduction in pest populations with minimal impact on their natural enemies. Mass trapping and mating disruption strategies using sex pheromones have significantly reduced the use of conventional insecticides, thereby providing sustainable and ecofriendly pest management in agricultural crops.

Insect pheromones are particular natural substances that satisfy contemporary pest control standards, such as being species-specific, non-toxic to mammals, safe for the environment, and a part of integrated pest management for agricultural pests. Thus, the practical use of insect pheromones-especially sex pheromones has proven to be extremely effective in managing low-density pest populations and reducing them over time with no negative effect on their natural enemies. Sex pheromone-based mass capturing and mating disruption techniques like PB Rope L and SPLAT have drastically decreased the need for traditional pesticides, allowing for environmentally friendly and sustainable pest management of agricultural crops.

Introduction:

Olfaction, or the sense of smell as it is more well known, is crucial to a species' ability to reproduce and choose food, especially when combined with gustation, the sense of taste (Hartevelt et al., 2015).

The term "insect olfaction" describes how chemical sensors let insects recognize and distinguish volatile substances for oviposition habitats, predator avoidance, mating partner detection, and foraging. For insects, it is therefore the most significant sense. The timing of the majority of significant insect activities depends on what the insects smell and when they smell it. In many insect species, such as the wasp Polybia sericea and the moth Deilephila elpenor, for instance, scent is necessary for both finding host plants and hunting prey. Sex pheromones are those that an organism releases in order to attract members of its own species, persuade them to mate, or carry out some other sexual reproduction-related task. Sex pheromones are primarily responsible for

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identifying females for reproduction, luring the other sex, and transmitting data about species, age, sex, and genotype (Howse et al., 2013). Sex pheromones are chemical messengers that facilitate intraspecific communication, which is crucial for reproduction (Wyatt 2003, 2014). Bombyx mori, a moth, was the first insect to have its sex pheromone scientifically characterized (Butenandt et al., 1959). Pheromones are released when calling female moths seek mates (Itagaki and Conner, 1988). Males can locate partners up to several miles away thanks to these female pheromones, which is crucial for night-flying moths. Additionally, they are crucial for mate recognition and, should signals and preferences differ throughout populations, could result in reproductive isolation and speciation (Schneider, 1992; Johansson and Jones, 2007). Pheromones are also produced by males in several moth species. These male pheromones have a restricted range of action and are crucial for mating and courting choices. Although research on male pheromones has not been as extensive as that on females, a variety of chemical kinds, scent-disseminating male structures, and behavioral roles have been identified. They can communicate information about mate quality and species identity and play a part in female acceptance (Conner and Iyengar, 2016).

Five decades of pheromone research have been driven by the goal of managing unpleasant insects in forestry, horticulture, agriculture, stored items, and as disease-carrying insects by means of species-specific behavior-modifying compounds. It has been found that hundreds of pheromones and other semiochemicals are utilized to defend plants and animals from insects as well as to track their presence and quantity. Tens of millions of lures are produced annually, covering at least 10 million hectares, for the purposes of bulk trapping and monitoring. At least one million hectares are managed for insect populations using attract and kill methods and air permeation. Here, we go over the most significant and common real-world uses. Because pheromones do not negatively impact natural enemies and are increasingly effective at low population densities, they have the potential to permanently reduce insect populations in ways that conventional insecticides are unable to. Controlling native and invasive insects is becoming a more pressing task due to a changing environment that includes increased growth season temperatures and altered rainfall patterns. Five decades of pheromone research have been driven by the goal of managing unpleasant insects in forestry, horticulture, agriculture, stored items, and as disease-carrying insects by means of species-specific behavior-modifying compounds. It has been found that hundreds of pheromones and other semiochemicals are utilized to defend plants and animals from insects as well as to track their presence and quantity. Tens of millions of lures are produced annually, covering at least 10 million hectares, for the purposes of bulk trapping and monitoring. At least one million hectares are managed for insect populations using attract-and-kill methods and air permeation intensified insecticide use will not provide a solution, but pheromones and other semiochemicals instead can be implemented for sustainable area-wide management and will thus improve food security for a growing population. Given the scale of the challenges we face to mitigate the impacts of climate change, the time is right to intensify goal-oriented interdisciplinary research on semiochemicals, involving chemists, entomologists, and plant protection experts, in order to provide the urgently needed, and costeffective technical solutions for sustainable insect management worldwide.

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Semiochemicals:

Chemical involved in communication are termed as semiochemical. It is classified as intraspecific and interspecific pheromone.

I.Intraspecific communication

Pheromone

A pheromone is a secreated or excreated chemical factor that triggers a special response in members of the same species. Pheromone are chemical capable of acting outside the body of the secreating indivisual to impact the behaviour of the receiving indivisual. The term "pheromone" was introduced by Peter Karlson and Martin Luscher in 1959, based on the greek word pherein (to transport) and hormone (to stimulate). They are also sometimes classified as ectohormones. These chemical messengers are transported outside of the body and result in a direct developmental effect on hormone levels or behavioural change.

Pheromone are of 2 types

I. **Releaser effect pheromone**:

These produce an immediate and reversible behavioural change in the receiving insects. They operate through the olfactory sensillae.

II. **Primer effect pheromone**:

These triggers a chain of physiological changes in the body of the insect and operate through gustatory sensillae. These regulate caste determination and reproduction in social insects.

Types of releaser effect pheromones

1. **Sex pheromones** : Many insects release sex pheromone to attract a mate, and these are well studied in many Lepidopterous (moths and butterflies). Bombykol is the first sex pheromone isolated and identified from silkworm Bombyx mori in 1959 by A. Butenandt and co-worker. Important sex pheromone which have a potential in pest management are dispalure (gypsy moth), gossyplure (Pink bollworm), grandlure(cotton grey weevil), looplure (Trichoplusia ni), Helilure (Helicoverpa armigera) and Litlure (Spodoptera litura).
2. **Agrregation pheromones**: These pheromones induce aggregation or congregration of insects for protection, reproduction and feeding. They also function in defense against predators, mate selection and overcoming host resistance by mass attack EG; Frontalin (Dendroctonus), Ipsenol (Ips), periplanone (Periplanone) and Dimethyldecanol (Tribolium)
3. **Alarm Pheromone :** These are primarily an "antipredator device, a warming to conspecific about the presence or the attack of an enemy. This warning elicits different behavioural such as dispersion or escape in different behaviour such as dispersion or escape in different insects. Ex; Terpenes (Aphids), Aldehydes (Hemipeterans), Formic acid (Ants) and Monoterpene hydrocarbons (Termites soldiers)
4. **Trail pheromones**: These are used to find mates, or to utilize food resources more efficiently.

   Eg; Caproic acid (Zootermopssis), hexanoic acid and heptonoic acids (Ants).
5. **Epidietic Pheromones**: These are compounds function in the regulation of population density

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by controlling the dispersion of indivisuals.

6. **Territorial Pheromones** : Secreated by males of some species and attract both males and females for e.g. male of bumble bees and carpenter bees demarcate the territory for foraging activity. Sex pheromone are useful in integrated pest management in three distict ways viz., monitoring of insect population, mass trapping and mating distruption (Prasad, 2015)

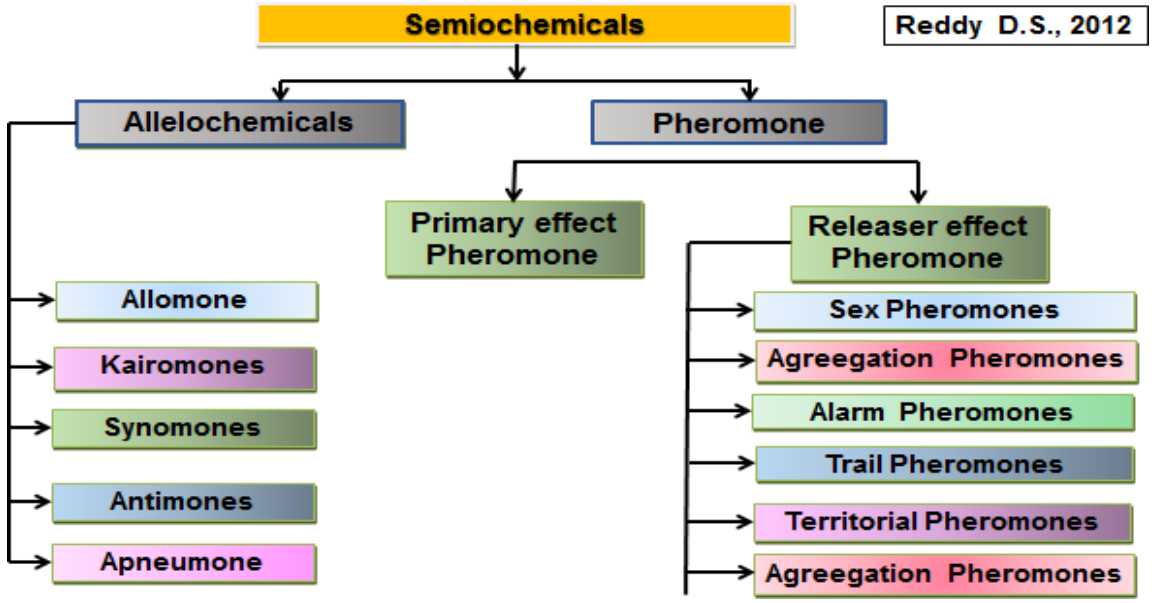

Image1:Classification of Semiochemicals

Pheromone Gland:

The ovipositor, located at the end of the adult female abdomen and the pheromonegland are closely related. The gland is made up of a single layer of epithelial cells that resemblesa band and surrounds the top portion of the ninth and final abdominal segment as well as thelower section of the eighth abdominal segment. In reaction to hormonal stimulus, these cellscreate, store, and release the components of pheromones (Image 2). The pheromone is releasedthrough a thin cuticle that is tightly packed with tiny hair-like projections covering the outside ofthisband (Vogel *et al*., 2010).

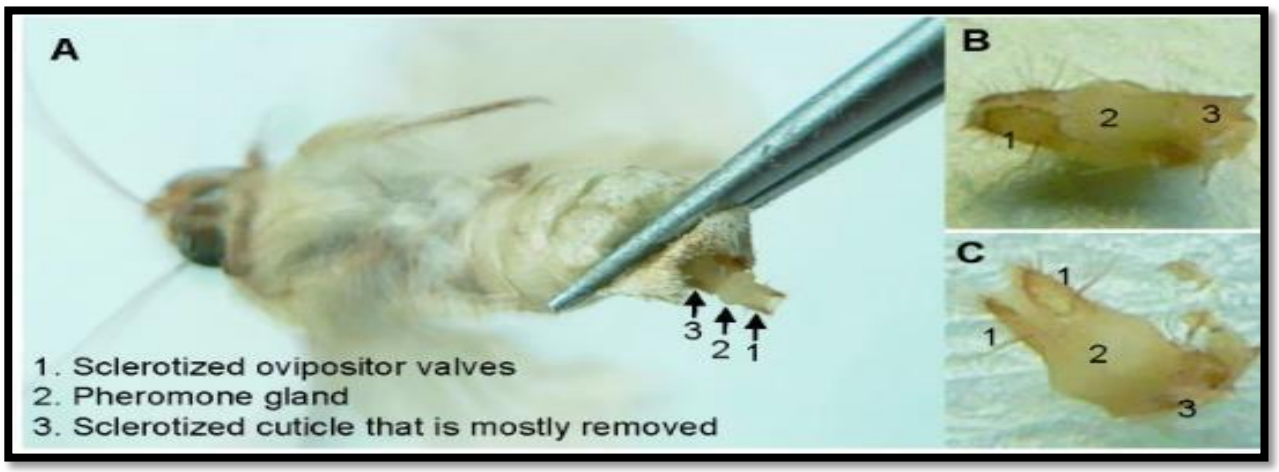

Image2: Pheromoneglandin silkworm

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(Figures courtesy :https://www.researchgate.net/figure/Dissection-of-Hv-sex-pheromone-gland-for-RNA-extraction-A-Gland-was-forced-out-by_fig1_41027410)

Pheromone Detection In Insect –Accessory Olfactory System:

The moth's sensory pathways during interaction with its surroundings heavily rely on the olfactory system. The antenna transmits chemical and to a lesser extent, anemometric data to the Antennal Lobe (AL), from where it is transmitted to the upper centers of the calyces in the mushroom body. Chemical detection is handled by ORNs at the antenna. The AL may have the job of amplifying and encoding this smell data, keeping the pertinent elements and encapsulating it for use by higher brain regions and motor systems. Component ratios, temporal features, quality, concentration, and identity of the odor are among the pertinent coding parameters. The lateral horn (LH) and mushroom body calyces (MB) receive the spatiotemporal output that the AL uses to encode some or all of these factors for sensory integration and memory. The moth's nervous system's ability to accomplish this could provide important insights into the fundamental processing ideas needed to tackle the particular odour coding problem. This is especially true when it comes to extracting pertinent information for the detection task, both spatially and temporally (Pearcea et al., 2004).

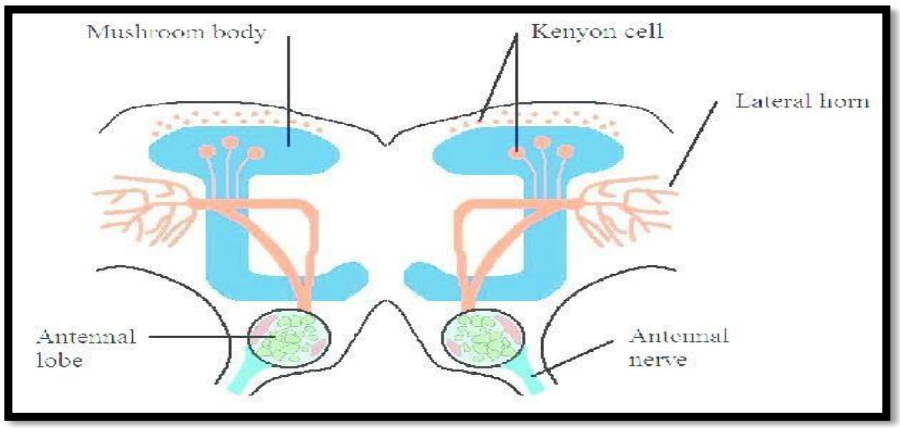

Image3 : Accessory olfactory system

(Figures courtesy : https://www.researchgate.net/figure/horizontal-section-through-the-brain-of-the-insect-showing-the-main-elements-of-the_fig2_50420641)

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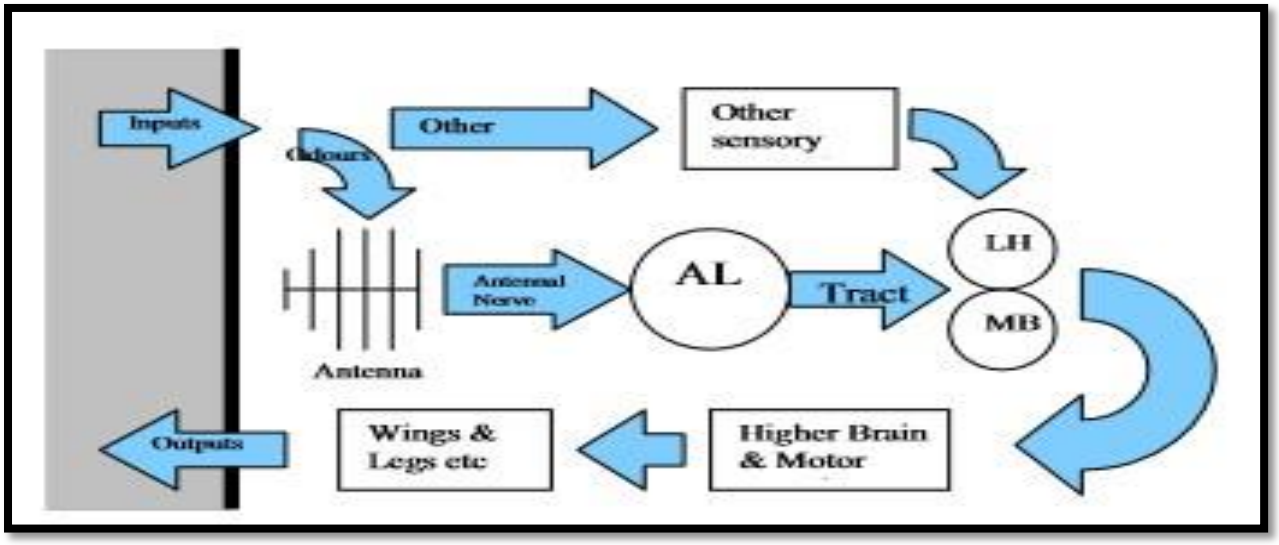

Image 4: Overall system context of the pheromone detection system in moths (shown incentre). AL is the antennal lobe, MB the mushroom bodies and, LHthe lateral horn. Thisschematicshowstheanimalinan„input/output"diagramwithrespecttoitsenvironment.
(Pearcea*etal*.,2004).
(Figures courtesy: https://www.researchgate.net/publication/225964081_Chemotactic_Search_in_Complex_Environments)

The olfactory detectors

On the antenna, the detection happens. Sensilla, which resemble hair-like cuticular structures and contain ORNs, are plainly seen in Image 5. One to three ORNs, which shoot a dendrite up into the hair, are housed in each sensillum. Dendritic receptor proteins on this site function as a lock for a specific odor "key." Therefore, the smell molecule's identifying location is the receptor protein. Since chemical cues are commonly found in blends, moths typically use multiple molecular cues for pheromone communication. Interfering with the blends can prevent appropriate behavioral responses, and varying the chemical composition, number of components, and ratio of concentrations of these blends appears to be important to the pheromone detection task since different species frequently use similar chemicals in different contexts. Due to this, ORN responses within the sensilla are frequently highly specialized to specific components of the blend and have very specific response patterns. For instance, even at very high concentrations, the two major pheromone components of S. littoralis do not excite each other's respective ORNs, allowing for extremely specific and selective responses. Thus, one crucial and evident aspect of moth pheromone reception is the activation of highly unique receptors for each pheromone. When a pheromone molecule contacts with the receptor protein, a transduction cascade sets off the production of a neurological signal. The ORN cell body, which is situated immediately below the sensillum's base, receives this signal. Action potentials are set up there and they go via the ORN axon to the AL, the moth brain's primary olfactory center (Pearcea et al., 2004).

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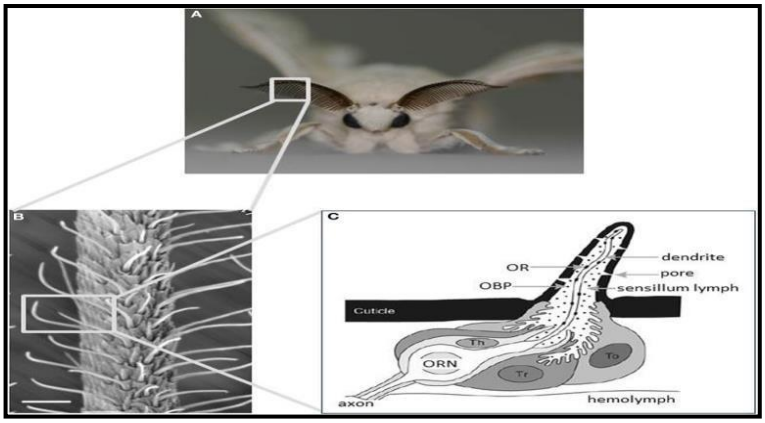

Image5:Antenal lobe

(Figurecourtesy:https://www.researchgate.net/figure/Main-olfactory-sensory-organs-of-the-silkmoth-Bombyx-mori-A-A-male-silkmoth-with-its_fig1_261764615)(Sakurai*etal.*,2014)

Antenal lobe:

Antennal lobe ORN axons terminate in structures – Glomeruli. ORNs innervate the glomeruli – spheroids of tightly packed dendritic and axonal branches. First-order synaptic neuropil are where ORNs, and the two major classes of AL neuron, principal neurons (PNs) (Image 6) and local interneurons (LNs) interconnect (Pearcea et al., 2004).

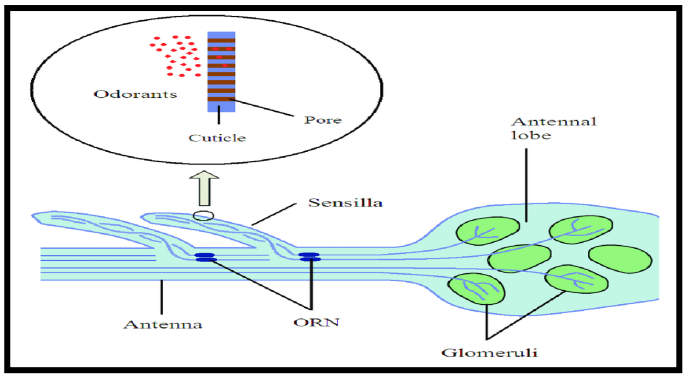

Image6:Antenal lobe

(Figurecourtesy:https://www.researchgate.net/figure/b-Insect-olfactory-sensilla-The-oRNs-are-compartmentalised-into-sensory-hairs-to-detect_fig4_50420641)

Behaviour ecology of pheromone mediated communication in moths:

Male moths can use incredibly low pheromone concentrations to identify partners over vast spatial scales (hundreds of meters). The quality of the pheromones emitted by the female is the primary determinant of this behavior, but the visual system also acts as a gate that is flight is only launched in the presence of visual signals, and visual cues also appear to be necessary for the target's actual localization and landing behavior. It has been shown, therefore, that eyesight is not necessary for the general search behavior. A particular plume shape is produced by the

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female moth's discharge of a sex attractant pheromone blend, which is conveyed downwind, as seen in (Image 7). Other species that produce comparable pheromones in the vicinity employ alternative night time periods to prevent incompatible mating behaviors and cross-pollination. When a male moth finds its unique pheromone blend, it tracks the filament of the intercepted plume as it glides slowly upwind. he plume that directs the male moth toward the female is composed of pockets that contain high pheromone concentrations surrounded by clear air. Its structure is highly intricate, erratic, and governed by intricate patterns. It is observed that the chemical plume has a filamentous structure, with the filaments moving downwind. As the plume moves away from the source, the clean air gaps widen and combine to form a plume that is made up of successive bursts of pheromones with varying concentrations and spacing. This structure varies in both location and time, causing changes in the concentration of its constituent parts due to the burst's longer duration and longer interpulse interval. It's interesting to note that individual air molecules can travel many meters in straight lines even in tumultuous wind conditions. It has been proposed that moth chemical search behavior is influenced by the following essential characteristics of chemical plumes: Because of the intricate dynamics and structure of chemical plumes, male moths must use more sophisticated search strategies in addition to gradient-based chemotaxis in order to locate a partner. In fact, at least two stereotyped behaviors are said to comprise the search behavior of male moths (Image 8):

Cast : A zigzag movement orthogonal to the wind direction in the absence of any pheromone filament

Surge: An upwind flight caused by contact with apheromone filament.

Surprisingly, the male moth frequently finds its plume closer to the source when it loses it. Furthermore, it consistently maintains an altitude in proximity to the plume, and it typically has a body that is not perfectly aligned with the direction of the wind. The male moth's speed declines and its casting frequency rises as it approaches the source. A landing near the pheromone source and a potential reward result from this (Pearcea et al., 2004).

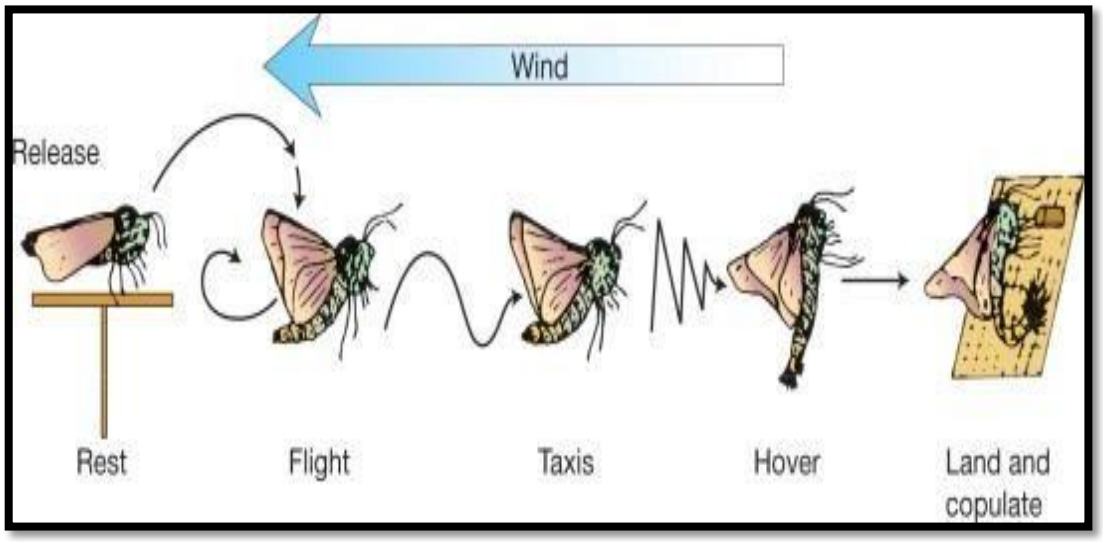

Image 7 : Typical responses of male noctuid moths to the sex pheromone released by female moths. **moths.**

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(Figure courtesy : https://www.sciencedirect.com/topics/earth-and-planetary-sciences/sex-pheromone)

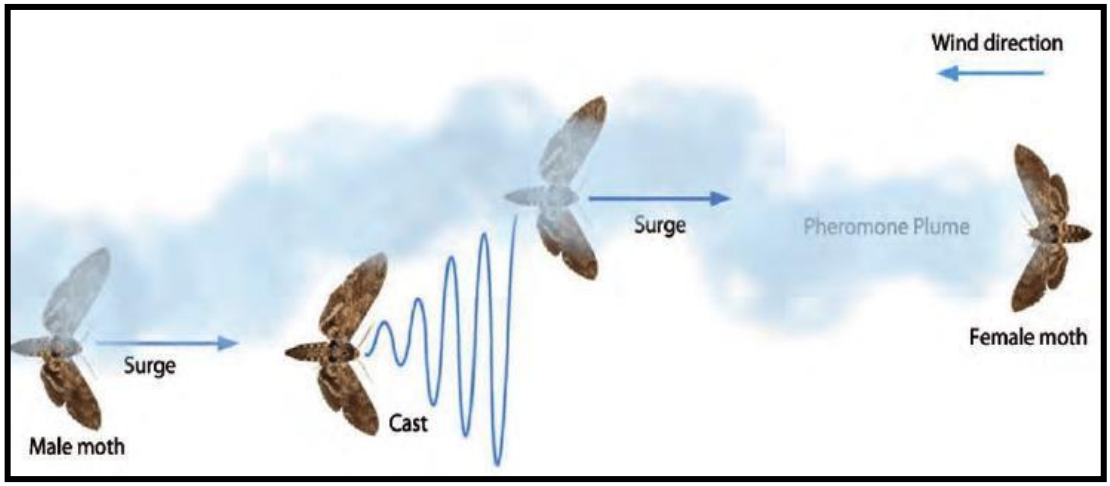

Image 8 : Illustration of the cast and surge male moth behaviour and the female pheromone plume

The attracted sex uses the concentration gradient to find the signaler. According to early research, a point source's scent diffuses in a cone-shaped plume that grows downwind; the plume's shape is dependent on airspeed and vegetation structure. Nevertheless, more recent research suggests that this plume is affected by turbulence in the airstream, which creates pockets of greater concentration or lack of the vapor, rather than being straight or homogenous over extended distances. An insect downwind would not perceive the plume as a continuous stream, but rather as odorous bursts. Many insects emit vapor in pulses which increases the heterogeneity in vapor concentration. plume's symmetrical expansion from the emission site is seen in Gaussian plume model (a). Concentration can be seen in each disc distributed typically around a meandering center line in the meandering plume model (b). Pheromone plumes have a highly filamentous structure, as shown by the most current research. plume's symmetrical expansion from the emission site is seen in Gaussian plume model (a). Concentration can be seen in each disc distributed typically around a meandering center line in the meandering plume model (b). Pheromone plumes have a highly filamentous structure, as shown by the most current research (c) Because the antennal receptors need occasional stimulation to avoid saturation and maintain upwind flight, pulses in emission and reception may make orienting easier. He however point out that direct upwind orienting may be challenging over extended distances due to the heterogeneous character of the pheromone plume (Image 9).

The attracted insect may miss the olfactory trail in areas with little to no odor.Openings in the vegetation canopy that provide warmer convection zones or "chimneys" thattransmit the pheromone across the canopy can further impede detection. Casting, or sweepingback and forth in an arcing pattern until the plume is met again, can help attracted insects boosttheir odds of finding it again. As most insects are tiny and have few pheromones available forrelease, partners need to be able to react to extremely low amounts. Male gypsy moth (*Lymantriadispar*) and silkworm (*Bombyxmori*) attractants can be released at molecular concentrations aslow as 100 molecules per milliliter of air. A female's release of less than 1 ug/sec can do

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this(Schowalter*et al*., 2006).

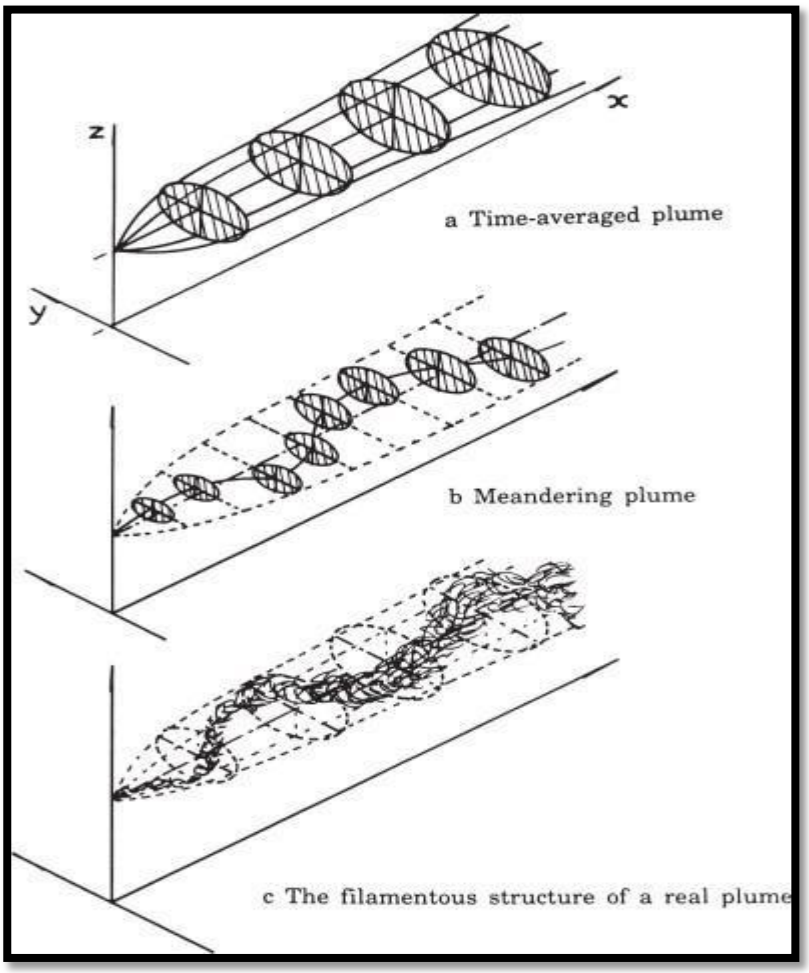

Image 9 : Models of pheromone diffusion from a point source.
(Schowalter et al., 2006)
(Figure courtesy : https://www.sciencedirect.com/topics/earth-and-planetary-sciences/sex-pheromone)

Other parameter that affects the release of pheromone in moth

In certain instances, temperature can also affect when pheromones emit. Although the pheromone is normally released at night, in colder climates the females can release it early in the day, even in the presence of sunlight. The responses of the male moth have been found to be influenced by specific chemical substances. A chemical found in the pheromones of other species called a behavioral antagonist affects the olfactory search for the pheromone source. In the presence of the behavioral antagonist, male moths often are unable to track a pheromone plume, even at concentrations as low as 1%. In these situations, it is possible to see a considerable rise in the latency to surge along with a decrease in the surge displacement and flight time. Receptors that are responsive to both the behavioral antagonist and the secondary pheromone component can recognize antagonists. Given that the antagonist of certain species is a pheromone component of other species, this seems to offer the best means of reducing the uncertainty regarding the species of the calling female (Pearcea et al., 2004)

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Pheromone produced in Moths

Table1:Pheromone produced in Moths

Important insect pests	**Pheromones**
American Bollworm,*Helicoverpa armigera*	(Z)-11-hexadecanal,(Z)-9-hexadecenal (Zhang *et al*., 2012)
Pink bollworm,*Pectinophora gossypiella*	(Z,Z)and(Z,E)7,11-hexadecadienyl acetate (1:1)(Hussain*et al*., 2021)
Tobacco cut worm,*Spodopte ralitura*	(Z, E)- 9,11- tetraddecadienyl acetate and (Z,E)-9,12- tetradecadienyl acetate (10:1) (Ding *etal*., 2022)
Diamond back moth,*Plutella xylostella*	(Z) 11- hexadecenal, 11-hexadecanyl acetateand(Z)11-hexadecenol(5:5:1)Chi*etal*.,2024)
Fall armyworm,*Spodoptera furgiperda*	Z9:14AC, Z11:16AC,Z7:12ACinratioof 87:12.5:0.5(Robert *et al*., 2019)
Tomato leaf minor, *Tutaabsoluta*	(3E,8Z,11Z)-3,8,11-tetradecatrienyl acetate (TDTA) (ChermitiB.andAbbes K., 2012)

Utilization of olfactory communication in IPM

I. Monitoring

Monitoring is a vital component of pest management programs and is an efficient means of identifying insect population trends. Behavioral manipulation based on pheromone has been created as a pest monitoring method. It uses a pheromone that is synthesized and combined with a dispenser and trap to attract and capture the intended insect. Still, the method can be adjusted to fit the needs of particular agroecosystems. The identification and tracking of significant insect pests in crops is one of the most common and effective uses of sex pheromones. Pheromone-baited traps are able to identify insect infestations early on in their existence. Trapping data collected across a larger region can be used to detect the emergence and spread of invasive pests as well as to offer information on the migration and dispersal of the target pest. One of the main uses of pheromone levels (ETL) of a pest damage is to choose the best time and method for

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controlling the infestation. The choice of insecticidal spray is made using the male catches in the traps because, in the case of PBW in particular, the damaging symptoms and life phases of the pest are not evident during the early stages of infestation. Apart from population monitoring, the data collected from trap catches over a crop season offer valuable insights on the life cycle, seasonal patterns, and other essential aspects of the biology and ecology of the pest insect. It would be possible to project insect damage using simulation modeling and prediction with the additional confirmation of weather and geography data. T. absoluta Meyrick (Lepidoptera: Gelechiidae) is a serious global pest of tomatoes. Homemade traps, such as translucent plastic cylinders, or water traps enticed with sex pheromone dispensers, have been used for mass trapping (Abbes et al., 2011; Lobos et al., 2013). The best placement for the traps is close to ground level, and they should ideally be filled with 0.5 mg of pheromone. When 48 traps were used per hectare, leaf damage was decreased more effectively than with traditional pesticide treatment. An comprehensive dataset of temperatures and PBW moth trap captures from India's cotton-growing states is used to create a degree-day-based model that forecasts the phenology of this hated pest. The model predicts the length of the cropping season and the temperature for a total of seven generations of PBW. The science of phenology examines how biological processes, such insect growth, are influenced by the weather. The ideal time to apply pesticides and other pest management measures can be predicted with the use of phenology models, sometimes referred to as degree-day models (Rameash, 2022).

II. Mass Trapping

Mass trapping is a direct control technique that lowers the target species' population density or pest damage by using a large number of pheromone traps. When the quantity of pheromone sources in both control approaches is equivalent, mass trapping is more effective than mating disruption. This is due to the fact that although traps permanently postpone sex, mating disturbance merely temporarily delays it. This technique was most successive at managing weevils as usually manage by aggregation and less at The effectiveness of the application can be influenced by a number of variables, including trap design and density, population size, the biology of the target pest, isolation, and immigration risk. The target pest's pheromone composition and production costs may also be important for an economically viable mass trapping strategy (Sayed et al., 2021)

III. Mating disruption

The mating disruption device uses sex pheromone to stop male moths from finding females, which stops the target insect from reproducing. Between the source of the pheromone and the possible mate, the insect in search of a mate becomes perplexed. In 1978, PBW was used to disrupt cotton in the Coachella Valley with pheromone composition that was registered with the U.S. Environmental Protection Agency. Many dispensing strategies for PBW mating disruption have been developed globally and tested in various cotton-growing regions. Various dispensation systems have been created globally to disrupt PBW mating, such as No Mate -PBW®, PB-ROPE®, Selibate® and Distrupt®. These systems have been tested in various cotton-growing regions, including the United States, Mexico, Israel, Pakistan, India and Egypt. The Central Insecticides Board & Registration Committee has approved the commercial use of PB Rope L® for the mating disruption of PBW on cotton, with a recommended dose of 25 hectares. For season-long control, the 140 mg

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gossyplure/dispenser in the PB Rope L® is tied around and kept until the final plucking. SPLAT (Specialized Pheromone and Lure Application Technology), in which gossyplure is prepared with wax and applied as dollops onto the base of leaf petiole of the top shoot of the cotton plant, is another promising formulation in the mating disruption of cotton PBW (Rameash, 2022)

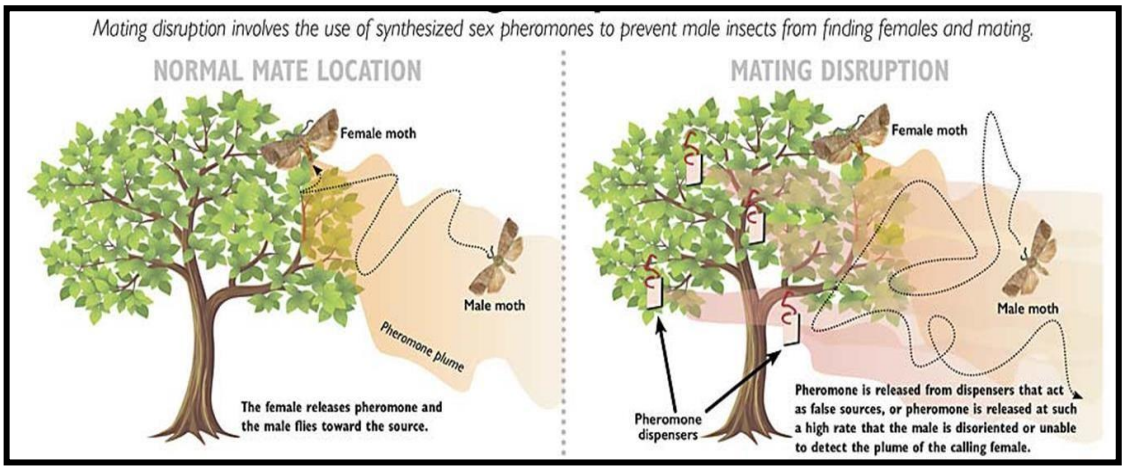

Image10:Mating disruption

(Figure courtesy : https://www.goodfruit.com/good-to-know-the-disruption-eruption/)

a. PB Rope L?

Since the PBW develops inside the boll, employing pesticides to provide sufficient control is challenging. The twisted tie known as PB-Rope L emits the same aroma that attracts male bollworms. The male adults are confused by this aroma, which keeps them from locating the female adults to mate with. Because of this, fewer eggs are deposited, which lowers the PBW population and lessens crop damage. Economically speaking, it has been determined that PBW can be effectively managed by using PB-Rope L in addition to the standard plant protection schedule. The recommended usage of PB-Rope L is 100–200 ropes/acre. If the pheromone trap counts are continuously higher than the 7 adults per day economic criterion, then higher rates ought to be utilized. Overly twisted ropes have the potential to restrict stem growth. Replace ropes with new ones after around 3.5 months as they will have run out of pheromone but the pest will still be active. Apply right before moth emergence in the field or when the cotton reaches the pin square stage. The ropes were affixed to the plant at a height of 10 to 12 cm, with 500 pieces per hectare. The ropes were spaced 4.5 meters apart and were intended to be placed at the pin square formation stage, which occurs 45 days following seeding (Patil et al., 2004). The active ingredient of PB-Rope L is (Z, Z)-7, 11-Hexadecadien-1-yl Acetate L: 46,70 % and (Z, E)-7, 11-Hexadecadien-1-yl-Acetate : 44, 15 % (Hussain et al., 2021). Moths catches in traps greatly reduced ($F= 368.8$, $P < 0.001$) in PB rope plots compared to control fields in all ecological zones. There were no significant differences in moth catches ($F= 2.35$, $P > 0.01$) among different Tehsils and cotton varieties. Moths catches in traps greatly reduced ($F= 368.8$, $P < 0.001$) in PB rope plots compared to control fields in all ecological zones. There were no significant differences in moth catches ($F= 2.35$, $P > 0.01$) among different Tehsils and cotton varieties. Moths catches in traps greatly reduced ($F= 368.8$, $P < 0.001$) in PB rope plots compared to control fields in all ecological zones. There were no significant differences in moth

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catches (F= 2.35, P > 0.01) among different Tehsils and cotton varieties. The percentage flower and boll damage in the control fields was significantly higher than those in the PB ropes fields. (Hussain et al., 2021).

Image 11: Correct placement of PB-rope L on a cotton plant.
(Figure courtesy: https://plantwisepluskнowledgebank.org/doi/full/10.1079/pwkb.20147800105)

b. SPLAT®(Specialized Pheromone and Lure Application Technology)

The SPLAT® (Specialized Pheromone and Lure Application Technology) emulsion is a novel controlled-release technology that can be tailored to distribute and shield a broad range of compounds such as phagostimulants, insecticides, and semiochemicals from degradation in a range of environmental conditions. Create novel SPLAT® products that interrupt mating, attract and kill, and repel pests in forest and agricultural environment. Using SPLAT (Specialized Pheromone and Lure Application Technology), gossyplure is combined with wax and applied in dollops to the base of the cotton plant's top shoot's leaf petiole. Pink Bollworm (Pectinophora gossypiella) is managed in cotton. with CREMIT PBW, a greenlabeled pheromone-based product. It is applied at placed on branch axils, ideally six inches below the crop canopy, in the form of tiny dollops (250 mg). Apply manually in a zigzag pattern with 400–500 dollops evenly spaced out per acre. SPLAT formulations based on phenomenology applied at the crop's source. Each SPLAT dollop delivered the necessary amount of sex pheromone to stop the mating process. The potent attraction of the pheromone that each SPLAT dollop emits has caused males to stray from females. SPLAT dollops continuously release large amounts of pheromone, in contrast to females. In contrast to conventional farmers' practices, which realized 22.34 q per ha even after four or five chemical sprays, area-wide management of pink bollworm applied at 500 g m/acre in four splits at 35-40, 65-70, 95-100, and 125-130 days after sowing recorded more than 80–90% control of pink bollworm with a maximum yield gain of 33.58 q per ha. Even 45 days later, 40% of the pheromone still present in the SPLAT-PBW treated fields prevented the male moths from mating. The natural enemies in the cotton environment were confirmed to be safe for all SPLAT dosages evaluated. PBW management's non-chemical method gives you a lot more control than traditional methods. Therefore, using SPLAT-PBW for insect family planning is now the most effective strategy to reduce the threat posed by pink bollworms

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(Sreenivas et al., 2021). The rice yellow stem borer Scirpophaga incertulas walker (Crambidae: Lepidoptera), is combated in the paddy environment by means of lure application technology (SPLAT-YSB) dispensers that release pheromone (Badariprasad et al., 2019).

IV.Push-pull strategy

The push-pull approach, which uses both an attractant and a repellent stimulus atthe same t ime The push-pull approach, which uses both an attractant and a repellent stimulus at the same time to elude pests, is a widely used sustainable substitute for conventional pesticides. Repellent stimuli are used to "push" insect pests away from the crop, while attractant stimuli are used to "pull" pests to other regions of the crop, with the goal of minimizing crop damage. In push-pull trials, sex pheromones can help determine when to introduce stimuli and when to perform other population-decreasing acts (200). The combined effect of host plant volatiles can strengthen the attraction of aggregation pheromones and courtship to herbivores. In push-pull trials against aphids, nepetalactone, one of the aphid sex pheromone component, and (Z)- jasmone a host-plant volatile that attracts aphid parasitoids, may be used to pull parasitoids into the crop (Powell and Pickett, 2003). Likewise the action of the lady beetle pheromones, pentacosane and tricosane, can push the parasitoids from surrounding areas into the field (Nakashima et al., 2004)

Insect Resistance to sex pheromones

There are currently very few reports on insects becoming resistant to sex pheromones. The pink bollworm moth, P. gossypiella (Saunders) (Lepidoptera: Gelechiidae), was the subject of the first study on the possible development of pheromone resistance by Haynes and colleagues. According to Haynes et al., (1984), there are contemporary alternative IPM strategies to stop the emergence of sex pheromone resistance, even though resistance to the P. gosspiella pheromone is still an opportunity when utilized extensively in controlling insect pests as a mating disruptant. However, resistance appeared unlikely to develop because artificial pheromones used to disrupt mating did not result in any insect deaths. According to Haynes and Baker, (1988) who found an effective defense against disrupting pheromones in insects would be a small alteration in pheromone production this may be due to selection pressure provided by the continuous application of mating disruptants for population control..

Conclusion

Compared to biological control and pesticide technologies, the development of insect pheromone-related technologies for monitoring endemic pest populations, identifying invading species, mass trapping for population reduction and distrupting mating patterns has been relatively recent in IPM. Recent developments in the application. Use of pheromone is being made in a number of areas, one of which is the understanding that mass trapping can be a very efficient and profitable application of theses behaviour-modifying substances. Novel luretrap technologies like SPLAT, PB Rope L are continue to be developed for many new pests. This unique techniques have less chance of development of resistance in insect to this pheromone and being eco-friendly nature. It can be better alternative and can be integrated into IPM starategies.

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Farmer should follow community level pest management by pheromone.

Futurescope:

A dopting this strategy at community level will help the farmer to overcome problem of pest damage. Need to prepare SPLAT based pheromone products in different crops.Need to work on long lasting pheromone traps by making research in plastics use in trap.There is emerging need to work on finding different new pheromone component and wide scope for development of new formulation based pheromone.

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CHAPTER- 8
BIOREMEDIATION STRATEGIES FOR GREEN AND SUSTAINABLE ENVIRONMENT

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Abstract

Bioremediation is a broad concept that includes many levels of multiscale complexity involved in the removal of hazardous waste from polluted locations.Bioremediation is defined broadly as any process in which a living or dead biological system (typically bacteria, microalgae, fungi and plants) is used to remove environmental pollutants from air, water, soil, gasses, industrial effluents, and other sources in natural or artificial settings.In comparison to traditional physicochemical treatment approaches, bioremediation may have significant benefits since it aspires to be sustainable, environmentally friendly, inexpensive, and scalable.The inherent capacity of organisms to adsorb, collect, and degrade prevalent and new contaminants has led to the use of biological resources in the remediation of polluted environments. Bioremediation has become a widely recognized remediation methodthat is improving consistently. Bioremediation integrates the tools of many disciplines. Opportunities for novel bioremediation approaches develop when advances in each field give birth to new cleanup needs. This chapter focuseson principle of Bioremediation, various*Ex situ* or *in situ* bioremediation techniques that can be used, depending on a variety of factors, such as cost, pollutant types, and concentration, their limitations and what the future holds for bioremediation in order to reduce the amount of pollution in the world.

Keywords: Bioremediation, *Ex-situ* or *in-situ* techniques, environmental pollutants, sustainable, microorganisms

Introduction:

The worldwide industrialization and the extensive consumption of chemical compounds such as petroleum products, solvents, pesticides, and heavy metals lead to soil, water, and air pollution. As a result, the public is increasingly concerned about the risk that these polluted settings provide to human and ecological health. They stay hazardous in the environment for much longer. Many of these contaminants are mutagenic for both humans and their environment. Heavy metals can accumulate in the brain, liver, and kidneys. Other consequences for animals include cancer, nervous system damage, reduced development, and even death. Heavy metals in soils impair food quality and quantity because they hinder nutrient absorption, plant development, and physiological metabolic processes. Furthermore, expanding population and rapid urbanization need the restoration of contaminated areas for redevelopment and effective exploitation (Megharaj*et al.*, 2014).

The traditional method of remediation, which involves simply removing soil from a contaminated site and dumping it in a landfill ('dig and dump'), is not a sustainable solution because it only moves the contaminant from one location to another and poses a significant risk due to the requirements for excavation, handling, and transportation of hazardous materials (Masindi*et al.*, 2021).

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Bioremediation uses living organisms, mainly microorganisms (bacteria, fungi and microalgae) to decompose environmental contaminants. Other names used for bioremediation are biotreatment, biorestoration and bioreclamation. It is a cost-effective and environment friendly method to purifycontaminated soil and water, and is emerging as an alternative to costly physicochemical remediation technologies. Numerous microorganisms, particularly bacteria, microalgae, and cyanobacteria, are capable of using harmful organic pollutants as sources of carbon, energy, and other nutrients. However contaminated sites are generally complicated because contaminants exist in combination, such as organics with inorganics or parent compounds with their degradation products. Therefore, successful bioremediation must take an integrated strategy that includes disciplines such as microbiology, engineering, chemistry, ecology, and geology. The main advantage of bioremediation is that on-site cleanup reduces site interruption and avoids shipping expenses. Another significant advantage of bioremediation is the conversion of the pollutant to carbon dioxide, water and biomass, which results in total and permanent elimination of the contaminant, hence reducing the risk and long-term obligation.

Mechanisms through which microorganisms and plants remove or transform environmental pollutants are being studied continuously. Still bioremediation research has largely focused on bacterial processes. Many hostile situations have degraded, through bioremediationincluding the removal of contaminants from acidic, hyperthermal, hypersalineor industrial waste (Krzmarzick,2018; Kaur*et al*., 2021). Use of more than one living organism will improve the efficiency and results. Recent research on bioremediation is heavily focused on inoculation of a polluted site with organisms or supplying nutrients to promote the growth. Many researchers employed bioremediation technology for the treatment of various pollutants, including organophosphate pesticides such as chlorpyrifos, methyl parathion, etc, andremoval of organic and inorganic pollutants (Sharma*et al*., 2021; Tripathi*et al*., 2011; Tripathi and Garg, 2014; Sonawane*et al*., 2022).Typical bioremediations involve oxidations that enhance the water-solubility of organic compounds and make them vulnerable to oxidation and hydrolysis that ultimately biodegrades hydrocarbons to harmless by products like carbon dioxide and water. Organic contaminants can be biodegraded, but heavy metals are oxidized or reduced. Hence heavy metals bioremediation needsto be further investigated.

Microbial enzymes benefits bioremediation approaches to break downhydrocarbons into less harmful compounds. Recent researches focus on role of genetically-modifiedmicroorganisms that could contribute to eliminate petroleum, naphthalene, toluene, benzene,and other xenobiotic chemicals.

Bioremediation outcome is affected with numerousreasons, including temperatureof the surrounding environment, oxygen availability, and available nutrition. The main challenge to bioremediations is rate as these processes are slow.Emerging environmental pollutants that are of synthetic or natural origin reach ecosystems mainly through anthropogenetic activitiesand cause harm to lifeforms like plants, animals, and humans. One of the mostcost-effective and promising and environment friendlybiotechnological innovations is bioremediation. (Megharaj*et al*., 2014). This chapter addresses principle of Bioremediation , microorganisms used in this approach, recent

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in situ and ex- situ approaches and their limitations including information on future prospects of Bioremediation for green and sustainable decontamination to control environmental pollution.

Principle of Bioremediation:

Bioremediation" is the term used to describe the process where pollutants in the environment are degraded or detoxified under controlled conditions by using biological organisms. It is the process of converting harmful organic pollutants into non-toxic byproducts like carbon dioxide and water or naturally-occurring inorganic compounds that are safe for use by humans, plants, animals, and aquatic life (Malik *et al.*, 2022). In order to speed up microbial growth and degradation, environmental conditions must often be controlled during the process. Table 1 depicts essential factors required for bioremediation. Living organisms and fertilizers can aid in the process of bioremediation, which occurs naturally and is encouraged (Ren*et al.*, 2018). Enzymes play a crucialpart in every stage of the metabolic process like family of oxidoreductases, lyases, transferases, and hydrolases.Non-specific and specific substrate affinities permit many enzymes to degrade a wide range of pollutants. Enzymatic action on the pollutants defines the success of bioremediation

Factors	**Desired conditions**
Microbial culture	Appropriate kinds of micro-organisms that can degrade all of the contaminants.
Oxygen	sufficient to facilitate aerobic biodegradation
Water	Soil moisture levels should range between 50-70% of its capacity to store water.
Nutrients	Nitrogen, phosphorus, Sulphuretc. to support microbial growth.
Temperature	Appropriate temperatures for microbial growth (0–40˚C)
pH	Best range is from 6.5 to 7.5
Site characterization and selection	Before proposing a bioremediation solution, appropriate remedial investigation work is required to determine the degree of the pollution.

Microorganisms used in Bioremediation

A variety of microbes from different environments are utilized in bioremediation. *Achromobacter, Alcaligenes, Xanthobacter, Arthrobacter, Pseudomonas, Bacillus, Mycobacterium, Corynebacterium, Flavobacterium, Nitrosomonas,* and other microorganisms are examples of such microbes. *Bacillus, Pseudomonas, Sphingomonas, Flavobacterium, Nocardia, Rhodococcus,* and*Mycobacterium* are aerobic bacteria that can degrade a variety of complex organic compounds. Degradation of pesticides, aromatic compounds and alkane hydrocarbons havebeen reported by these aerobic microbes (Kour*et al.*, 2021)Amphibious bacteria are becoming increasingly prevalent for the bioremediation of polychlorinated biphenyls, chlorine compounds,trichlorethylene and chloroform that degrade and convert pollutants to fewer toxic forms. Several bacteria, such as sulfate-reducing bacteria, Pseudomonas andAeromonas have been used in the bioremediation process under anaerobic conditions (Tegene and Tenkegna, 2020).

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Various techniques for Bioremediation

Bioremediation techniques can be classified as (i) *in situ* techniques, which directly treats polluted sites, (ii) *ex situ* techniques that are applied to excavated materials. Some examples of bioremediation related technologies are phytoremediation, bioventing, landfarmingbioattenuation, composting (biopiles and windrows), andbiosparging. Other remediation techniques include thermal desorption,bioleaching, rhizofiltration, soil washing, vitrification and, air stripping.In order to enhance the growth and metabolism of the microorganisms in these approaches, additional pH buffers, nutrients, vitamins and minerals are added. In some cases, specialized microbial cultures are added (biostimulation). The end goal of bioremediation is to remove or reduce detrimentalpollutants to improve quality of soil and water (Azubuike*et al.*, 2016). Bioremediation can be used in a plethora of ways, and some of the most commonlyused strategies are presented below (Figure 1).

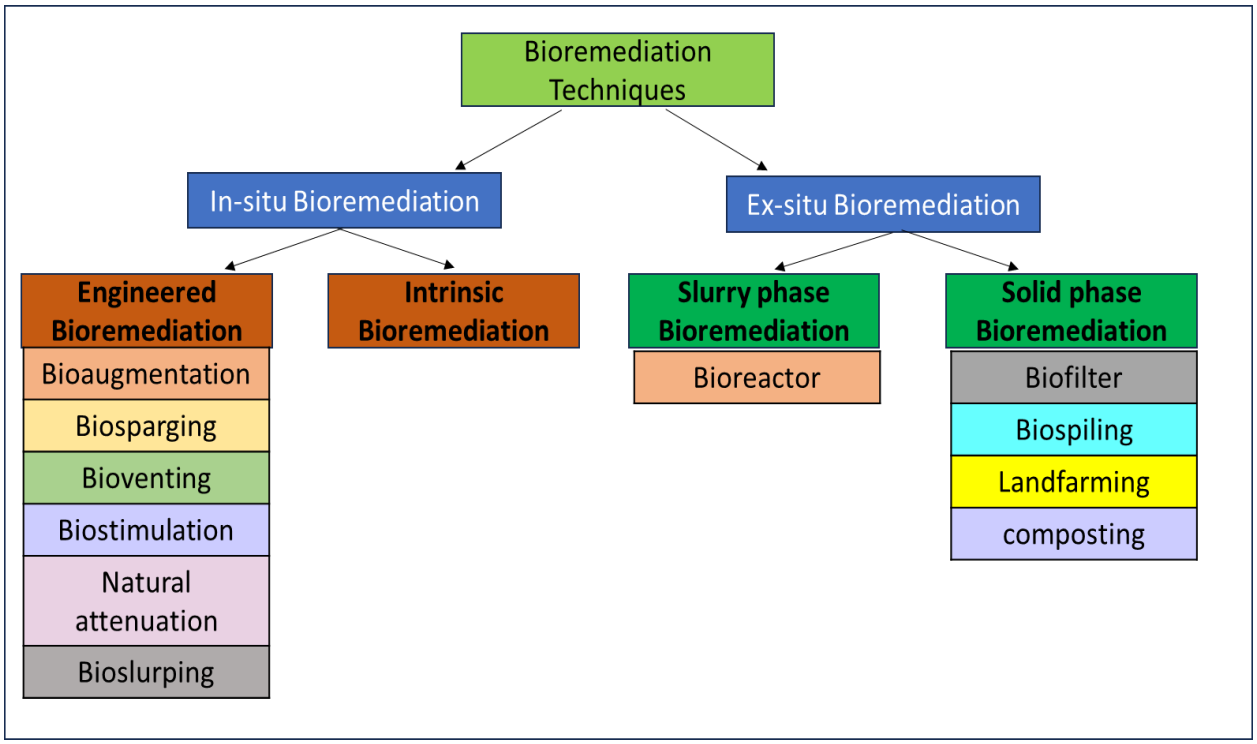

Fig. 1 Various Bioremediation strategies

A. *In-situ* Bioremediation strategies:

In-situ bioremediation strategies involve treating contaminated sites directly without removing the contaminated material from the polluted site. These techniques utilize naturally occurring microorganisms or introduce specific microbial cultures to degrade or transform contaminants in place.These techniques are applied to polluted soil and groundwater at settings with minimal disturbance. Bioventing, biosparging, intrinsic bioremediation,phytoremediation and natural attenuation are few examples of *in situ* bioremediation techniques. *In-situ* bioremediation can be are of two different types: intrinsic and engineered. *In-situ* bioremediationcan be expensive due to specialized equipment but it is preferred over ex-situ methods as it often causes minimal disturbance to the soil structure. Each *in-situ* bioremediation strategy has its advantages, limitations, and applicability depending on the specific contaminants, site conditions, regulatory requirements, and remediation goals. Additionally, a combination of techniques may be employed to optimize

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remediation efficiency and achieve desired outcomes. Regular monitoring and ongoing optimization are often necessary to assess the effectiveness of in-situ bioremediation strategies and make adjustments as needed. *In-situ* bioremediation methods have successfullypreserved sites polluted with,toxic metals, paints,chlorine and hydrocarbons (Sharma, 2019; Azubuike*et al*., 2016).

a. Intrinsic *in-situ* bioremediation:

Intrinsic bioremediation techniques involve utilizing naturally occurring microorganisms present in the environment to remove pollutants from soil, water, or air. These techniques are often favored due to their cost-effectiveness and minimal environmental disruption. Intrinsic in situ bioremediation can be performed using anaerobic reductive dechlorination, bioaugmentation, biostimulation, amendment delivery, biofilteration, biosparging,aerobic treatment and bioslurping techniques.These intrinsic bioremediation techniques can be applied individually or in combination, depending on the nature and extent of contamination, site-specific conditions, and regulatory requirements.In situ bioremediation has been used as a method for the biological treatment of clogged groundwater, using a stimulation–optimization approach.Microbial inoculants can be utilized for the *in- situ* treatment of heavy metals. Cr (VI) interacts with Fe (II) ions also through the redox reactions, and the release of iron in soluble forms promotes the reductive reactions (Zhang *et al*., 2021). The major limitation of intrinsic *in-situ* bioremediation is that the site has to be very permeable soil.

b. Engineered *in-situ* bioremediation

Engineered in situ bioremediation refers to the deliberate design and implementation of bioremediation techniques within a contaminated site to enhance the natural processes of microbial degradation. This technique employs microorganisms that have undergone genetic engineering in order to optimize remediation efficiency. In this method, a specific microorganism is brought into the contaminated site that is accompanied by providing the physicochemical conditions for optimal growth of microorganisms (Kumar, 2018).Success of engineered in situ bioremediation depends on careful planning, implementation, and ongoing management.

b1. Bioventing:

Bioventing is another form of bioremediation used to treat contaminated soil and groundwater. It's specifically designed for sites contaminated with petroleum hydrocarbons, such as gasoline or diesel fuel. In bioventing, air (usually containing oxygen) is injected into the contaminated soil to enhance the activity of naturally occurring aerobic microorganisms, which metabolize the contaminants. These microorganisms require oxygen to break down the hydrocarbons into harmless byproducts like water and carbon dioxide. The process is typically implemented by installing wells or pipes into the contaminated soil, through which air is injected. The injected air helps to create optimal conditions for microbial activity, facilitating the degradation of the contaminants over time. Bioventing is considered a cost-effective and environmentally friendly remediation technique, particularly suitable for sites where traditional excavation and removal methods may be impractical or too disruptive. It's often used in combination with other remediation methods to achieve comprehensive cleanup of contaminated sites. This method promotes the natural *in-situ* aerobic biodegradation of organic contaminants, such as petroleum hydrocarbons, phenols, and other

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reduced pollutants by enhancing the microbial metabolism.However, this approach has the problem of only working at the lowest depths of the polluted soil environment (García *et al*., 2010).

b2. Biostimulation

Biostimulation primarily aims to improve the activity of indigenous microorganisms by providing them with nutrients, electron acceptors (e.g., oxygen, nitrate), or other growth-promoting substances that are insufficient in the soil. Common nutrients include nitrogen, phosphorus, and potassium, which can stimulate microbial activity and increase the rate of pollutant degradation. Biostimulation is done by indigenous microorganisms that are well-adapted to the environment, and are already well dispersed spatially which makes it more beneficial approach. Still the challenge is to deliver additives so they are easily available to the subsurface microbes.Specific to marine oil spills, nitrogen and phosphorus have been key nutrients in biodegradation bacteria can in principle be used to degrade hydrocarbons. (Yadav *et al.,* 2018).

b3. Bioslurping

Bioslurping is a comparatively innovative technique for soil and groundwater remediation that combines elements of vacuum-enhanced recovery (also known as soil vapor extraction) with in situ bioremediation. It's particularly effective for sites contaminated with light non-aqueous phase liquids (LNAPLs), such as petroleum hydrocarbons. Bioventing and bio stimulation are used in conjunction with vacuum-assisted pumping, and soil vapors extraction (SVE) in this method for restoration of soil and groundwater. Liquid is removed from the free product layer with the help of a “slurp” that spreads into the layer. LNAPLs are pumped to the surface by the machine, where they are separated from the surrounding air and water. This method is cost-effective on storage, disposal, and treatment as it requires less ground water, even though it’s not ideal for remediation in low-permeable soils. Bioslurpingnecessitates 25 feet of excavation below the ground surface and then the contaminants floating on the water can be removed(Tong, 2018; SookhakLari*et al*., 2019).

b4. Biosparging

Biosparging is an environmental remediation technology that involves injecting air or oxygen into contaminated soil and groundwater to enhance the activity of indigenous microorganisms that degrade organic contaminants. This process promotes the biodegradation of pollutants, such as petroleum hydrocarbons, by providing the necessary oxygen for microbial activity.The injected air or oxygen increases the bioavailability of contaminants by aerating the soil and groundwater, which stimulates microbial growth and metabolic activity. As microorganisms break down the contaminants, they convert them into less harmful byproducts, such as carbon dioxide and water. Biosparging is often used in conjunction with other remediation techniques, such as bioventing and soil vapor extraction, to address a wide range of organic contaminants in soil and groundwater. It is particularly effective for sites contaminated with petroleum products, such as gasoline, diesel fuel, and oil. Overall, biosparging offers a cost-effective and environmentally friendly solution for remediating contaminated sites, allowing for the restoration of soil and groundwater quality (Johnson *et al*., 2001).

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Bioattenuation, also known as natural attenuation or intrinsic bioremediation, is a process by which natural biological and chemical reactions in the environment reduce the concentration and toxicity of contaminants without human intervention. This process relies on naturally occurring microorganisms, plants, and geological formations to degrade, transform, or immobilize contaminants in soil, sediment, or groundwater.

b5Bioattenuaton

Bioattenuation, also known as natural attenuation or intrinsic bioremediation, is a process by which natural biological and chemical reactions in the environment reduce the concentration and toxicity of contaminants without human intervention. This process relies on naturally occurring microorganisms, plants, and geological formations to degrade, transform, or immobilize contaminants in soil, sediment, or groundwater. In bioattenuation, microorganisms present in the environment metabolize organic contaminants, such as petroleum hydrocarbons or chlorinated solvents, using them as a food source and breaking them down into less harmful byproducts like carbon dioxide, water, and inorganic compounds. This microbial activity can occur under aerobic (with oxygen) or anaerobic (without oxygen) conditions, depending on the specific contaminants and environmental conditions.

Bioattenuation processes can also involve physical and chemical mechanisms, such as adsorption, dispersion,volatilization, dilution and microbial degradation, which further contribute to the reduction of contaminant concentrations in the environment. One of the key advantages of bioattenuation is its passive and sustainable nature, requiring minimal human intervention and often being more cost-effective compared to active remediation techniques. However, the effectiveness of bioattenuation depends on various factors, including the type and concentration of contaminants, environmental conditions (such as temperature, pH, and moisture), and the presence of suitable microbial populations (Kumar, 2018).

B. Ex-situ Bioremediation strategies:

Ex-situ bioremediation is a process of treating contaminated soil, water, or other substances by removing them from their original location and treating them in a controlled environment. This method is often used when the contaminants are too difficult to treat in situ (in their original location), or when it's not feasible to do so due to various reasons like accessibility or safety concerns. In ex-situ bioremediation, the contaminated material is excavated and transported to a treatment facility where it can be treated using biological processes. These processes typically involve the use of microorganisms, such as bacteria, fungi, or plants, to degrade or neutralize the contaminants. The conditions in the treatment facility can be optimized to enhance the activity of these microorganisms, such as controlling temperature, pH, and nutrient levels.

Common techniques used in ex-situ bioremediation include composting, landfarming, biopiling, bioreactors, and soil washing. Each technique has its advantages and limitations depending on the type and extent of contamination, as well as the specific site conditions(Azubuike*et al*., 2016).

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1.Biopiles

Biopiling is a bioremediation technique used to clean up contaminated soil or groundwater. It involves creating piles, or "biopiles," of contaminated material and then stimulating the growth of microorganisms that naturally break down the contaminants. These microorganisms can include bacteria, fungi, and other microbes that metabolize pollutants, such as petroleum hydrocarbons or heavy metals, into less harmful substances. Biopiling is often used in conjunction with other remediation techniques and can be a cost-effective and environmentally friendly way to clean up contaminated sites. Biopiles are used to reduce petroleum pollutants by introducing aerobichydrocarbons to contaminated soils.However, the extent of weathering can change the chemical make-up by making the materials more hydrophobic, limits the potential of the biopiling method for biodegradation (Azubuike et al., 2016).

2. Windrows:

Windrows are long, narrow piles of compostable materials that are arranged in rows and turned periodically to aid in the composting process. They are commonly used in composting operations, particularly in large-scale facilities for organic waste management.

The process of creating windrows involves piling organic materials, such as yard waste, agricultural residues, food scraps, or manure, into long, narrow rows. These rows are typically several feet high and wide, allowing for proper aeration and decomposition of the materials.

Turning the windrows regularly is essential to ensure even decomposition and proper aeration throughout the pile. This turning process helps mix the materials, introduces oxygen into the pile, and redistributes heat generated by microbial activity. The frequency of turning depends on factors such as the type of materials being composted, environmental conditions, and the desired composting time.

Windrows provide an efficient and effective way to compost large volumes of organic waste, producing valuable compost that can be used to improve soil fertility and structure. They are often used in commercial composting facilities, municipal composting programs, and agricultural operations. Additionally, windrows can be covered with a layer of finished compost or a composting fabric to help retain moisture and heat, further promoting decomposition (Cózar et al., 2021)

3. Landfarming:

Landfarming is a technique used for the remediation of contaminated soil. It involves the treatment of contaminated soil by spreading it out over a designated area of land and periodically mixing it to encourage biodegradation of the contaminants by naturally occurring microorganisms in the soil. The process typically begins by excavating the contaminated soil and spreading it in a layer over the land. Various amendments may be added to the soil to enhance microbial activity and promote the breakdown of contaminants. These amendments can include organic materials such as compost, manure, or agricultural residues, as well as nutrients like nitrogen and phosphorus, which can stimulate microbial growth. Over time, the soil is periodically turned or tilled to incorporate oxygen and ensure uniform distribution of contaminants and amendments. This promotes aerobic conditions favorable for microbial degradation. The process may take several months to years, depending on factors such as the type and concentration of contaminants, soil properties, and climate conditions (Kumar, 2018).While landfarming is a relatively low-cost and sustainable remediation option, its

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effectiveness can be influenced by factors such as soil type, climate, and the presence of inhibitory substances. Additionally, careful monitoring is required to ensure that contaminants are adequately degraded and that the remediation process does not cause adverse environmental impacts, such as groundwater contamination or emissions of volatile compounds (Azubuike et al., 2016).

4. Biofilters:

Biofilters are air or water filtration systems that use living organisms, such as bacteria, fungi, or plants, to break down pollutants. In biofiltration, contaminants are removed or transformed by biological processes, converting them into less harmful substances like water and carbon dioxide. Biofilters offer several advantages, including being environmentally friendly, cost-effective, and sustainable compared to traditional filtration methods. They can also be tailored to target specific contaminants and are relatively low maintenance. However, their efficiency can be influenced by factors like temperature, pH, and nutrient availability, and they may require regular monitoring and optimization to maintain their effectiveness. Additionally, biofilters may have limitations in treating certain types of pollutants or high pollutant concentrations (Azubuike et al., 2016).

Conclusion andFuture Prospects:

Bioremediation stands out as a sustainable technology with immense potential for addressing environmental contamination while promoting ecological restoration. Its ability to harness the natural processes of microorganisms and plants to degrade or neutralize pollutants offers several advantages over traditional remediation methods that often rely on energy-intensive processes or produce harmful by-products. With ongoing research, technological innovation, and collaborative efforts, the future of bioremediation looks bright, offering hope for cleaner environments and healthier ecosystems for future generations. Researches are going on engineering microbes with enhanced capabilities to degrade specific pollutants. These genetically modified organisms could be tailored to target various contaminants more effectively.Nanomaterials are being explored for their potential in bioremediation. Nanoparticles can be designed to adsorb pollutants and enhance microbial activity, improving the efficiency of remediation processes.Bioaugmentation involves introducing specialized microbial cultures into contaminated sites to enhance biodegradation. Biostimulation involves modifying environmental conditions to stimulate the growth and activity of indigenous microorganisms. Future research may focus on optimizing these techniques for different types of pollutants and environmental conditions.Bioremediation can be integrated with other remediation technologies such as phytoremediation, electrokinetic remediation, and chemical oxidation. Combining these approaches can provide synergistic effects and improve overall remediation outcomes.Advances in computational modeling and environmental monitoring technologies enable better prediction and tracking of bioremediation processes. Real-time monitoring can provide valuable insights into microbial activity and contaminant degradation rates, facilitating more efficient remediation strategies.Research into bioremediation techniques suitable for extreme environments, such as deep-sea oil spills or contaminated Arctic regions, could expand the applicability of bioremediation to previously inaccessible areas. As bioremediation technologies evolve, it's crucial to address ethical concerns surrounding the use of genetically modified organisms and ensure compliance with environmental regulations to minimize potential risks and unintended consequences.

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CHAPTER- 9
MICROARRAY: AN APPROACH FOR DISEASE DIAGNOSIS

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Abstract

DNA microarrays (collections of DNA probes arranged on a shared base) have recently enlarged the spectrum of commercially available laboratory-ready kits in molecular biology. They are powerful new tools for the investigation of global changes in gene expression profiles in cells and tissues. Their assembly process is automatized and the DNA microarrays are further miniaturized. The DNA microarrays are used in search for various specific genes (e.g. connected with an infectious agent) or in gene polymorphism and expression analysis. They will be widely used to investigate expression of various genes connected with various diseases in order to find causes of these diseases and to enable their accurate treatment. Since the DNA microarray assembly technology has been based on methods widely used in the semiconductor industry, we can expect a rapid onset of the routine use of this revolutionary device.

Keywords:DNA Microarray, gene polymorphism, expression analysis, treatment, semiconductor

Introduction:

Microarrays are a modern technology in which more than 1000 nucleic acids are bound to a single surface and used to quantify the relative abundance of nucleic acid sequences in a mixture through hybridization and then to quantify the hybridization events.

Nucleic acid arrays or in simple terms DNA arrays are a group of technologies in which specific DNA sequences are synthesized in a 2-D (or sometimes 3-D) array on a surface in such a way that the DNA is covalently or non-covalently attached to the surface.. Roger Bumgarner, Associate Professor, Department of Microbiology, Box 357080, University of Washington, Seattle WA 98195;)

DNA microarrays have the ability to measure the expression of thousands of genes simultaneously(Application of DNA microarray technology in determining breast cancer prognosis and therapeutic response,Donal J Brennan et al. Expert Opin Biol Ther. 2005 Aug.)

Applications of microarray for various diseases diagnosis

The application of diagnostic assays is becoming increasingly important in the management of ensuing diseases. It is essential to have a rapid, accurate, and reliable diagnostic method in order to identify the condition and determine the appropriate course of action, which can reduce the death rate and related expenses.

(1)DNA Microarray for Genetic Disorder Diagnosis:

It goes without saying that inherited genetic abnormalities create genetic illnesses. The two types of illnesses are caused by mutations in the DNA sequence and abnormalities in the chromosome. The latter can result in altered splicing, reduced protein synthesis, and/or altered amino acid sequences in proteins, all of which can lead to distinct illness manifestations. In addition to this, SNPs proved

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to be valuable indicators for tracing the genes responsible for various diseases, particularly those that manifest at different phases of life. Conventional techniques for detecting mutations include restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism (SSCP), and gel-based sequencing of polymerase chain reaction (PCR) amplified material. Despite being effective, these sequencing processes are arduous and time-consuming.

Sequence variants and genetic disorders are being extensively screened using conformation-sensitive gel electrophoresis (CSGE) [1.30] and capillary electrophoresis (CE) [4] to meet the requirement for more effective mutation screening. Additionally, SNP screening and typing, genetic counseling, and other diagnostic domains have all benefited from the development and application of DNA microarray technology. In actuality, the advancements in microarray technology have greatly benefited the high-throughput investigation of genetic mutations and SNPs, leading to the collection of substantial numbers

(2) Detection of chromosomal arnormalities:

It is the reason of genetic disorders chromosome abnormality due to the deletion or duplication of a chromosome, deletion or duplication of a part of chromosome, or a bareak, translocation, or inversion in the chromosome. The diseases include due to chromosome abnormalities are of several types such as

Down's syndrome (associated with chromosome 21),

Edwards syndrome (chromosome 18),

Patau syndrome (chromosome 13), Tumer syndrome (XO),

and Klinefelter syndrome (XXY), leading to irreversible physical and mental abnormalities and death, and even fetal death.

Kang and coworkers developed a DNA microarray fordetecting chromosomal abnormalities causing various genetic disorders like -Down's syndrome, Patau syndrome, Edward syndrome, Turner syndrome, Klinefelter syndrome, alpha-thalassemia retardation-16, Charcot- Marie-Tooth neuropathy 1A, Cri-du-chat syndrome etc (J.-J. Kang, et al. 2007: US20070048742A1). They used the bacterial artificial chromosome chip (BAC chip) to diagnose chromosomalabnormalities.

(3)detection of mutations causing monogenic diseases:-

Monogenic diseases are largely grouped into two types according to etiological classification: dominant and recessive disorders.In the case of Avellino corneal dystrophy, heterozygous mutation causes severe loss of vision as one gets older, whereas in homozygous mutation brings complete loss of vision sooner. In heterozygous mutation does not cause a disease. To identify heterozygous carriers of autosomal recessive disorders, Cheung et al. invented a cDNA microarray that exhibits different genetic signatures from the heterozygous mutation carriers and normal individuals . DNA microarray was fabricated to cover all reported 16 substitution mutations causing WD. It was used to successfully diagnose nine WD patients.

(4)DNA microarray for the diagnosis of infectious diseases:-

Infectious diseases have been a major cause of deaths in the 20th century, such as COVID-19. Humans have been fighting infectious diseases caused by such persistent pathogens for years. The emergence of infectious diseases has become more serious due to many more such pathogens, such as acquired immune deficiency syndrome (AIDS) caused by HIV virus.

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Other virus such as Nipah virus encephalitis, avian influenza, dengue fever and West Nile oncophylitis; re-emerging pathogens such as malaria, measles, foodborne pathogens; and antibiotic-resistant superbacteria such as methicillin-resistant Staphylococcus aureus, vancomycin-resistant S. Aureus and multidrug-resistant Mycobacterium huberculosis .Making the situation worse.

- **Detection of Pathogenic Bacteria:**There have been several reports on the development of microarrays for the detection of bacteria.The developed DNA microarray, named PathoChip™, was evaluated by applying a variety of clinical isolates from blood, stool, cerebral spinal fluid, pus, and urine. Based on the 23S rDNA and 16S-23S rDNA ISR sequence analysis, the bacteria- and bacterial species-specific probes were designed by considering relations between the probe properties and hybridization efficiency.Detection of pathogens in food samples was also carried out by using DNA microarrays. DNA microarray to overcome the disadvantages of whole-genome DNA-DNA hybridization. This diagnostic microarray was also combined with a ligase detection reaction (LDR), which is suitable for identifying SNPs and point mutations, as well as pathogen detection because of its powerful discriminatory power of DNA bases.

- **Detection of Pathogenic Fungi:**The infectious diseases caused by pathogenic fungi have been increasing because of the increase of chemotherapy for hematological malignancies, high-dose corticosteroid treatment for organ transplant recipients, and the spread of AIDS over the past decade example, Penicillium marneffei is the third most common cause ofinfections in human . Detecting pathogenic fungi may require several weeks, owing to slow growth. Specifically, in the case of immunosuppressed patients, it is very difficult to detect circulating antifungal antibodies in blood .

  Most of the diagnostic tests are directed to genus- and species-specific or single-copy genes. Several groups introduced the broad-spectrum detection system with probes designed from internal transcribed spacer (ITS) regions of the rRNA genes. Lindsley et al. The methods of detecting a dimorphic fungus, including differentiating a dimorphic fungus from other fungi. They identified the presence or absence of fungi in blind samples by using a dimorphic fungal probe, species-specific probe, microbe- specific probe, and a nucleic acid sequence corresponding.

Novel and future applications of microarrays in toxicological:

- **Transcription rate analysis**

The nuclear run-on assay has been employed to assess whether a gene is increased In its transcriptional rate in a certain circumstance. microarray would mean just an increase in the number of genes being Probed. The principle of the assay would then be that nuclei would be isolated from untreated And exposed cells or tissues. These nuclei would then be allowed to finish making .

- **mRNA translation**

All of the above microarray based assays give information about the upstream events that can lead to differential mRNA levels. The subject of the determination of gene expression by measurement of mRNA levels microarrays have potential application both in recognition andUnderstanding of

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toxicity from xenobiotics. These technologies have exciting potential in toxicology but also bring With then the challenge of data analysis and then integration for real success to be Achieved.

❖ **Data analysis**

DNA microarray technology has progressively matured to generate extremely large quantities of data, with some studies generating expression data on 40.000 transcripts. The aim of DNA microarray data analysis is the identification of a set of differentially expressed genes in various sample subgroups, which can be used to distinguish between these subgroups on a molecular level. The DNA microarray data itself, and is aimed at the discovery of novel, unbiased patterns in the data related to the intrinsic similarity of the gene expression profiles derived. Several types of analysis have been applied to DNA microarray data. These include clustering methods such as hierarchical clustering, k-means or self- organising maps, and dimension reduction methods like, principal component analysis correspondence analysis.

Future prospects:

During the past two decades, DNA microarray-based techniques have been predominantly applied to identification of biomarkers, detection of pathogens, and identification of disease-causative agents. In many cases, they allowed accurate and rapid results, but their wide-spread use has been hampered for the limited identification capability. To increase its practical usage in diagnosis, it should become more affordable, convenient to practice, accurate, and maybePortable.Additionally, DNA microarray systems may be combined with other techniques such as CpGIsland detection or chromatin immunoprecipitation. For the successful diagnosis of diseases, it have must specific probes, which can be considered as the contents of chips! Genome projects on many infectious organisms are generating unprecedentedly large amount of sequence information, which can be used to design more specific probes for the diagnosis of more diverse infectious agents. With all these advances, it is expected that the DNA microarray or its sister technologies will play an increasingly important role in disease diagnosis in the near future.

Conclusions:

Gene analysis by microarray offers newModels for approaching disease diagnosis,Prognosis, and treatment . Stratification of patients into relevant ‘molecularCategories’ of disease can be expected toImprove the precision and outcomes of clinical medicine. In theAbsence of clinical benefit in non-responderPatient subgroups and provide a more accurate prediction of patient survival. Finally, Detailed molecular understanding of diseasePathways and molecular-genetic markers of Therapeutic response is likely to uncoverMore targets for therapeutic intervention.The microarray has proven to be a valuable tool in the effort to translate the large amounts of available genomic sequence data into meaningful insights about biological function.

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CHAPTER- 10
POSTBIOTICSANDTHEIRACCOMPANIEDHEALTHBENEFITS

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Abstract

Presently, the health market is facing enormous demand of products which confer healthbenefits apart from providing nutrition to the consumers. The market has evidenced a greatriseindemandsofprebioticsandprobiotics.Thehealthbenefitsofboththebioactivecomponents have been acknowledged by the consumers and scientifically proven throughintense experiments. Market has high demands of pre and probiotics but the establishment ofpostbiotic still requires profound experimental evidence. The present chapter deals with thestatusof postbioticsin health marketas compared topreand probiotics.

Keywords:prebiotics,probiotics,health,nutrition,bioactive

Introduction:

Now days a new term is coined for products which are formed after the interaction ofprebiotics and probioticsreferred as Postbiotic. Postbiotics are referred to those compoundswhich are formed in the intestine when the gut bacteriaferment the prebiotic fiber suchbioactive compounds offershealth benefits similar to probiotic bacteria. Figure – 1 explainssomeof postbiotics which implicatehealthbenefits.

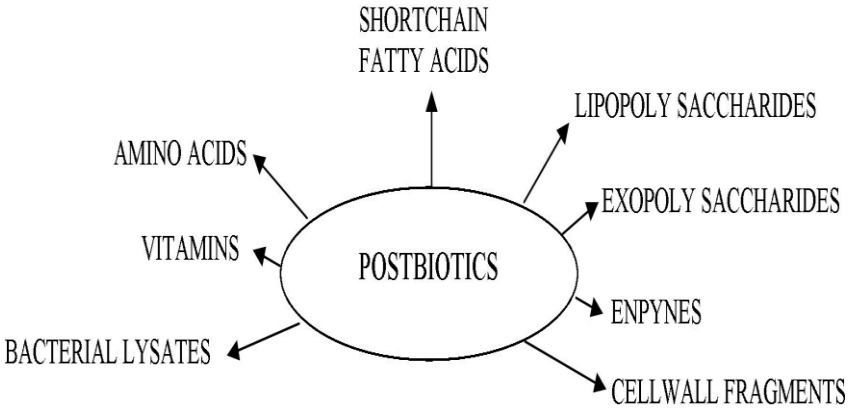

Fig.1:Somecommonpostbiotics

Postbiotic is a comparatively new concept and presently not very easily available in themarket as

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prebiotics and probiotics are.The most important benefits found associated withpostbioticsaregiven in Figure-2.

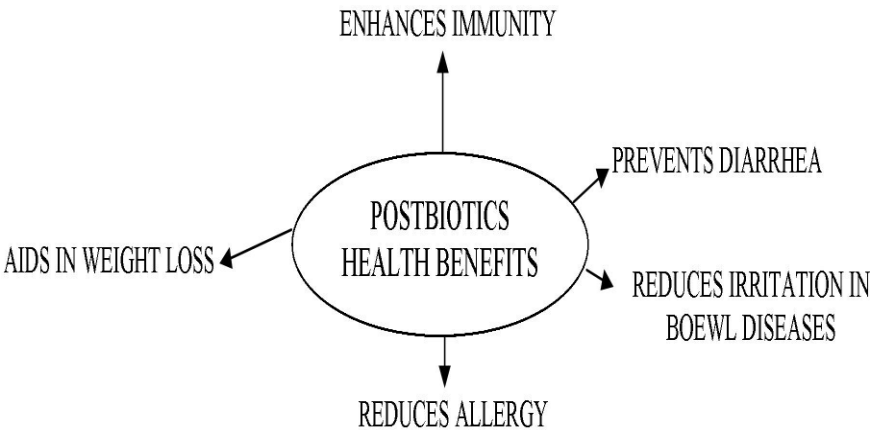

Figure-2:BenefitsassociatedwithPostbiotics

Sources:

Aspostbioticsarecompoundsformedduringinteractionofprobioticswithprebioticsthereforetheirsourcesare alsoshared.Themostcommonlyacknowledgedsourcesareyoghurt,Sauekraut,Meso,Cheeses.Kafir,Sourdo ugh,Buttermilkandpickles.

Somecommonsynonymsusedforpostbioticsarereferredin Figure-3

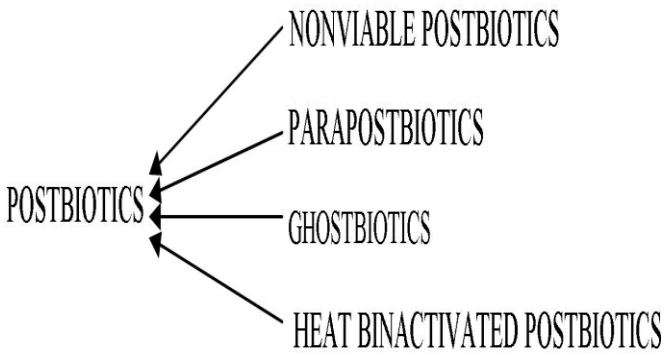

Figure-3:Somecommontermsforpostbiotics

Definition:

The most widely accepted definition of postbiotics, was provided by International ScientificassociationforProbioticsandPrebiotics.Postbioticsaredefinedas“Apreparationofinanimate microorganisms and or their components that confer health benefit on the host”.Thisdefinition has addedclarity to theconceptof postbiotics.

Postbiotic therefore imply to the functional bioactive compound produced in matrix of thecellsduringtheprocessofgrowthandutilizationofprebiotics.Postbioticstakeintoconsiderationallnonvia blemetabolitescomponentsformedbythecellconferringhealthbenefits.Theymayincludeextracellularexte nsionslikepilli,peptides,peptidoglycantiechoic acid, cell lysates, exopolysaccharides, short chain fatty acids etc. Table-1 highligthsadvantagesand disadvantages of postbiotics.

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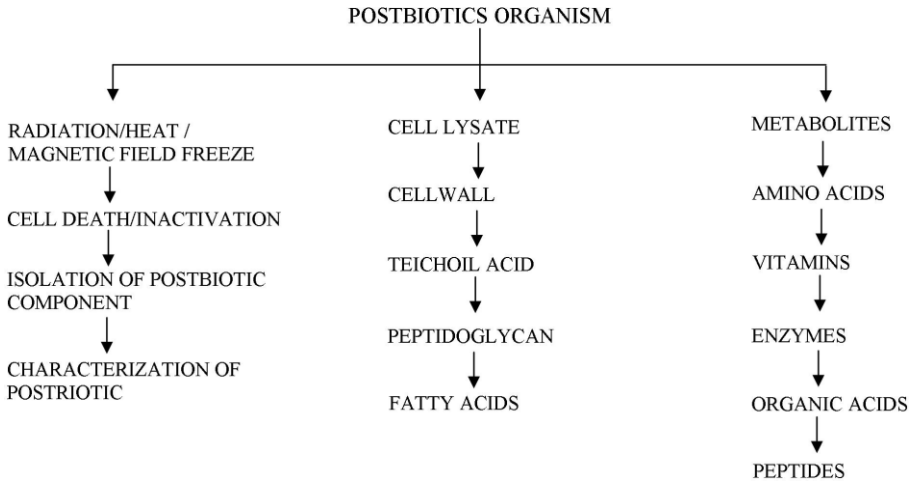
Table-1:AdvantagesandDisadvantagesofPostbiotics

Advantages	Disadvantages
Theyhavespecifictarget	Theyarehighlycostly
Theyarestable	Undesirableamineformation
Shelflifeis more	Goodinstrumentationsupportrequired.
Generallysafe	SensitivetowardsalkalinepH.Translocationf romgutto blood is notfeasible

Synthesisofpostbiotics:

As earlier stated postbiotics are non viable metabolites generated by fermentation processcatalyzedby probiotics.o



Influenceonoverallhealth

Thesedentarylifestylehasinculcatedmetabolicdisturbancessincefewdecades.Suchdisorderscan be handled by adequate physical activity with monitored diet intake.Probioticshave played an important role in rejuvinating the immunesystem and maintaining themetabolic balance to a certain level. The probiotics require monitored storage conditions. Theviable nature make probiotics very sensitive product. Studies have revealedthat postbioticsmaybeanalternativeforhealingcertainailments asgiveninTable-1.

Table-2:MetabolicdisordersandPostbiotics.

Ailmen t	Postbioticeffect	References
1.Inflammation	metabolites cellcomponents orothermoleculeswhichcausesrelief.	Lyonsetal.,2010
2.Bacterialinfection	antimicrobial peptidesorganicacid s othermetabolites	Petersenetal.,2011

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	inhibitthegrowthoractivityofharmfulbacteria.	
3.Immunomodulations	theymayenhancetheactivityofimmunecells regulatetheproductionofcytokines helpstomaintainabalancedimmuneresponse.	Kingmaetal.,2011
4.Cancer	modulatingtheimmunesystemproducinganti-inflammatorycompounds affecting the gut microbiota compositionhasimpactcancerdevelopmentor progression.	Gourbeyreetal,2011
5.Hypertension	short-chainfattyacidscausesbloodpressureregulation. promotetherelaxationofbloodvesselwalls potentiallyleadingtoimprovedbloodflow	Schiavietal.,2011

AdvantagesofProbioticOverProbiotic

Probiotichealthbenefitsarewellestablishedandgroundedtotheirinfluenceongutmicrobiota. The limitations of probiotic usage in health and pharmaceutical products are theviability controls. This has lead to the scientific community to focus on nonviable post bioticsratherthanviableprobiotics.Thenonviablepostbioticshavecomponentsderivedfromprobiotic. These conceptsare the emerging horizons of health associated market. Postbioticsalsoareundertheumbrellaofhealthpromotingfoodsandarefocusedbyscientificcommunity. The approach of postbiotics is technologically acceptable butin most caseshealth benefits are not so well established as for probiotics. The postbiotics is actually amixture of metabolic intermediates synthesized during digestion of prebiotic fibre. The cellfree concortiummay contain cell wall lysates, metabolites, surface proteins trichroic acid,peptidoglyican which can render same health benefits as live viable probiotic confer to thehost.Figure- 4 indicates theadvantagesof postbiotics.

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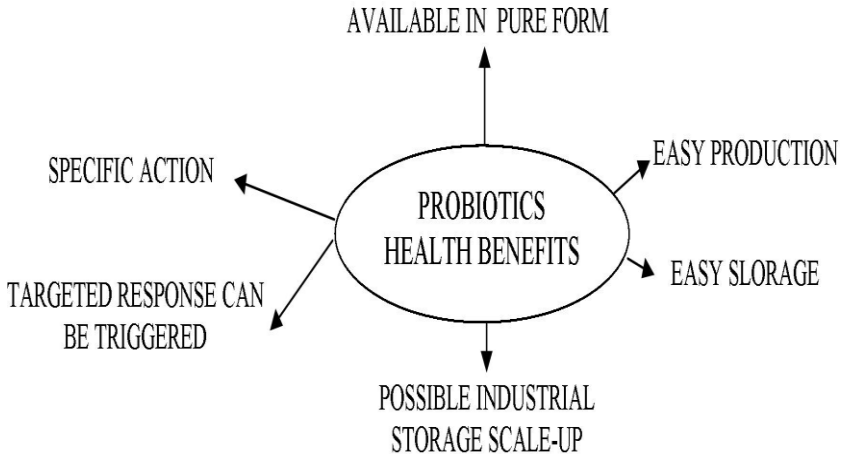
Figure-4:Advantagesofpostbioticstotheconsumers



Conclusion:

Microbiota of gut is the hub of all health related features of host. Any health promoting foodwhether pro, pre or postbiotics, finally are evaluated on basis of their functionalities inintestine and their beneficial effects on host. Health industryisin search of safer replacementfor probiotics. Further studies can befocused on interactions between postbiotics and gutmicroflorato unveil newmechanisms leading to improvedhealth.

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CHAPTER- 11
LIGNINOLYTIC ENZYMES FOR BIOMASS MANAGEMENT

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Abstract

Lignocellulosic biomass is globally distributed and the least expensive bio resource for the synthesis of value added chemicals and metabolites. For their degradation ligninolytic enzymes play a vital role. Ligninolytic enzymes such as Cellulase, Cellobiose dehydrogenase, Laccase, Lignin Peroxidase, Manganese peroxidase and many morehavenumerouscommercial applications.Butinstabilityoftheenzymesisthemajorissueinimplementations oftheseenzymes. Microorganism such as Bacteria and Fungus majorly contribute for ligninolytic enzymatic production. Lignocellulose degradation can be achieved by wood-degrading fungi thereby reducing the physical and chemical stability of wood. Hindrancesinproductionincludelowsecretionsbythemicroorganism,enzymegetting d degradedinthe solventaswellasinstabilityathightemperature andpH. Thus designing of bioreactor with fully automated control system will aid in high productivity. Genetic engineering for improvement in enzyme yield and stability can be primarily focused. Production of enzyme consortium may be another way for enhancing the progressive and fast ligninolytic digestion. Industrial demand of enzyme can be achieved by proper discussion that can open new horizons for constructing a better enzyme based fermentation process.

Keywords: Lignocellulosic biomass, Laccase, Lignin Peroxidase, Manganese peroxidase, microorganism.

Introduction:

Increase in population results in increase in anthropogenic activities. Many activities are of environmental concern, as they results in change of climate, levels of fossil fuels, accumulation of waste, and increase in pollution. Thus the present scenario focuses on generation of renewable resources which can solve the problems of energy crisis. Energy generation from waste especially the accumulated lignocellulosic wasteobtained from agriculture and agro-food processing industries are the prime focus of this chapter. More emphasis can be given to circulating the economy. Circulating economy focuses on reusing the bio waste of first industry as the raw material for the second industry for the production of value added products. This will provide a strategy for approaching to the zero waste.[M. J. Taherzadeh, 2019; V.Sharma et al., 2022a; L. F.Rojas, 2022]

Energy production from non- renewable natural resources can be an alternative source. Huge amount of Lignocellulosic biomass are generated from crop waste, agro industrial waste, plant waste as well as weed biomass can be used as a raw material for the production of energy. Generally this biomass left unused by the industries or are burnt for clearing the land, which results in increase of pollution. Lignocellulose comprises of cellulose, hemicellulose, lignin which can be degraded for the production of organic acids, bioplastics, biofuel,

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hydrogels, resins, vallins etc. [V. Sharma et al., 2022a, V. Sharma et al., 2022b, M. M. Devi et al., 2020;M. Kaur et al.,2022]

Processing of lignocellulose under natural environmental conditions is very slow and doesn't result in value added products. Components of lignocellulose viz cellulose, hemicellulose and joined by lignin. Thus presence of lignin does not allow complete degradation of lignocellulose. Treatment of Lignocellulosic waste with chemical, physical or physicochemical methods is observed to be cost effective. It may also result in the production of some biochemical inhibitors which hinder the complete degradation. To overcome such issues bioremediation and use of enzymes has been preferred. Ligninolytic enzymes attack cellulose and hemicellulose and convert them into simpler forms which are easily degradable. [A Paszczynski et al, 2000; S. Kwon et al., 2021]

The major constituent in the cell wall is lignocellulose. It comprises of cellulose, hemicelluloses and lignin. Cellulose is a homopolysaccharide composed of glucose linked by β-1, 4-glycosidic bonds and can be degraded by enzymes that have the capacity to cleave the glycosidic bonds while hemicellulose is a hetero-polymer and lignin is an aromatic polymer. [J Silva et al., 2021]

Lignin is the only substance that can be completely degraded into water and carbon dioxide by microbes. Nevertheless, due to the presence of polyphenolic groups in lignin, it requires many years to degrade naturally. Many fungal species from the phylum Basidiomycetes contribute majorly to lignin degradation. The known lignin degraders are white rot, brown rot and soft rot fungi. The enzymes secreted by white-rot fungi have the ability to break down Cα - Cβ, β- aryl, C1-Cα of lignin. Low molecular weight chelators catalyze the degradation of lignin**.** [I. Afanasevna et asl., 2022; V. Sharma et al., 2018; A Iram et al., 2020;S. Sharma et al., 2021, S. Hernandez Saldarriaga et al., 2020]

Lignin degrading enzymes such as Laccase, Lignin Peroxidase and Manganese Peroxidase has the potential to degrade lignin, thereby releasing cellulose and hemicellulose for complete degradation.Many auxiliary enzymes enhance the degradation processes of complex carbohydrate into simpler forms. Enzymatic degradation also proved to overcome the issues of biochemical inhibitors, thereby showing effective degradation.[R.R. Singhania et al., 2022; n. Annamalai et al., 2016]

Many bacteria, fungus has been reported to produce ligninolytic enzymes extracellularly and is getting emphasis due to its commercial importance. Solid state fermentation (SSF) is getting importance for the production of these commercial enzymes. SSF may provide future variation for improved production of enzymes. This may include increase in the concentration of end products, increased in product stability, decrease in catabolic suppression, production of water insoluble substrates etc. [R.R. Singhania et al., 2017]

Ligninolytic enzymes:

Globally distributed and the least expensive bio resource for the synthesis of value added chemicals and metabolites is Lignocellulosic biomass. They are observed as waste of agriculture productivity. Lignin present in plant cell walls hinders the saccharification of cellulose and hemicellulose. This can be achieved by using pretreatment methods helping in transforming complicated carbohydrate into sugar which further act as a feed for production of biofuels, hydrogels, biofilms, drug delivery and other organic acid production. To

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hydrolyze lignin and turn it into usable compounds, a range of ligninolytic enzymes are employed. [V. Sharma et al., 2022, A.T. Martinez et al., 2017; Y. Arfi et al., 2014]

Cellulases are hydrolyzing enzyme which produces glucose, cellobiose and cellooligosaccharides. They are categorized as endoglucanases, exoglucanases or cellobiohydrolases and β- glucosidases.These enzyme works together in two steps. The first steps involves the breakdown of cellulose by endoglucase and exoglucanase thereby converting it into liquid phase which is then converted into glucose molecules by β-glucosidases. Numerous bacteria and fungi generate the three enzyme endoglucanases, cellobiohydrolases, and β-glucosidases. On the other hand, fungi that work with cellulose also create cellobiose dehydrogenases and lytic polysaccharide monooxygenases. Numerous organisms, including bacteria (both aerobic and anaerobic), fungus, archaea, and protozoa, produce endoglucanases. However, fungi like *Aspergillus sp*. and *Trichoderma* produces these enzymes for commercial purposes.Numerous bacteria and fungi can manufacture cellobiohydrolases; however, glycosyl hydrolase family cellobiohydrolases are predominantly synthesised by glycosyl hydrolase family cellobiohydrolases in bacteria and fungus. Cellobiohydrolases are believed to remove cellulose chains from the microcrystalline cellulose structure by effectively degrading microcrystalline cellulose. Cellobiose competitively inhibits Cellobiohydrolases.[A.T. Martinez et al., 2017, H. Ostby et al., 2020, L. Luo et al., 2016]

Two cofactors such as heme and flavinfavours the activity of Cellobiose dehydrogenase. Proteolytic cleavage is in beween a flavodehydrogenase domain and heme group. The enzyme is supported by lytic polysaccharide monooxygenase enzyme. Lytic polysaccharide monooxygenase enzyme is reported to have an important role in cellulose degradation with cellobiose dehydrogenase. Oxygen present in its active site has to be reduced thereby activating the enzyme. This can be achieved by using artificial reductant such as Ascorbic acid producing aldonic acid. [S.Sharma et al., 2018; L. Santibanez et al., 2021]

Hemicellulasesenzymes hydrolyze the main chain, such as endo-β-1,4-xylanases (xylanases) and endo-β-1,4-mannanases (mannanases), and debranching enzymes that help remove substitutions from the polysaccharide backbone, like deacetylases, arabinosidases, and galactosidases, are examples of hemicellulolytic enzymes.Endoxylanase enzyme endo-1,3-beta-xylanase (EC 3.2.1.32) hydrolyzes glycosidic linkages between xylans at carbon 1 and 3, producing xylobiose, xylotriose, and xylotetrose, breaks down xylan to form xylooligosachharides. α-D-xylosidexylohydrolase (EC 3.2.1.177) hydrolyzes the terminal residues of non-reducing alpha-D-xylose, releasing α-D-xylose.[P. Binod et al., 2019;F.J. Contesini et al., 2017]

Glucohydrolase families 10 and 11 comprise endoxylanases. While GH11 enzymes preferentially target longer xylan chains and do not release xylose, GH10 endoxylanases may generally use smaller substrates and synthesize xylose. Although xylanases can be produced by a variety of species, including bacteria, fungi, archaea, marine algae, protozoa, etc., the majority of xylanases reported for industrial use are filamentous fungi [A.Karnaouri et al., 2019; G Yao et al., 2016].

β-D-Xylosidaseenzyme act on the nonreducing end of the xylooligosaccharides such as xylose residues. Xylanase β convert short stretch of oligomers to monomers. It has been observed that the affinity of oligosaccharide is inversely related with the degree of

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polymerization. Three distinct categories can be distinguished among arabinofuranosidases. Type A enzymes primarily function on ρ-nitrophenyl-α-l-arabinofuranoside and arabino-oligosaccharides; type B hydrolyzes arabinoxylan to yield xylose and l-arabinose; and the third type, referred to as 1 → 4-β-d-arabinoxylanarabinofuranohydrolase, is incredibly selective for arabinosidic linkages in a variety of arabinoxylans, releasing l-arabinose upon interaction. Furthermore, on a lot of synthetic substrates, the third type is inactive [C.Weng et al., 2021; R Datta et al., 2017]

Esterase enzyme removes ferulic acid from O-5 of arabinose of glucuronoarabinoxylan .Acetoxylanesterase has a strong specificity for O-acetyl-4-O-methyl-D-glucurono-D-xylan. Glucuronoylesterases are recently identified members of the CE15 family of esterases that catalyse the hydrolysis of the ester link between lignin and 4-O-methyl-D-glucuronic acid residues in lignin-carbohydrate complexes (LCCs). α-Glucuronidase are usually secreted intracellularly which belong to GH67 family. They are membrane associated with short glucuronic acid sometimes substitutedwithxyloolgosaccharides whereas extracellularly secreted glucoronidase belongs to the family GH115. [L. Luo et al., 2016; R.Rahamnpour et al., 2016; X jiang et al, 2016]

Laccases are dimeric or tetrameric molecules with four copper atoms. The enzyme has reported to degrade diphenols,polyphenols, aliphatic and aromatic amines as well as nonphenolic compounds. Lignin is depolymerized by manganese peroxidase enzyme by chemical reactions such as oxidation and reduction. Chelators such as oxalate, malonate aids in increasing the enzyme activity and degrade phenolic compounds. Lignin peroxidase take action on phenolic and additionally on non phenolic compounds. They doesnot require chelators as they posses high redox potential. The degradation is chemically divided into oxidation and then two consecutive reduction.[C.Weng et al., 2021, C. Chio et al., 2019, L. Pollegioni et al., 2015]

Dye- decolorizing Peroxidase utilizes free radicals as intermediate and contain heme group. These enzyme degrade non-phenolic lignin related substrate results in production of p-anisaldehyde. β-aryl ether linkages is the main linkage observed in lignin. It is cleaved by B-Etherase by attacking glutathione group which is broken down to residues of hydroxypropiovanillone finally producing Vanillin. Two guaiacylgroup are linked together with biphenyl bonding. On degradation of one methoxy group present in 5, 5′-dehydrodivanillate results in production of hydroxyl group which is thereby decomposed and produce 5- carboxyvanillic acid and 4-carboxy-2-hydroxypentadienoic acid. The products generated are fully hydrolysed and produce vanillic acid. [S. Saini and K.K Sharma 2021; M. Andlar et al., 2018; I. Nurika et al., 2020;M.Marinovic et al., 2018]

Production of ligninolytic enzymes:

Complete digestion of agricultural waste can be achieved by ligninolytic enzymes. These enzyme are naturally produced by Bacteria and Fungus. With gain in commercial requirement of ligninolytic enzyme it gained attention. Thus the production of these enzymes in quantity and quality will be the center of attention. In comparison to bacterial enzymes fungal enzymes is more beneficial as they produce them extracellularly and can be easily recovered.[A. Karnaouri et al., 2019; H.Zabed et al., 2019]

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Microorganism such as Bacteria and Fungus majorly contribute for ligninolytic enzymatic production. Lignocellulose degradation can be achieved by wood-degrading fungi thereby reducing the physical and chemical stability of wood. Fungus excretes extracellular as well as intracellular enzymes which not only reduce polysaccharide complexicity but also assist in opening of phenyl rings. High ligninolytic enzymes are observed in soft-rot fungi, white –rot fungi and Brown-rot fungi. [A.T.Martinez et al., 201;,L.Limaye et al., 2017]

Cellulose enzyme preferentially degrades cellulose resulting in microscoipic voids in the secondary cell walls of plant biomass. Laccase and peroxidases aids in degrading lignin. Lignin degradation help in increase of pulp which are required in pulp and textile industries as well as in production of Biofuels. Pretreatment agricultural biomass and saccharification can overcome the conversion hinderance in conversion of complex sugars to monomeric sugars.*Serpulalacrymans* was able to utilise five distinct agricultural wastes—cocoa pods, sugarcane bagasse, rice straw, maize leaves, and corn cobs—to produce monosaccharides and phenol. The findings showed that maize leaves produced the most total reducing sugars (207.37 mg g−1) and rice straw produced the most total soluble phenols (0.140 mg g−1). [H. Zabed et al., 2019;R.S.. Granja-travez et al.,2020]

White Rot fungi confirmed to secrete Cellulose, Hemicellulose and lignin degrading enzymes thereby completely degrading the biomass to Carbon dioxide and water. Depending on their mechanism of action and substrates, they are divided into two groups: selective delignifiers and non-selective delignifiers. While non-selective delignifiers concurrently break down all of the Lignocellulose biomass polymers, selective delignifiers primarily hydrolyze lignin and barely affect cellulose and hemicellulose. Fungal ligninolytic enzymes are produced by Trichoderma, Neurospora and Aspergillus species. [J.mei et al., 2020; P.Leite et al., 2021]

Bacterias with ligninolytic enzyme productivity showed higher growth rate and high metabolic activity which results in decrease in pretreatment duration. They degrade lignin into simpler phenolic compounds which are further processed by hydrolysis activity. Few bacteria also report to produce enzyme responsible for breakdown of lignin, cellulose and hemicellulose. Lignin-degrading bacteria have been identified as *ActinobacteriaMicrobacteriumphyllosphaerae, RhodococcusjostiiRHA1, α-ProteobacteriaOchrobactrum sp., and γ-Proteobacterium Pseudomonas putida* KT2440. Additionally, the bacteria showed significant titers of the enzymes laccase, lignin peroxidase, and manganese peroxidase. [J. Liu et al., 2020; R.S. Granja- Travez et al., 2020; J. Mei et al., 2020]

Table: Representing the microorganism with the type of feeding material

Name of ligninolytic enzymes	**Type of microorganism**	**Name of microorganism**	**Feed (Raw material)**
Cellulase	Fungi	*Aspergillus niger ITV02*	Wheat straw
		Lentinussquarrosulus	Kans grass
		Trichoderma reesei Rut	Kans grass
		Aspergillus aculeatus	Partheniumhysterophorus
		Aspergillus assiutensis	Sugarcane bagasse

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	Bacteria	*Bacillus sp.*	Wheat straw
		Cellulomonassp	Wheat straw
		Paenibacillusillinoisensis	Wheat straw
		Bacillus cereus	Wheat straw
		Paenibacillusbarcinonensis	Wheat straw
Endoglucanase	Fungi	*Penicillium aurantiogriseum*	Corn stover
		P. citrinum	Cellulose pulp and cassava peel
		Aspergillus sp	Cellulose pulp and cassava peel
		T. reesei	Cellulose pulp and cassava peel
		Pycnoporussanguineus	Sugarcane bagasse
	Bacteria	*Ruminiclostridiumthermocellum*	Corn straw, corn cobs, rice straw, poplar sawdust
Cellobiohydrolase	Fungi	*Penicillium aurantiogriseum*	Corn stover
B- glucosidase	Fungi	*Penicillium aurantiogriseum*	Corn stover
		P. citrinum	Cellulose pulp and cassava peel
		Aspergillus sp	Cellulose pulp and cassava peel
		T. reesei	Cellulose pulp and cassava peel
		Pycnoporussanguineus	Sugarcane bagasse
	Bacteria	*Ruminiclostridiumthermocellum*	Corn straw, corn cobs, rice straw, poplar sawdust
Exoglucanase	Fungi	*Pycnoporussanguineus*	Sugarcane bagasse
	Bacteria	*Ruminiclostridiumthermocellum*	Corn straw, corn cobs, rice straw, poplar sawdust
Laccase	Fungi	*Geobacillus sp.*	Corn stover
		Trameteshirsuta F13	Beechwood sawdust
		Lentinussquarrosulus	Kans grass
		Trichoderma reesei Rut	Kans grass
		Trichoderma asperellum	Sweet sorghum stover
		Pycnoporussanguineus	Sugarcane bagasse
		Marasmielluspalmivorus	Eucalyptus globulus wood
		Penicillium echinulatum	Eucalyptus globulus wood

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	Bacteria	*Chromohalobactersalexigens*	Almond shell
		Bacillus ligniniphilus	Rice straw
		Thermus sp.	Eucalyptus
xylanase	Fungi	*P. citrinum*	Cellulose pulp and cassava peel
		Pycnoporussanguineus	Sugarcane bagasse
		Aspergillus sp	Cellulose pulp and cassava peel
		T. reesei	Cellulose pulp and cassava peel
		Marasmielluspalmivorus	Eucalyptus globulus wood
		Penicillium echinulatum	Eucalyptus globulus wood
		Aspergillus aculeatus	Partheniumhysterophorus
		Aspergillus assiutensis	Sugarcane bagasse
	Bacteria	*Ruminiclostridiumthermocellum*	Corn straw, corn cobs, rice straw, poplar sawdust
		Micromonosporasp	Rice straw, corn straw, wheat straw, soybean straw
		Streptomyces sp	Rice straw, corn straw, wheat straw, soybean straw
		Saccharomonospora	Rice straw, corn straw, wheat straw, soybean straw
		Mycobacterium sp.	Rice straw, corn straw, wheat straw, soybean straw
		Bacillus sp.	Wheat straw
		Cellulomonassp	Wheat straw
		Paenibacillusillinoisensis	Wheat straw
		Bacillus cereus	Wheat straw
		Paenibacillusbarcinonensis	Wheat straw
Manganese peroxidase	Fungi	*Trameteshirsuta F13*	Beechwood sawdust
		Pycnoporussanguineus	Sugarcane bagasse
		Acinetobactersp	Corn straw
	Bacteria	*Micromonosporasp*	Rice straw, corn straw, wheat straw, soybean straw

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		Streptomyces sp	Rice straw, corn straw, wheat straw, soybean straw
		Saccharomonospora	Rice straw, corn straw, wheat straw, soybean straw
		Mycobacterium sp.	Rice straw, corn straw, wheat straw, soybean straw
Lignin peroxidase	Fungi	*Pycnoporussanguineus*	Sugarcane bagasse
	Bacteria	*Acinetobactersp*	Corn straw
		Micromonosporasp	Rice straw, corn straw, wheat straw, soybean straw
		Streptomyces sp	Rice straw, corn straw, wheat straw, soybean straw
		Saccharomonospora	Rice straw, corn straw, wheat straw, soybean straw
		Mycobacterium sp.	Rice straw, corn straw, wheat straw, soybean straw

Production of commercially active enzymes by microbes can be achieved by degradation of lignocellulose biomass. They favor growth and high enzyme productivity. Solid state fermentation (SSF) is budding technique followed in Asian and Western Countries. It uses solid biomass as a feed for the growth of microorganism. The enzymes thus produced are recovered by downstream processing. For SSF-based synthesis by Bacterial and fungul enzymes, lignocellulose biomass including rice bran, brewer's wasted grain, coffee husk, grape pomace, wood chips, oilseed cakes, wheat bran and corn straw have been utilized. Various parameters such as pH, temperature, oxygen content and moisture content also plays an important role in enzyme production. Thus designing of bioreactor with fully automated control system will aid in high productivity. [P.Leite et al., 2021; J. Lui et al., 2020]

Future prospects:

Ligninolytic enzymeshavenumerouscommercial applications.Butinstabilityoftheenzymesisthemajorissueinimplementations oftheseenzymes. Hindrancesinproductionincludelowsecretionsbythemicroorganism,enzymegetting degradedinthe solventaswellasinstabilityathightemperature andpH. Although production of these commercial enzymes may solve many problems but still for large scale utilization in biofuel production and for production of other bi-products obstacles has been noticed. Thus refinement in production protocols has to be focused. Poor degradation of ligninolytic substrate is due to the presence of lignin which majorly hinders the fast processing by cellulose enzyme. Consequently, more technical approaches are required for attaining the level of commercial essentiality. Genetic engineering for improvement

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in enzyme yield and stability can be primarily focused. Production of enzyme consortium may be another way for enhancing the progressive and fast ligninolytic digestion. Industrial demand of enzyme can be achieved by proper discussion that can open new horizons for constructing a better enzyme based fermentation process.

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CHAPTER- 12
NUCLEIC ACID ISOTHERMAL AMPLIFICATION TOOLS FOR DETECTION OF PLANT PATHOGENS

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Abstract

Plant diseases are causing huge crop loss in both field and storage conditions which leads to huge economical loss and ultimately *gross domestic product* (GDP) reduction. Effective protective measures for managing the diseases are necessary. For managing of diseases effectively we need to detect and diagnose pathogens which are associated with diseases. Nucleic acid amplification techniques are used as leading methods in detection and analysis using a small quantity of nucleic acids. The polymerase chain reaction (PCR) is the most widely used method for DNA amplification for detection and identification of pathogens. However, using PCR-based methods for in-field diagnostics is a challenge and sometimes nearly impossible because it requires a thermo cycling machine to separate two DNA strands and then amplify the required fragment. Novel developments in molecular biology are amplifying DNA in isothermal conditions without the need of a thermo cycling machine. Common isothermal amplification methods include loop-mediated isothermal amplification, recombinase polymerase amplification, rolling circle amplification, nucleic acid sequence-based amplification, and helicase-dependent amplification. Nucleic acid isothermal amplification techniques are novel, sensitive, less time consuming, cost effective and also eco-friendly than any other molecular based methods of pathogen detection. Here, we summarize approaches for in-field diagnostics of phytopathogens based on different types of isothermal amplification and discuss their advantages and disadvantages.

Keywords: molecular diagnostics, recombinase polymerase amplification; rolling circle amplification, Nucleic acids, loop-mediated isothermal amplification

Introduction:

Plant diseases have been identified as a significant factor contributing to substantial crop losses in both field and storage settings, thereby impacting the economy of nations. Consequently, the implementation of efficient management strategies is imperative in order to mitigate these losses. Proper detection and diagnosis are crucial components of effective management practices. Nucleic acid amplification techniques play a crucial role in the detection and analysis of small quantities of nucleic acids. Among these techniques, the polymerase chain reaction (PCR) stands out as the most widely utilized method for DNA amplification, as well as the detection and diagnosis of various diseases. However, PCR necessitates the use of a thermocyclic machine to separate the two DNA strands and subsequently amplify the desired fragment. In contrast, novel techniques such as Loop-

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mediated Amplification (LAMP), Rolling Circle Amplification (RCA), Nucleic acid sequence-based amplification (NASBA), Recombinase polymerase amplification (RPA), and helicase-dependent amplification (HDA) have emerged in the field of molecular biology. These techniques enable DNA amplification under isothermal conditions, eliminating the requirement for a thermo cycling apparatus.

Isothermal DNA/RNA detection methods that utilize an amplification strategy (isothermal detection concepts summarizes the different detection platforms that are covered hear).

Sl. No.	Platform	Amplified component	Amplification catalyst
1.	LAMP	Probe	Enzymatic
2.	RCA	Probe	Enzymatic
3.	NASBA	Complementary sequence of target (RNA)	Enzymatic
4.	RPA	Target and the complementary sequence	Enzymatic
5.	HDA	Target and the complementary sequence	Enzymatic

1. Loop-mediated amplification (LAMP)

Loop-mediated amplification (LAMP) is an isothermal technique that has demonstrated amplification levels comparable to that of PCR. LAMP also achieves a high degree of target specificity, which can be attributed to the utilization of two sets of primers that span six distinct sequences of the target. These primers consist of forward primers (inner and outer) and backward primers. The inner forward primers (F1c-F2, where 'c' represents 'complementary') and outer forward primer (F3) are complemented by similar counterparts in the backward primer set. The DNA polymerase employed in LAMP is Bst DNA polymerase, as it possesses strand displacement activity, unlike tag polymerase, which is not used due to its endonuclease activity.

The LAMP process consists of three distinct stages: initiation of starting material, amplification through cycling, and elongation and recycling. The F2 region of the inner primer initially binds to the target at approximately 60 degrees Celsius, followed by extension through the action of a DNA polymerase. Initially, the outer primer F3 binds to the target strand at F3c, causing the polymerase to extend F3 and displace the newly synthesized strand. The displacement of the strand leads to the formation of a stem-loop structure at its end, which is a consequence of the hybridization between F1c and F1 regions. Eventually, the reverse primer set can hybridize to this displaced strand, resulting in the generation of a new strand with stem-loop structures at both ends through the activity of the polymerase. The exponential amplification cycle is initiated by the introduction of dumbbell-structured DNA, which undergoes repeated extension and strand displacement to generate strands containing multiple inverted repeats of the target DNA. The LAMP technique is capable of amplifying a small number of target DNA copies to an astonishing 109 within a time frame of less than

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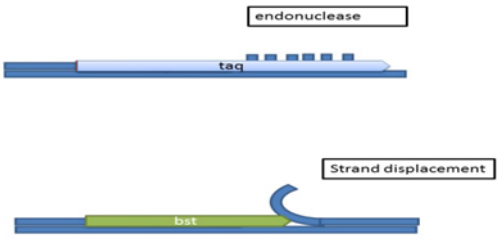
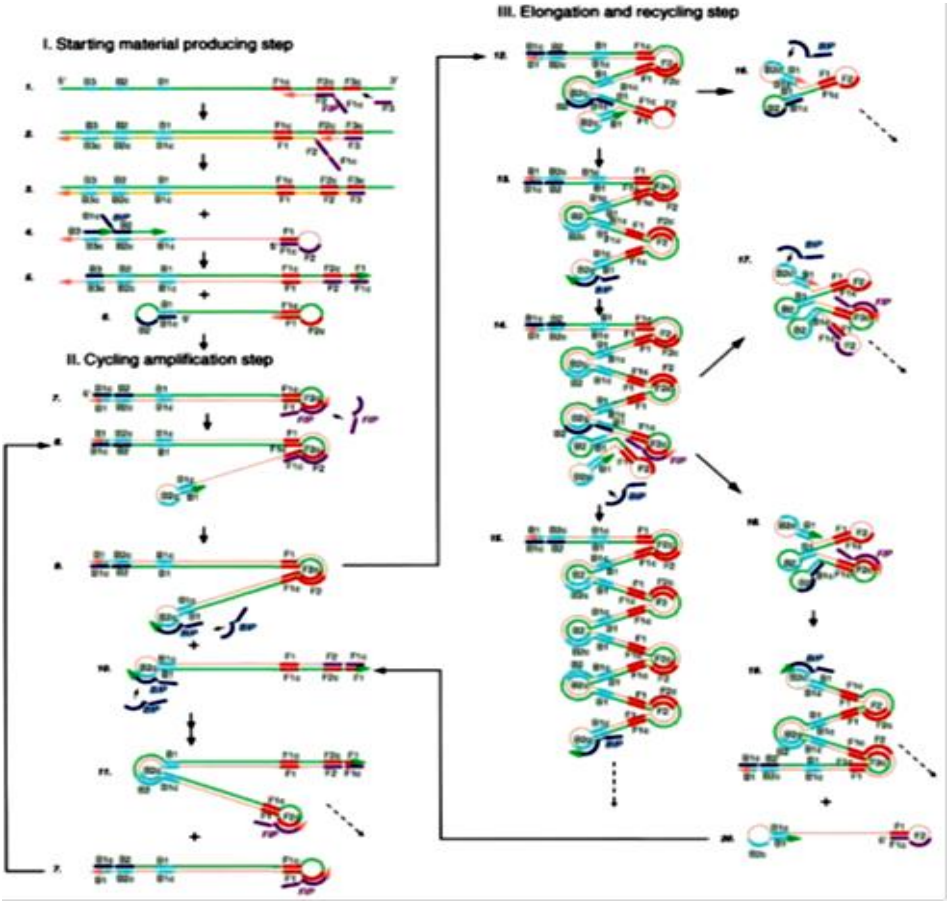
one hour, even in the presence of substantial quantities of non-target DNA. LAMP has found utility in the detection of diverse viral pathogens. However, a notable drawback of LAMP lies in the complexity associated with designing primer sets, as they need to cover six distinct regions of the target DNA.



Difference of Tag and Bst polymerases enzyme.



Mechanism of Loop-mediated amplification (LAMP). Four probes (F1c-F2, F3, R1c-R2, R3) are used for this method. F1 is complementary with F1c (c stands for complementary sequence).

2. Rolling circle amplification (RCA)

During the 1990s, it was documented that DNA polymerase had the ability to employ circular templates in order to generate an elongated linear strand. This elongated strand would contain numerous duplicates of a specific sequence, and this phenomenon was termed rolling circle amplification (RCA). RCA can be categorized into three distinct types: (1) linear template with a single primer on a circular template, (2) circular template, and (3) multiple random primers.

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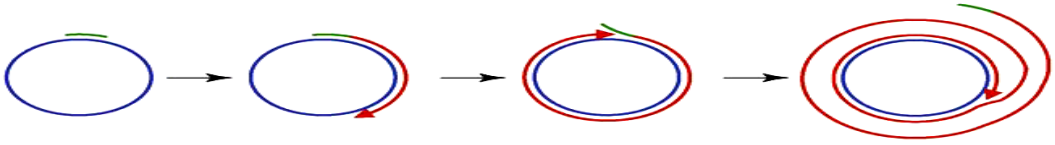
In the linear template - single primer approach, two methods are utilized: padlock probes and rolling circle amplification, along with target sequence recycled rolling circle amplification (TR-RCA). TR-RCA involves RCA linked to the ligation of a padlock probe (in the presence of a target analyte) to enable the sensitive detection of nucleic acids. The padlock probe is a single probe that consists of 30- and 50-end sequences that interact with target sequences in a juxtaposed manner to facilitate ligase-mediated circularization of the padlock probe if the 50-end is phosphorylated. Following the circularization of the padlock probe, a primer and DNA polymerase are employed to generate long repeating DNA sequences, which can subsequently be identified using various detection methods. The recycled rolling circle amplification (TR-RCA) method is a variation of the RCA technique that does not rely on ligases like padlock probes. In TR-RCA, the circular DNA template needed for amplification is pre-circularized, with the primer-binding site hidden in the dumbbell's duplex region. Upon target binding, the duplex region opens up, allowing the primer to bind and initiate the RCA process. RCA has found widespread use in nucleic acid detection, including measuring mRNA expression levels and detecting microRNA in various settings. Circular template methods with a hyperbranched rolling circle amplification (RCA) have been employed to enhance the sensitivity of RCA. To achieve this, both forward and reverse primers are utilized. In the conventional RCA process, the forward primer generates a multimeric single-stranded DNA (ssDNA), which serves as the template for multiple reverse primers. Subsequently, the DNA polymerase extends the reverse primer, displacing the downstream DNA during the extension process, resulting in the formation of a branched or ramified DNA complex. The amplification process terminates once all the ssDNA strands have been converted into double-stranded DNA (dsDNA). This amplification technique is referred to as hyperbranched RCA.

In the Circular template and multiple random primers, Oligonucleotide primers that are complementary to the amplification target circle are hybridized to the circle. The polarity of polymerization is indicated by arrowheads, which highlight the 3-ends of the DNA strands. The original primer sequences within the product strands are denoted by thickened lines. By adding DNA polymerase and deoxynucleoside triphosphates (dNTPs) to the primed circle, each primer is extended, and the newly synthesized strands are displaced due to the elongation of the primer behind them. Subsequently, secondary priming events can occur on the displaced product strands of the initial rolling circle amplification step.

Different types of RCA:

1. Linear template and single primer.

2. Circular template



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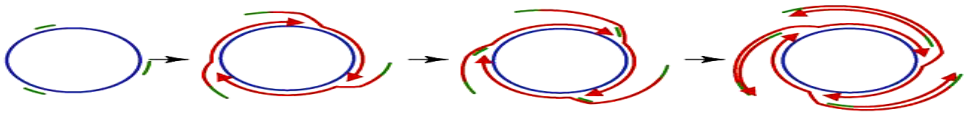
3. Circular template and multiple random primers.



3. Nucleic acid sequence-based amplification (NASBA)

Nucleic acid sequence-based amplification (NASBA) represents an isothermal amplification technique developed for the purpose of detecting RNA targets. In this approach, the forward primer (P1) is bifurcated into two segments, one of which is complementary to the 3'-end of the RNA target while the other aligns with the T7 promoter sequence. Upon binding of P1 to the RNA target (RNA (+)), reverse transcriptase (RT) extends the primer to generate a complementary DNA (DNA (+)) strand via the action of RNase H, subsequently degrading the RNA strand of the RNA-DNA (+) hybrid. Subsequently, the reverse primer 2 (P2) binds to the DNA (+), and a reverse transcriptase (RT) synthesizes double-stranded DNA (dsDNA) containing a T7 promoter sequence. Following this initial step, the system progresses into the amplification stage.

The T7 RNA polymerase is responsible for synthesizing numerous RNA strands from a double-stranded DNA template. In the process, a reverse primer known as P2 binds to the newly formed RNA molecules during step 6. Following this, the reverse transcriptase (RT) extends the reverse primer, while the RNAse H enzyme degrades the RNA portion of the RNA-cDNA duplex, resulting in the formation of single-stranded DNA (ssDNA) during step 8. The newly generated complementary DNA (cDNA), referred to as DNA (+), then serves as a template for the primer P1, initiating a cycle of amplification. This amplification process, known as NASBA, can achieve a remarkable 10 million-fold increase in the amount of target RNA within a span of 1 to 2 hours. NASBA has been successfully commercialized and has proven effective in amplifying and detecting a wide range of target RNA sequences using this technique.

4. Recombinase polymerase amplification (RPA)

Recombinase, a protein, facilitates the process of hybridization between short oligonucleotide primers and the corresponding target sequence. In the context of recombinase polymerase amplification (RPA), the recombinase-primer complexes play a crucial role in initiating the amplification cycle. These complexes scan the duplex template for regions that are homologous to the primers. Upon locating the specific site, the enzyme unwinds the double strands, enabling the primer and target sequence to form a hybridized structure. This process is facilitated by single-stranded DNA (ssDNA) binding proteins, which bind to the DNA that has been "melted" or denatured. The primer, a short DNA sequence, is then elongated with the newly synthesized strand displacing the old strand. This newly synthesized double-stranded DNA can then serve as a template for the next cycle of amplification, resulting in exponential amplification of the target DNA.Although rapid and sensitive, one potential issue with this process is the presence of background noise. To address this, a primer is used that contains a specific cleavage site for the double-strand-specific Escherichia coli endonuclease IV (Nfo). This endonuclease recognizes and cleaves a specific type of DNA lesion called a

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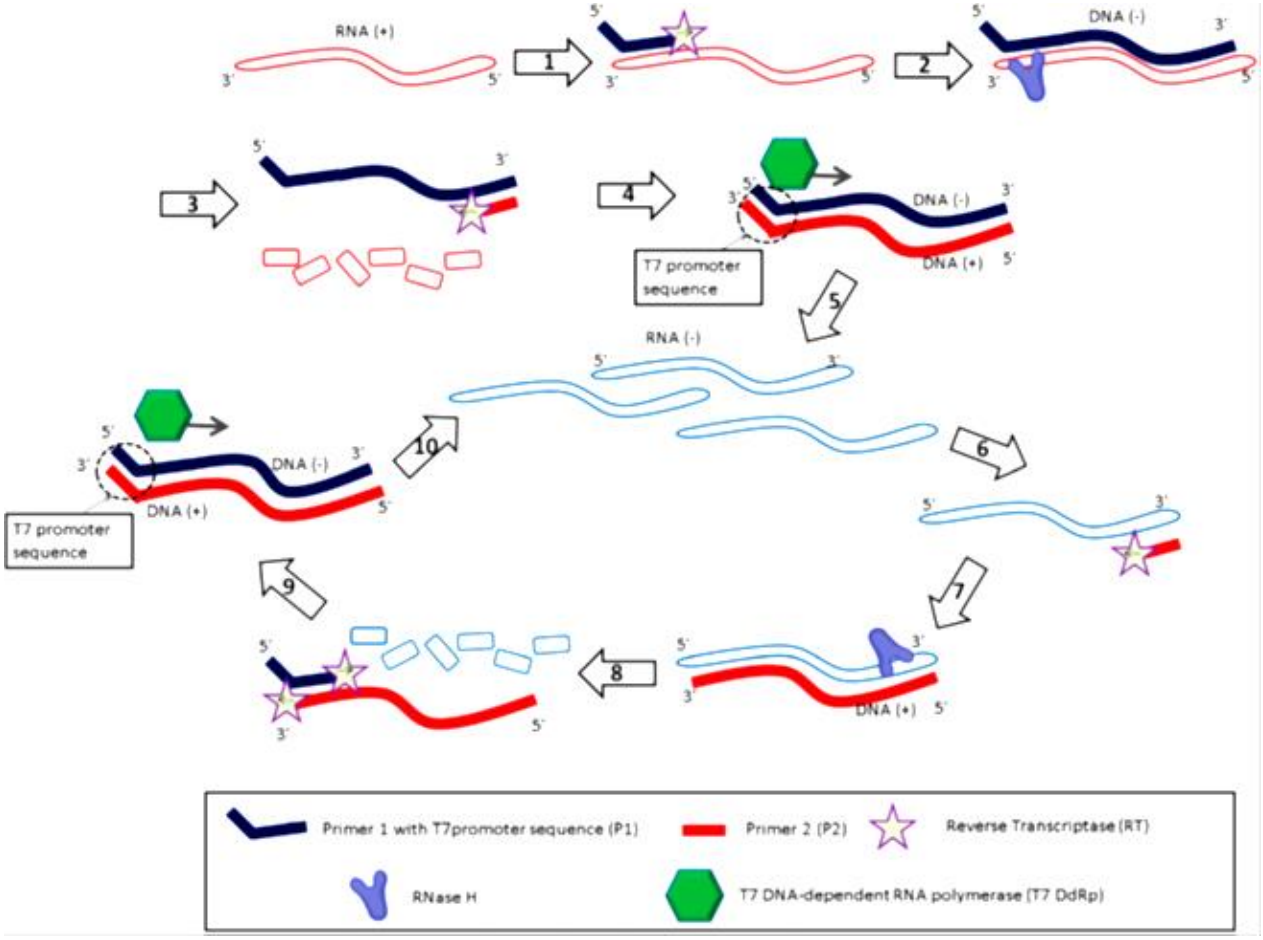
tetrahydrofuranabasic site in the double-stranded DNA. The primer can only be used for the DNA polymerase extension step after it has been cleaved by the endonuclease, revealing a 30-OH end. To further enhance the detection of the cleavage reaction, the primer can be labeled with a fluorophore and quencher. This allows for the measurement of fluorescence, which is enhanced when the cleavage reaction occurs. The endonuclease cleavage step in this process serves as an additional proofreading step to eliminate background noise.



Nucleic acid sequence-based amplification (NASBA). RT = Reverse Transcriptase, T7 DdRp = T7 DNA-dependent RNA polymerase. Steps 1 to 5 are the initial phase to amplify original RNA (+) target to RNA (). Steps 6 to 10 are the amplification phase to amplify RNA .

However, it is important to note that the reaction conditions for this process, known as recombinase polymerase amplification (RPA), are stringent. This means that the detection of the target DNA using crude samples could be problematic.

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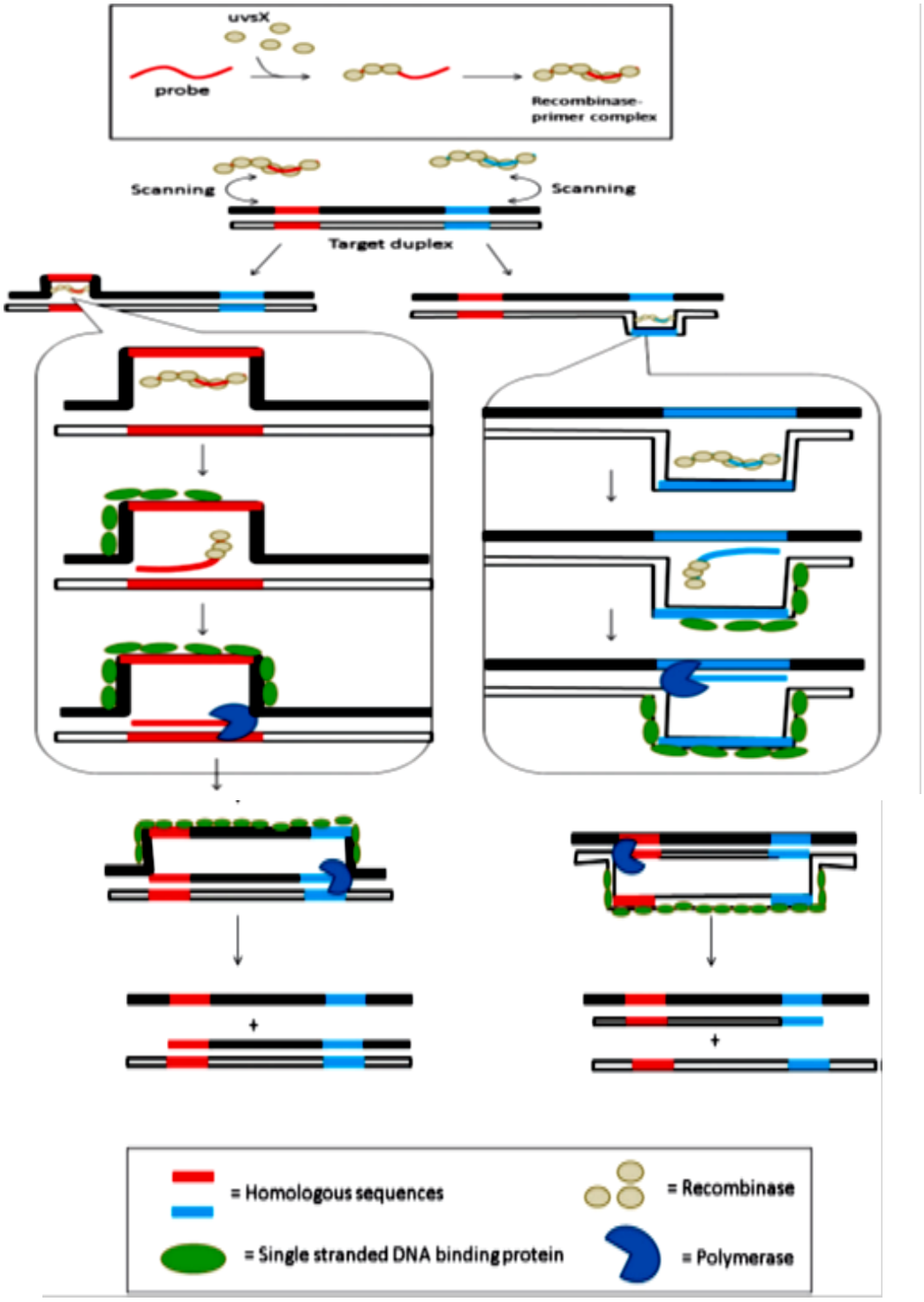

Recombinase polymerase amplification begins with the formation of the nucleoprotein complex, consisting of an oligonucleotide probe and a recombinase. The primer–recombinase complex then scans the duplex DNA until a sequence that is complementary to the primer is reached, at which point the recombinase aids the formation of a primer–DNA complex. DNA polymerase can then access the replication fork and extend the primer.

5. Helicase dependent amplification (HAD)

Helicase-dependent amplification (HDA) is an isothermal amplification technique that relies on a polymerase and helicase to amplify DNA without the need for thermal cycling. In this

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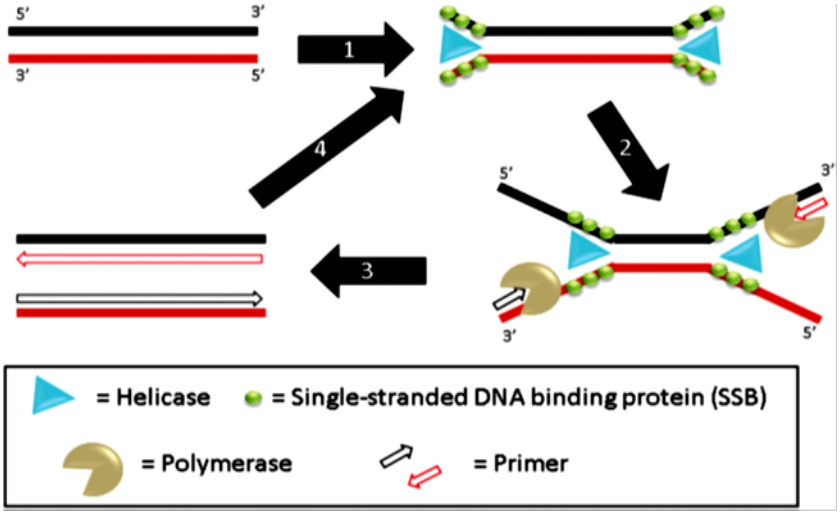
method, helicases unwind double-stranded DNA, while single-stranded DNA binding proteins stabilize the resulting single strands (step-1), which then hybridize to primers (step-2). Subsequently, DNA polymerase extends the primers (step 3), and the newly formed duplexes serve as templates for the next cycles (step 4). The use of a thermally stable helicase allows the process to be carried out at a constant temperature of 60°C, enhancing sensitivity and specificity while eliminating the requirement for additional proteins. A helicase-polymerase fusion complex has been developed, demonstrating the ability to amplify a 1.5 kb target with similar specificity to separate DNA polymerase and helicase. HDA is suitable for amplifying targets in complex biological samples, such as crude bacterial lysates, achieving million-fold amplification.



Helicase-dependent amplification (HDA). During step-1, helicase unwinds DNA duplex to generate ssDNA regions. This process is aided by SSB (single-stranded DNA binding proteins) and accessory protein. The primers can then bind to the ssDNA regions, extended by polymerase (steps 2 and 3). The newly formed duplexes can then be amplified again via steps 1–3.

Advantage and disadvantage of Isothermal amplification methods.

Advantage

- Nucleic acid sequence-based amplification (NASBA), - amplification of more than 10^9 copies in just 90 min.
- Rolling circle amplification (RCA) for detection characterization and for recombination.
- Loop-mediated isothermal amplification (LAMP) is rapid, sensitive, can be seen by eye, making LAMP well-suited for field diagnostics.
- Nicking enzyme amplification reaction (NEAR) -extremely rapid and sensitive & detection of small targets.
- Helicase-dependent amplification (HDA) requires only two primers.
- Strand displacement amplification (SDA)-exponential amplification

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Disadvantage

- NASBA - specificity of the reactions is dependent on thermolabile enzymes.
- RCA - analysis of restriction fragments generated mitochondrial sequences.
- LAMP –many primers & not useful for cloning.

Conclusion:

Sensitivity of this assay is more then compare to PCR because this assay can able to detect fiptomole, wear as PCR can up to picomole only. This can be analysed by serial dilution of DNA up to 10 fold.These methods have Specificity in detection of specific pathogen by using specific primers. Short time consuming then any other molecular / serological based methods. It takes approximately one hour to know the result was as PCR require three hour. Cost effective, initial investment is less because of no requirement of thermo cycler which is a coasty instrument.Amplification can get even simple DNA extraction methods. Were as PCR requires CTAB / Phenol chloroform based method of DNA and these methods are Eco-friendly.

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CHAPTER- 13
Cryo-EM an Emerging technique for the Elucidation of 3D Structure of Biomolecules

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Abstract

Cryo-Electron Microscopy is an emerging approach for the better elucidation and understanding the 3D structure of Biomolecules. In past 10 years Cryo-EM had become a promising appeal in solving the structures due to advancement in the hardware as well as the software involved in this technique. The perspective of this method is to combine all the 2D images of different orientation obtained from the electron microscopy into a single 3D image. The technique not only advances in the field of structural biology but also fills the gap that other standard techniques like X-ray Crystallography and NMR does not. The chapter includes the basic understanding of the methodology involved in Cryo-EM and the 3D Reconstruction of the image. Furthermore it also discuss some advance applications that have been going on currently so as to enhance the basic knowledge and perception of this emerging approach.

Introduction

Field of Structural Biology was originated in the midway of the last century. It is the branch of study that deals with the structural elucidation of bio macromolecules thereby understanding their function. Currently there are 3 techniques that are generally used by the structural Biologists: X-ray Crystallography, NMR Spectroscopy and Cryo-EM.As of October 17, 2019, the crystal structures in the PDB database account for about 89% of the total, the NMR structures account for 8.2%, and the EM structures account for 2.5%[1](Figure: 1). X-ray Crystallography is mostly relevant for proteins with molecular weight of 10-150 kDa whereas NMR is frequently used to study protein with low molecular weight of less than 80kDa.

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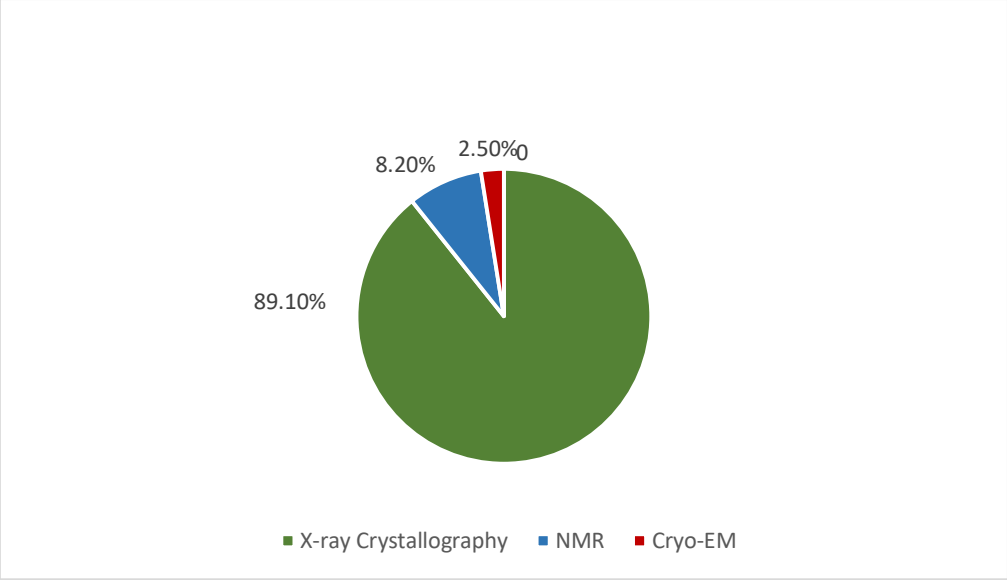

Figure: 1 Structural determination from three different methods

The advantage Cryo-EM holds over X-ray Crystallography and NMR are:

- The major advantage of Cryo-EM over X-ray Crystallography is it does not require any crystal.
- Cryo-EM technique also minimizes the chance of damage by radiation and can maintain the native form and functional state of the sample.
- Different multiple Conformational states can be captured in one experiment.
- It is appropriate for the structural analysis of membrane proteins such as GPCR and their complexes [1].

General Procedure for Structural Analysis by Cryo-EM

The extensive used approach in the process of structural analysis by Cryo-EM method are as follows:

(a) **Gene Expression**: Generally gene that codes for desired protein is amplified *in vitro* (with the help of Thermal Cycler) and inserted into the plasmid of host vector (DH5α is basically used as host vector for transformation). The amplified plasmid DNA is then isolated and inserted into expression vector.

(b) **Protein Expression**: Desired protein is expressed with the help of an expression vector (BL21 is used as expression vector) by IPTG induction. The expressed protein is then isolated and subjected to purification techniques.

(c) **Protein Purification**:Cryo-EM requires high purity, homogeneity, and integrity of the sample, as the isolated protein sample might have contain other proteins along with the desired protein, the sample needs to be purified. Chromatography techniques such as Affinity Chromatography, Size Exclusion Chromatography & Ion Exchange Chromatography are used for purification purpose. SDS PAGE can be frequently used

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to monitor the purity of the sample after each chromatography techniques. The sample is said to be pure if molecular sieve show a single peak and symmetrical distribution [1].

(d) **Negative Staining& Grid Preparation**: The sample molecules are submerged in the layer of heavy metal salt solution[1]. Then the sample is loaded (approx. 3µl)on the metal grid, generally copper or gold coated grids are used. A thin layer of sample is prepared on the metal grid.

(e) **Freezing**:With the help of Vitrobot the sample is then immediately plunge frozen into the liquid ethane (Liquid ethane is used because of its higher specific heat capacity). The advantage of this step is that the sample can be close to its "natural" state.

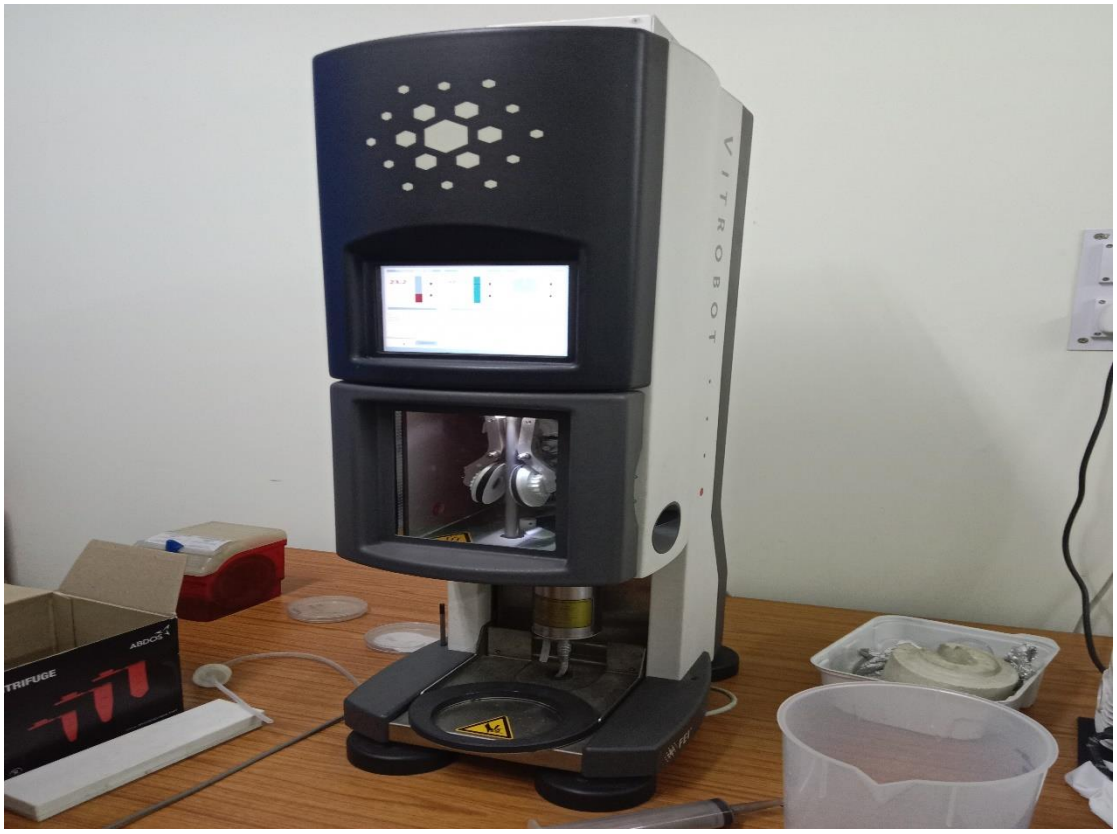

Figure: 2 Vitrobot used for quick freezing the sample

(f) **Imaging:** The sample is then loaded in Cryo-Electron Microscope for imaging. Previous imaging or screening of the sample can be done under normal TEM before subjecting the sample for final imaging under Cryo-EM.

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Figure: 3 Cryo-Electron Microscope, Pfizer's Groton, Connecticut [12].

(g) **Data Collection & 3D Reconstruction of Image:** Data is collected in the form of frames or movies. **Relion software** is used for the 3D Reconstruction of the image. The obtained frames are then import and subjected for the correction due to beam induced motion, furthermore the movies or frames are exposed to CTF estimation (for better resolution by phase changing) after which the movies are now known as micrograms. The micrograms are then manually picked by keeping certain criteria in mind such as: (1) Choosing approx. 500-600 per total microgram, (2) Avoid overlapped microgram, (3) Choose from high defocusing.

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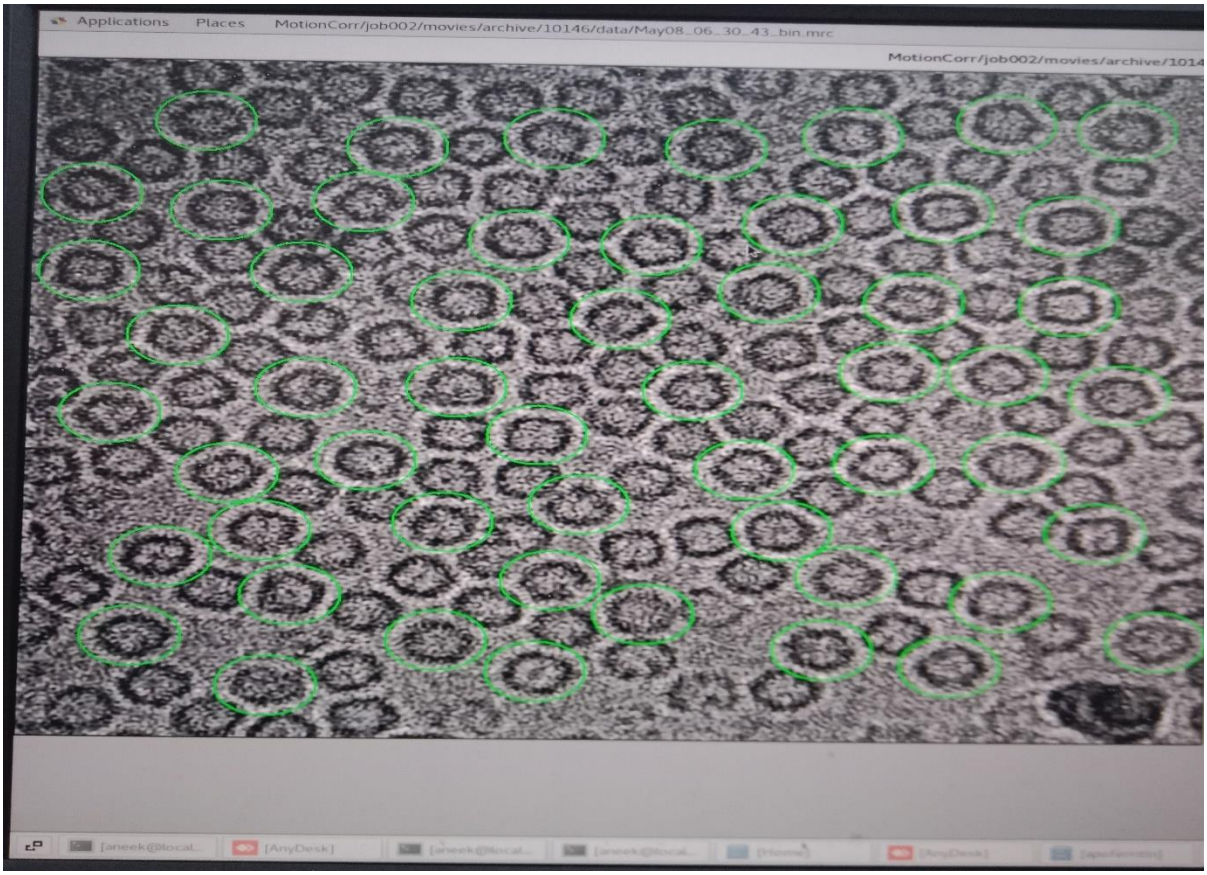

Figure: 4 Image shows the selection of micrograph in Relion software

Manually picked micrographs are then subjected to particle extraction followed by auto picking. Auto picked micrographs are then underwent 3D Model Reconstruction process where system tries to make 3D model by using 2D class values. Several steps including High resolution 3D Refinement and Mask Creation are done before the final Model Building. The 3D refined model after post processing is then fitted into the atomic model which is taken as the reference (from the repositories such as: Empiar, EMDB, PDB). Software such as **UCSF Chimera** is used for 3D image viewing. **Phoenix** and **COOT** are used for manual correction whereas **α-fold** can be used for the prediction of unknown structures.

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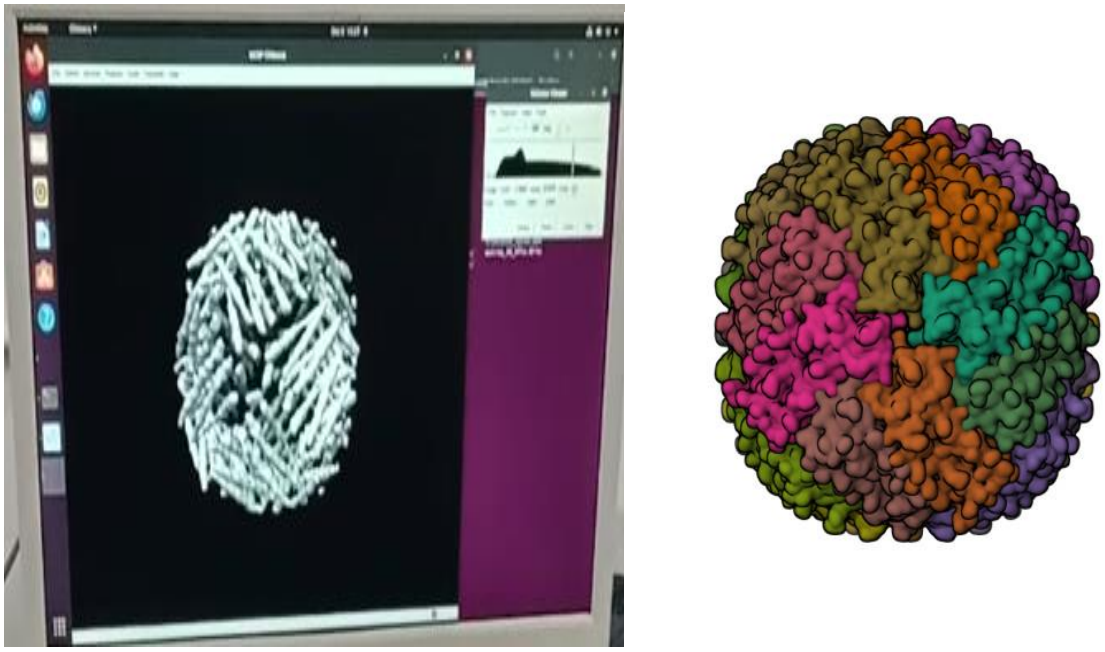

(a) (b)

Figure: 5 (a) Shows image of Apoferritin during refinement process, (b) Shows the image of Mouse Apoferritin after final 3D modelling (Apoferritin is the model protein used in Cryo-Electron Microscopy)[13].

Factors that influence the Resolution of the Structure

- Homogenous Protein sample.
- Proper grid preparation
- Ice formation during plunge freezing must be thin (balanced), neither thick otherwise the molecule will be present in the same plane, which will hinder nor too thin otherwise it will burn the sample.
- During imaging molecules must have all the orientations for better reconstruction of 3D structure.
- Visual biasing must be avoided.
- During imaging elastically scattering of electrons are needed (so that they will not transfer energy to the sample).
- Sometimes during final 3D reconstruction some part of atomic model doesn't fit in with Cryo-EM electron density map, in such cases manual correction can be done with the help of softwares.

Applications

Cryo-ET (Electron Tomography)

Electron Tomography is used in the case of molecules that have preferred orientation (eg: Mitochondria). In this imaging technique string of images are collected with every image taken at a different angle relative to the direction of the incident electron beam. Furthermore the images are combined computationally to form "tomograms". ET imaging requires sample

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to be thin enough for the incident of electron beam to be transmitted, typically not much more than~ 0.5 microns even for the microscopes capable of operating at 300 kV [2]. Other applications of Cryo-ET includes imaging of thin regions of mammalian cells [4] and investigation of a variety of filamentous and cytoskeletal assemblies [5, 6].

Application is SBDD

SBDD is a streamlined drug design method rest on molecular recognition of the three-dimensional structure of ligands and target proteins, with the aim of finding and optimizing small-molecule drugs[7]. Generally, the steps involved in SBDD include structure determination of the target protein, cavity identification, ligand database construction, ligand docking and lead discovery.

Application in Antibody Drug Development

After immunizing animals or humans with labelled antigens to obtain antigen and polyclonal antibody complexes, Cryo-EM is used to analyse the complex structure to confirm key epitopes and build epitope models. Considering the complexity of the manual matching process between density maps and amino acids, they also developed an algorithm tool for identifying antibody sequences based on density maps and matched and scored them with the antigen-binding specific B-cell next-generation sequencing database, thus quickly obtaining epitope information and monoclonal antibody structural models. Recently, the structures of B-cell receptors have also been resolved by Cryo-EM, which promotes the development of antibody-based therapeutics[9, 10].

In case of Membrane Proteins

Membrane proteins are the important protein involved in most of the biological process. It is estimated that about 60% of the drug targets are membrane proteins [1]. However purification and crystallization of membrane protein is very tough process, many membrane proteins do not form a crystal at all as the result it is very difficult to solve their structure with the traditional methods like X-ray Crystallography. This problem can be solved by using Cryo-EM as it does not require any crystal.

Viral Particles

The size of virus particles is larger than that of proteins, so it is more suitable to analyze their 3D structure with Cryo-EM. In 2018, Yuan *et al*, first reported the atomic resolution structure of the nucleocapsid of herpes simplex virus type 2 (HSV-2) of the herpesvirus α family.

Conclusion

Structure defines the function of all the biomolecules, to understand the behaviour and function of any biomolecule one must first understand its structure. Here comes the field of structural biology in play. Standard techniques like XRD & NMR have been used for the 3D structural determination of various molecules. Cryo-EM have now become an emerging techniques that that not only provide 3D structural information of the molecule but also holds many advantages over traditional methods. With the development of technology and computational biology Cryo-EM may even surpass X-ray Crystallography. In India there are

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only few national level labs that have the state of art facility of Cryo-Electron Microscopy such as CCMB Hyderabad, IISC Bangalore, IISER Trivandrum, NCBS Bangalore, IIT Delhi, and AIIMS Delhi.

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CHAPTER- 14
SYMBIOSIS: ANMAGNIFICENT NATURAL PHENOMENA

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Abstract

Nature is an excellent example of balance. This balance maintains the environment and is a prime factor supporting life on this planet since years. Interdependency of biotic and abiotic factors is the most vital balance which successfully circulates energy and elements and maintains proper exchange mechanism. Apart from recycling of elements the interactions of plant-plant, microbe-plant, microbe-animal, plant animal and animal-animal are building blocks of food chain and food web maintaining the species population balance. The most admirable relationship is symbiosis where both the partners are benefitted due to the association. Symbiosis has greater prospects than only being an environmental phenomena. This chapter deals with symbiosis and its nature, variation and applicability.

Keywords: microbe, environment, symbiosis, recycling, relationship

Introduction:

Frequently, ongoing relationships between creatures of several species are referred to as symbiosis. Symbiosis is everywhere and have been crucial in the development of many modern forms. Cnidarians and green algae form a symbiotic relationship that results in corals, which enables the construction of complete reefs. Large-scale elementary nitrogen (N_2) fixation by legume plants and rhizobial bacteria is a crucial process for the availability of Nitrogen in many terrestrial ecosystems. Lichens are often found in difficult conditions where neither symbiont can survive on their own. They are the result of a symbiosis between fungus and algae or cyanobacteria.The majority of terrestrial plants have a symbiotic relationship with soil fungus called arbuscularmycorrhizal fungi (AMF), which give the plants nutrients in exchange for carbohydrates. It is likely that these symbiotic organisms helped terrestrial plants colonise the land. These few ecologically significant examples show situations when both symbionts benefit; these mutually beneficial symbioses are also known as mutualisms. [1-2]

The advantages of symbioses rely on the surrounding environment; therefore each one contact may be advantageous, commensal, or pathogenic.The definition can be limited to plainly discernible, mutualistic symbioses that have coevolved. The degree and duration of physical interaction between the partners influences how symbiosis is defined. While vertically transmitted symbionts can involve ongoing communication throughout their life cycle, horizontally transmitted symbioses are sometimes inherited and frequently reform. At the extreme, the eukaryotic cell organelles chloroplasts and mitochondria were once

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symbionts that were vertically transmitted over the course of several billion years. Contrarily, the brief contact between plants and insect pollinators is frequently not regarded as a symbiosis. Only 'intimate' microbial symbionts will be covered.[3-4]

Single-cell species such as microorganisms are assumed to haveinitially. Over Time, plants and animals established and flourished. As each new kingdom of life came about, the Ecosystem on Earth became more complex and the bionic components became modifiedin a broad definition, as “the living together in an intimate association of two or more dissimilar organisms.” Symbiosis can result in a relationship in which both organisms benefit. Nitrogen Fixation by legumes is a consequence of microbes that fix nitrogen and plants that supply simple- carbons.

Plants and fungi have established a cooperation in which the plant provides nutrients and ‘the fungi provide alkaloids to deter predation and allow for greater drought tolerance. More generally, plants and herbivores have essentially co-evolved such that the action of herbivores on plants can lead to greater diversity and dispersion of seed. [5-6]

Complex cellulose degradation of plant materialby herbivores is accomplished by specialized bacteria in gastrointestinal compartments that are optimally maintained by each host animal for bacterial growth. Within the mammalian digestive-tract, commensal microorganisms can provide energy, amino acids, and vitamins for the host, and provide protection against parasitic microorganisms. [7-8]

Microbes and parasitism/pathogenicity with plants and animals:

Parasitism, in its strictest definition, describes an interaction of one organism surviving at the expense of the other. However, when microbes are involved, the level of interaction is less clear and the definition less strict. To some extent, the definition of a parasite may depend on the point the depth of understanding of the symbiosis. In bio-trophic parasitism, one organism surviveat the expense of the other may but in reality it is an interaction in which both Organisms benefit, but the full symbiosis is not known. In addition, some interactions may be mutualistic (both organisms benefit) at certain times, but parasitic at other times. In contrast, pathogenic organisms represent examples of necrotrophic parasitism, but not all pathogenic microbes cause death of the host. Regardless, pathogen interaction represents greater loss to the host and needs to be considered differently than a parasitic interaction. Among plants and animals, particularly those of agricultural importance, a number of parasitic and pathogenic microbes are important relative to disease and host health. In animals a number of pathogens may survive and multiply in one host as a commensal and not cause disease, but can cause disease or death in another host. Understanding these zoonotic pathogens is important not only to animal health, but also to providing a safe food and water supply to humans. [9-10]

Symbioses between microbes are probably common and functionally important in a variety of biological systems, although they are hard to find. Symbioses can be relatively uncommon or dynamic, intercellular connections can be delicate, and the majority of microorganisms are still uncultivated. As a result, conventional techniques like microscopy are ineffective for the exact identification of novel interactions, their metabolic underpinnings, and the species involved. The field of microbial ecology has been completely transformed by high-throughput metagenomic sequencing of entire microbial communities. However, evidence for direct links between microbial species cannot be obtained from bulk samples, and genomic signals from symbionts can get buried in sequences from abundant organisms. As a result,

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characterizing symbioses between naturally occurring bacteria requires a specialized methodology. In order to characterize uncultivated symbionts using genomic and metagenomic methodologies, this chapter describes strategies for integrating fluorescence-activated cell sorting to extract and separate uncultivated symbionts with molecular biology techniques for DNA amplification.[26]

Plant Microbial Parasites and Pathogens:

Plants are the most abundant form of terrestrial life and are plagued by numerous opportunistic Organisms colonizing the leaves, stems, and roots. Fungi are associated with spotting, rusting, wilting and rotting of plants. Fungi in the phylum of Ascomycota (commonly called Ascomycetes) are diverse group known for a sac structure and include important decomposers in nature and sources for important medicinal uses. Species associated with plant disease include *Aspergillus, Fusarium, Thielaviopsis, Uncinula,* and *Verticillium*. Fungi in the phylum of Basidiomycota (commonly called Basidiomycetes) are a diverse group known for a “club” or “fruiting” structure, and include plant Disease-causing species of Rhizoctonia, Phakospora, and Puccinia. Oomycetes are small eukaryotic organisms, or protists, that are fungal-like, include species of Pythium and Phytophthora, and are associated with rusts, rots, and blights. Numerous bacteria cause diseases in plants, but species belonging to *Agrobacterium, Burkholderia, Clavibacter, Erwinia, Phytoplasma, Pseudomonas, Spiroplasma,* and *XanthomonasSpecies* can cause significant damage or death to plants. Disruption of Plant colonization is important for control of many diseases caused by microbes. [11-12]

Symbiosis is a close, sustained, and particular relationship between organisms of two or more different species. This definition, which broadly applies to a variety of connections of a positive, neutral, or destructive nature, is basically identical to the original idea of symbiosis coined by Anton de Bary in 1879. Accordingly, a relationship that benefits both partners is known as "mutualism," a relationship that is destructive to one party is known as "parasitism," and a relationship that benefits one partner while being mainly neutral for the other is known as "commensalism." There is another definition of symbiosis that only refers to mutualistic interactions and is often used by scientists in Europe. [27]

Animals and Microsporidia:

Microsporidia are unicellular organisms and intracellular parasites found in all major animal groups.Microsporidia are a common parasite in insects and fish, and a particular problem for farm-raised fish. Infection is associated with chronic, persistent illness and the parasite, although not directly lethal, has been shown to result in 30% mortality in farmed salmon. In most cases, the host exhibits reduced weight, vigor, and fertility. In addition to fertility issues, transmission can be vertical to the offspring, particularly in insect and crustacean hosts, and this parasite can change the sex of hosts via suppression of androgenic gland development. These organisms represent a large group of microbes that are re1ated to fungi phylogeneticalIy, but they are atypical fungi in cell structure. Formerly thought of as protists are called microspora, these eukaryotic organisms lack mitochondria (they have mitosomes), are non-motile, and form spores with thick cell walls that can survive outside the hosts for years. Shifts in pH can prime the spores to germinate and inject the microsporidia into the

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host cell, which is typically a mucosal epithelial cell. Once colonized in the host, the parasite can exploit the host cell for nutrients and energy. [13-14]

In coral:

Corals are the primary designers of ecosystems, building massive, complicated reefs that harbour a variety of marine species. A dynamic partnership with microorganisms, collectively known as the coral holobiont.This relationship includes a mutually beneficial symbiosis with photosynthetic dinoflagellates (*Symbiodinium* spp.), as well as enduring alliances with a variety of bacterial, archaeal, fungal, Protistant and viral partners. This coral holobiont's combined genomes create a coral hologenome, and interactions between genes within the hologenome eventually determine the coral phenotype. [29]

History:

In an 1878 lecture, Heinrich Anton de Bary introduced the term “symbiosis” in a biological context to describe intimate associations between different types of organisms. Symbiosis is ubiquitous in the natural world, especially when considering microbial symbionts such as the human microbiome(s). Due to this pervasiveness, most species have co-evolved with symbionts in the past and continue to do so in the present. Symbioses have been hypothesized to influence the evolution of sexual recombination, complexity, diversity, evolvability and cooperation, among many other behaviors. Indeed, symbiosis in all of its varieties is essential to our understanding of how life on our planet evolved, how it may continue to evolve, and how its evolutionary mechanisms can be harnessed to solve other problems. [15-17]

Deep decarbonization and effective water utilisation are made possible more easily by symbiotic infrastructure systems than by standalone upgrades to each type of infrastructure. Using sludge and reused water from municipal wastewater treatment as alternative fuels and water sources for coal power generation, linking the coal power and wastewater treatment sectors in China.A geo-database with information on 2,400 coal-fired power plants and 4,200 municipal wastewater treatment facilities was studied to conduct an integrated analysis utilizing. Such infrastructure symbiosis yearly provides 8.6 Mt CO_2 equivalent of greenhouse gas (GHG) abatement, which is equivalent to 29% and 0.28% of GHG emissions from the coal power and wastewater treatment sectors, respectively. [18]

The symbiosis saves 3.0 billion of freshwater yearly or 62% of the freshwater used by the coal power sector and generates 7.5 (3.4-12) billion CNY in cost savings. For sludge co-combustion and water reuse, respectively, 32% and 44% of all plant-level links can be used to achieve around 80% of the carbon, water, and economic advantages. Infrastructure symbiosis offers significant chances for both economic and environmental advantages. The cost-effective implementation of policies to encourage the development of infrastructure that integrates energy and water make it easier for China to meet its water and climate goals. [19]

Symbiosis refers to the phenomenon of organisms living in close association with one another. Symbiosis research has intellectual roots in Natural Philosophy and Natural History schools where it associates with sociocultural and political ideas on the organizational structure of society, as well as with theorizing on the nature and (re)distribution of ‘common goods,’ and the ‘division of labor’ in the ‘economy of nature.’ Symbiosis research was introduced in zoology and botany from the mid-eighteenth century onwards. [20]

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Theory of endobiogeny:

When two or more creatures coexist for their mutual and ultimately intrinsic benefit, this is known as symbiosis. The human and the microbiome, which are commensal organisms that coexist with humans, have a symbiotic relationship. The diverse flora plays a part in maintaining the organism's structural integrity and preventing the introduction of noncommensal organisms. They function in the areas of immunity, metabolism, adaption and adaptability. They also serve structural and functional functions. Receptors for hypothalamus and pituitary hormones are found in the intestines, where they modify the colonic flora's activity to change the rate of nutrient and fluid absorption. This is a crucial mechanism for adjusting food availability to the needs of the organism's endobiogenic processes. More precisely, the human being is an epiorganism made up of both the actual human and commensal flora.[21]

The evolution:

Symbiosis appears to be commonplace when learn about nature. The most striking examples of symbiosis' role in evolution are endosymbiotic relationships, in which prokaryotic organisms have established themselves as regular "inhabitants" of eukaryotic cells. The major examples of this are mitochondria and chloroplasts, which have been proven to have descended from prokaryotes. Other examples have recently been discovered in a number of smaller populations, including certain insects. Because the most usual, traditional instances of parasitism and mutualism frequently show a variety of coevolved traits in the involved species, these phenomena have also had wide-ranging and varied impacts on nature and evolution in general.[22]

Comparatively speaking to other types of symbioses, commensalism, amensalism, and synnecrosis have gotten comparatively less study attention. These are symbioses when all interacting species suffer significantly unfavourable effects or where the fitness of one species is unaffected by the contact. Natural selection is projected to be unfavourable to several of these symbioses, therefore they are not predicted to frequently occur in nature. [23]

Commensalism:

The host and the commensal do not interact physiologically or depend on one another during commensalism. The phrase literally translates to "eating at the same table." In other words, commensalism is a sort of symbiosis in which the commensal has access to the host's substances through spatial closeness. Each partner can live on their own. Although some nonpathogenic species, such as protozoa, are sometimes referred to as commensals, this interpretation is inaccurate because these organisms are parasites because they are physiologically dependent on the host. Hermit crabs and the sea anemones they transport on their borrowed shells are an illustration of commensalism. [24]

Endosymbiosis:

Perhaps more than any other mechanism, endosymbiosis has served as the main character in the story of eukaryotic evolution. The most important factor in the genesis and development of complex life on Earth has been primary endosymbiosis, which is the reception of a prokaryote by another living cell. The genetic fusion of an initially non-photosynthetic

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alphaproteobacterium and then a photosynthetic cyanobacterium into a eukaryotic cellular framework has profoundly affected and changed the biogeochemistry and biodiversity of the globe, from the land to the ocean to the atmosphere. [25]

Contemporary biology and modern biological philosophy both place a strong emphasis on symbiosis. The development of the hologenome concept of evolution, the idea that holobionts are the basic building blocks of natural selection in evolution. The hologenome concept of evolution provides the philosophical foundations of arguments. It also traces the debates back in time to their historical infancy, to the time when biologists first began to notice the relationship between the concepts of symbiosis and biological individuality. [28]

Symbiosis in in computer science and engineering:

Nature-inspired algorithms are those used in computer science and engineering to build functioning models by drawing ideas from living objects and mimicking their behaviours. A novel and promising metaheuristic algorithm is called the SOS algorithm (symbiotic organisms search). The symbiotic relationship that develops between various species in an ecosystem serves as the foundation for it. To live in their environment, organisms form symbiotic relationships such as mutualism, commensalism, and parasitism. Since then, Standard SOS has undergone numerous modifications, either through hybridization or as improved iterations of the original algorithm. The majority of these alterations were inspired by engineering construction projects and other fields like medicine. [30]

In recent decades, numerous metaheuristic algorithms have been put out to address optimisation issues. As a result, optimisation strategies have emerged as a result of metaheuristicresearches that have outperformed traditional gradient-based methods. A new SI-based metaheuristic algorithm that was motivated by nature is the Symbiotic Organisms Search algorithm (SOS)[19].

The SOS algorithm replicates the cooperative behaviour seen in different animals in an ecosystem. The upgraded and enhanced SOS encounters several difficulties while attempting to address complex optimisation issues that call for additional synergy. Therefore, by looking inside to the characteristics of the Basic (SOS) algorithm, these algorithms, adjusted or expanded, can still be improved. [31]

Conclusions:

Modern agriculture has led to significant improvements in efficiency of food production. The use of fertilizers, herbicides, fungicides, pesticides, antibiotics, and growth promoters has been instrumental in feeding the world. However, natural selection has established interactions that are often overlooked or disregarded. These symbiotic relationships could potentially reduce our use of synthetic agents and promote a more sustainable productive agricultural system. In addition, dedicating research efforts to better describe and understand beneficial symbiosis would allow more opportunities to exploit mutualistic relationships when they arise. Planting crops in particular rotations and with minimal tillage may sustain rhizosphere interactions that promote healthiness and growth of plants. Herbivores, such as the ruminant, have historically benefitted from a variety of symbiosesand there are many opportunities to enhance our understanding of these relationships to better utilize non-cultivatable land for animal production.

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CHAPTER- 15
GENOMICS AND ITS APPLICATIONS IN BIOTECHNOLOGY

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Abstract

Genomics is the multidisciplinary science to understand the structure, function and evolution of genes and their interactions by identification, molecular characterization and cloning of whole genomes for the ultimate goal of understanding phenomics of organisms. Overall genomics includes sequencing of genomes, determination of complete set of genes, analysis of transcripts and proteins encoded by an organism, their interactions and understanding of metabolic pathways. Thus, genomics not only deals with the generation of the genetic information, but also understanding mechanisms by which this information is used by an organism. Genomics is often divided into structural genomics, functional genomics and comparative genomics. Structural genomics deals with the construction of high resolution genetic and physical map and determination of the complete genome sequence or the complete set of transcripts produced by an organism. Functional genomics characterizes all the genes present in the genome in one go.The information generated through genomics have great impact on other biological sciences like medicine, environment and agriculture.

Keywords: genomics, metabolic pathway, medicine.

Introduction:

Genomics is an interdisciplinary field of biology focusing on the structure, function, evolution, mapping, and editing of genomes. A genome is an organism's complete set of DNAs, including all of its genes as well as its hierarchical, three-dimensional structural configuration. (franklin*et al.*,1953) (Rossi *et al*, 2008) In contrast to genetics, which refers to the study of individual genes and their roles in inheritance, genomics aims at the collective characterization and quantification of all of an organism's genes, their interrelations and influence on the organism. Genes may direct the production of proteins with the assistance of enzymes and messenger molecules. In turn, proteins make up body structures such as organs and tissues as well as control chemical reactions and carry signals between cells. Genomics also involves the sequencing and analysis of genomes through uses of high throughput DNA sequencing and bioinformatics to assemble and analyse the function and structure of entire genomes. (SatzingerH ,2008). Advances in genomics have triggered a revolution in discovery-based research and systems biology to facilitate understanding of even the most complex biological systems such as the brain. The field also includes studies of intragenomic (within the genome) phenomena such as epistasis (effect of one gene on another), pleiotropy (one gene affecting more than one trait), heterosis (hybrid vigour), and other interactions between loci and alleles within the genome. (Kadakkuzha*et al* ,2019)

Brief History:

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Genomics is a concept that was first developed by Frederick Sanger in early 1970s, who first sequenced the complete genome of a virus and of a mitochondrion. (Pevsner, 2009). In 1972, Walter Gilbert and his research group became the first to sequence a gene. They sequenced the gene of Bacteriophage MS2.They shared half of the 1980 Nobel prize in chemistry for independently developing methods for the sequencing DNA. In 1995, Hamilton O. Smith and his team became the first to sequence a genome of a free-living organism – that of Haemophilusinfluenzae.

Types of genomics:

I. Structural genomics: Itdetermine the structure of every protein encoded by the genome.
II. Functional genomics: Itcollect and use data from sequencing for describing gene and protein functions.
III. Comparative genomics: Itcompare genomic features between different species.

Structural genomics:

Structural genomics seeks to describe the 3-dimensional structure of every protein encoded by a given genome. This genome-based approach allows for a high-throughput method of structure determination by a combination of experimental and modelling approaches. (Brenner *et al*,2000) The principal difference between structural genomics and traditional structural prediction is that structural genomics attempts to determine the structure of every protein encoded by the genome, rather than focusing on one particular protein. With full-genome sequences available, structure prediction can be done more quickly through a combination of experimental and modelling approaches, especially because the availability of large numbers of sequenced genomes and previously solved protein structures allow scientists to model protein structure on the structures of previously solved homologs. (Marsden *et al*,2007) Structural genomics involves taking a large number of approaches to structure determination, including experimental methods using genomic sequences or modelling-based approaches based on sequence or structural homology to a protein of known structure or based on chemical and physical principles for a protein with no homology to any known structure. As opposed to traditional structural biology, the determination of a protein structure through a structural genomics effort often (but not always) comes before anything is known regarding the protein function. This raises new challenges in structural bioinformatics, i.e. determining protein function from its 3D structure. (Brenner *et al.*,2001)

Functional genomics:

Functional genomics is a field of molecular biology that attempts to make use of the vast wealth of data produced by genomic projects (such as genome sequencing projects) to describe gene (and protein) functions and interactions. Functional genomics focuses on the dynamic aspects such as gene transcription, translation, and protein–protein interactions, as opposed to the static aspects of the genomic information such as DNA sequence or structures. Functional genomics attempts to answer questions about the function of DNA at the levels of genes, RNA transcripts, and protein products. A key characteristic of functional genomics

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studies is their genome-wide approach to these questions, generally involving high-throughput methods rather than a more traditional "gene-by-gene" approach.
A major branch of genomics is still concerned with sequencing the genomes of various organisms, but the knowledge of full genomes has created the possibility for the field of functional genomics, mainly concerned with patterns of gene expression during various conditions. The most important tools here are microarrays and bioinformatics.

Comparative genomics:
Comparative genomics is a field of biological research in which the genomic features of different organisms are compared. The genomic features may include the DNA sequence, genes, gene order, regulatory sequences, and other genomic structural landmarks. (Touchman ,2010). In this branch of genomics, whole or large parts of genomes resulting from genome projects are compared to study basic biological similarities and differences as well as evolutionary relationships between organisms. The major principle of comparative genomics is that common features of two organisms will often be encoded within the DNA that is evolutionarily conserved betweenthem. (Hardison,2003). Therefore, comparative genomic approaches start with making some form of alignment of genome sequences and looking for orthologous sequences (sequences that share a common ancestry) in the aligned genomes and checking to what extent those sequences are conserved. (Xia X ,2013). Based on these, genome and molecular evolution are inferred and this may in turn be put in the context of, for example, phenotypic evolution or population genetics. (Hardison, 2003).

Genome sequencing:
Sequencing means to determine the primary structure of an unbranched biopolymer. Gene sequencing is the technique that allows researchers to read the genetic information found in DNA. Sequencing involves determining the order of bases. (Shendure*et al*,2008)
History**:** Firstsequenced genome was $_{\varphi}$X 174 bacteriophage in 1977 (5,375 bp) by Fred Sanger.
• In 1995: Haemophilus Influenza; (1,830,137 bp) Mycoplasma genitalium; (580,000 bp).
• In 1996: Saccharomyces cerevisiae; (12,068,000 bp) • In 1997: Escherichia coli; (4,639,221 bp).
• In 1999: Human chromosome; (53,000,000 bp).
• In 2000: Drosophila melanogaster; (180,000,000 bp).
• In 2001: Human; Working draft; (3,200,000,000 bp).
Methods of genome sequencing:

1. **Sangar's genome sequencing**:

It is a method of DNA sequencing that involves electrophoresis and is based on the random incorporation of chain-terminating dideoxynucleotides by DNApolymerase during in vitro DNA replication. After first being developed by Frederick Sanger and colleagues in 1977, it became the most widely used sequencing method for approximately 40 years. (Daniels*et al*,2021)It was first commercialized by Applied Biosystems in 1986. More recently, higher volume

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Sanger sequencing has been replaced by next generation sequencing methods, especially for large-scale, automated genome analyses.

Procedure:

The classical chain-termination method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, normaldeoxynucleotide triphosphates (dNTPs), and modified di-deoxynucleotide triphosphates (ddNTPs), the latter of which terminate DNA strand elongation. These chain-terminating nucleotides lack a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a modified ddNTP is incorporated. The ddNTPs may be radioactively or fluorescently labelled for detection in automated sequencing machines. (Sanger *et al*,1977)

The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP), while the other added nucleotides are ordinary ones. The deoxynucleotide concentration should be approximately 100-fold higher than that of the corresponding dideoxynucleotide (e.g. 0.5mM dTTP: 0.005mM ddTTP) to allow enough fragments to be produced while still transcribing the complete sequence (but the concentration of ddNTP also depends on the desired length of sequence). Putting it in a more sensible order, four separate reactions are needed in this process to test all four ddNTPs. Following rounds of template DNA extension from the bound primer, the resulting DNA fragments are heat denatured and separated by size using gel electrophoresis. In the original publication of 1977, the formation of base-paired loops of ssDNA was a cause of serious difficulty in resolving bands at some locations. This is frequently performed using a denaturing polyacrylamide-urea gel with each of the four reactions run in one of four individual lanes (lanes A, T, G, C). The DNA bands may then be visualized by autoradiography or UV light, and the DNA sequence can be directly read off the X-ray film or gel image.In the image on the right, X-ray film was exposed to the gel, and the dark bands correspond to DNA fragments of different lengths. A dark band in a lane indicates a DNA fragment that is the result of chain termination after incorporation of a dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP). The relative positions of the different bands among the four lanes, from bottom to top, are then used to read the DNA sequence (figure 1).

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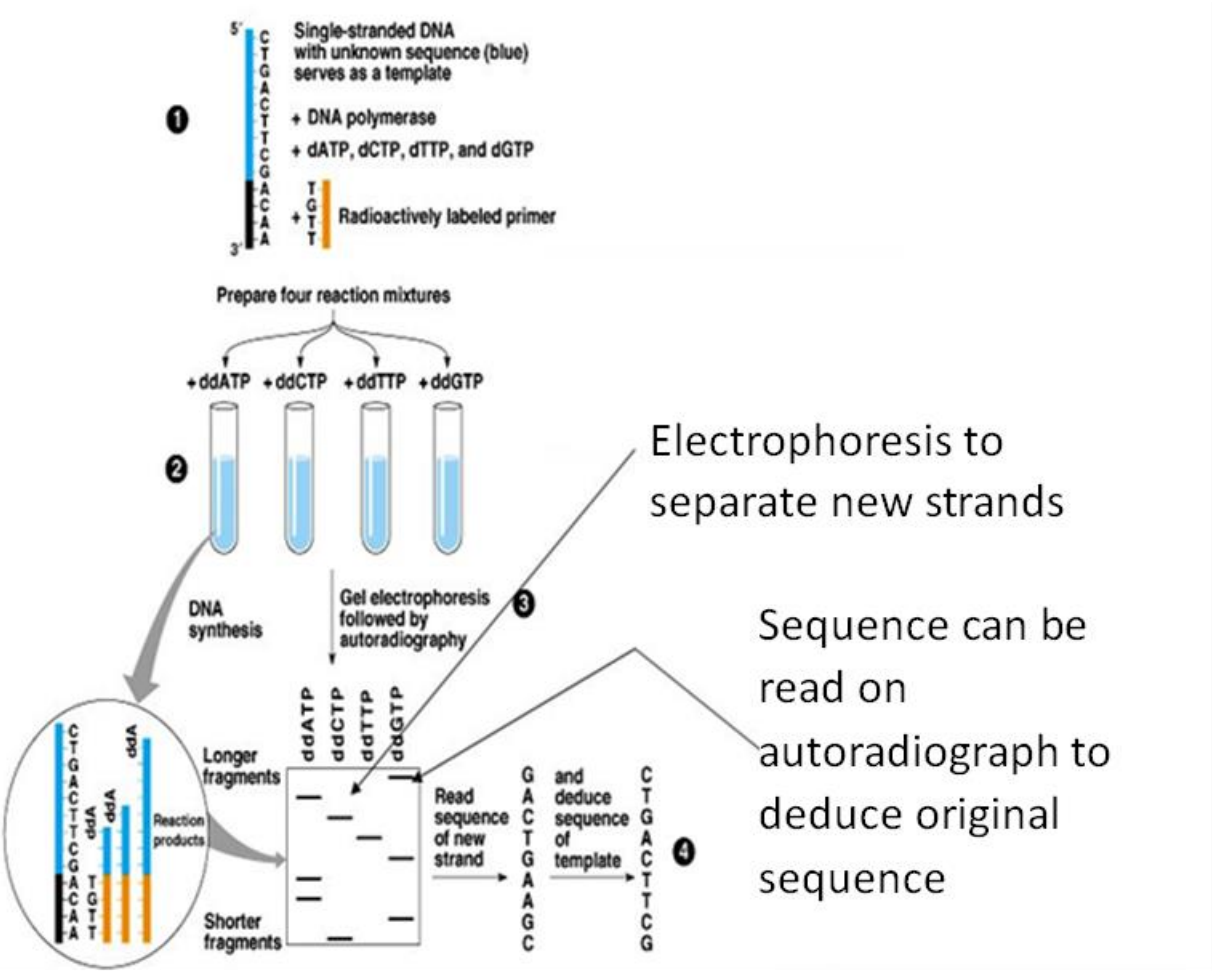

Figure1: Sangar's method of gene sequencing.

2. **Maxam gilbert genome sequencing**:

It is a method of DNA sequencing developed by Allan Maxam and Walter Gilbert in 1976–1977. This method is based on nucleobase-specific partial chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides.Maxam–Gilbert sequencing was the first widely adopted method for DNA sequencing, and, along with the Sanger dideoxy method, represents the first generation of DNA sequencing methods. Maxam–Gilbert sequencing is no longer in widespread use, having been supplanted by next-generation sequencing methods.

Procedure:

Maxam–Gilbert sequencing requires radioactive labelling at one 5′ end of the DNA fragment to be sequenced (typically by a kinase reaction using gamma-^{32}P ATP) and purification of the DNA. Chemical treatment generates breaks at a small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T). For example, the purines (A+G) are depurinated using formic acid, the guanines (and to some extent the adenines) are methylated by dimethyl sulphate, and the pyrimidines (C+T) are hydrolysed using hydrazine. The addition of salt (sodium chloride) to the hydrazine reaction inhibits the reaction of thymine for the C-only reaction. The modified DNAs may then be cleaved by hot piperidine; $(CH_2)_5NH$ at the position of the modified base. The concentration of the modifying chemicals is controlled to introduce on average one modification per DNA molecule. Thus, a series of

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labelled fragments is generated, from the radiolabelled end to the first "cut" site in each molecule.

The fragments in the four reactions are electrophoresed side by side in denaturing acrylamide gels for size separation. To visualize the fragments, the gel is exposed to X-ray film for autoradiography, yielding a series of dark bands each showing the location of identical radiolabelled DNA molecules. From presence and absence of certain fragments the sequence may be inferred (figure2).

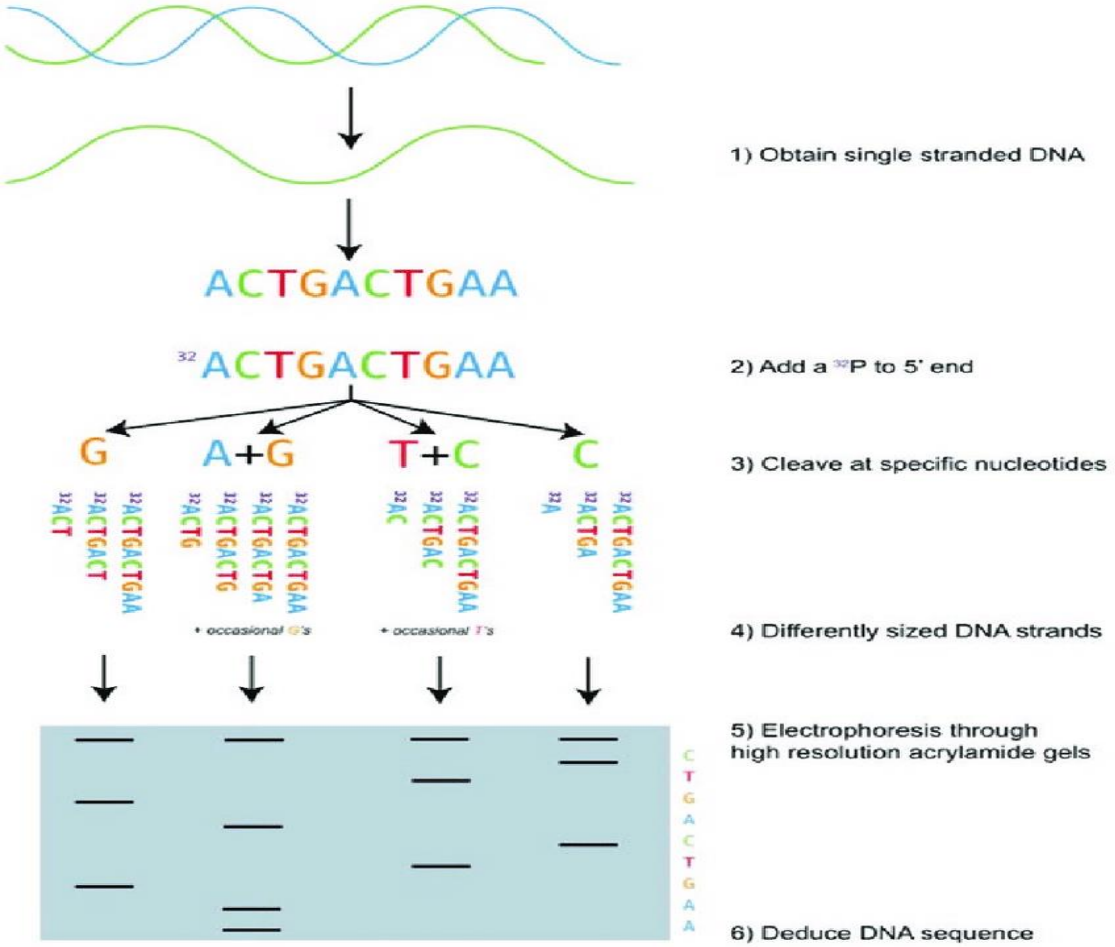

Figure2: Maxam-Gilbert sequencing (Preetha*et al*,2019)

Genome mapping:

Gene mapping or genome mapping describes the methods used to identify the location of a gene on a chromosome and the distances between genes.Gene mapping can also describe the distances between different sites within a gene. ("Gene mapping - Glossary Entry"2013)

The essence of all genome mapping is to place a collection of molecular markers onto their respective positions on the genome. Molecular markers come in all forms. Genes can be viewed as one special type of genetic markers in the construction of genome maps, and mapped the same way as any other markers. In some areas of study, gene mapping contributes to the creation of new recombinants within an organism.

Gene maps help describe the spatial arrangement of genes on a chromosome. Genes are designated to a specific location on a chromosome known as the locus and can be used as molecular markers to find the distance between other genes on a chromosome. Maps

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provide researchers with the opportunity to predict the inheritance patterns of specific traits, which can eventually lead to a better understanding of disease-linked traits. (Ladejobi*etal*,2016)

Two approaches to generating gene maps (gene mapping) include physical mapping and genetic mapping. Physical mapping utilizes molecular biology techniques to inspect chromosomes. These techniques consequently allow researchers to observe chromosomes directly so that a map may be constructed with relative gene positions. Genetic mapping on the other hand uses genetic techniques to indirectly find association between genes. Techniques can include cross-breeding (hybrid) experiments and examining pedigrees. These techniques allow for maps to be constructed so that relative positions of genes and other important sequences can be analysed.

Genetic mapping:

The first steps of building a genetic map are the development of genetic markers and a mapping population. The closer two markers are on the chromosome, the more likely they are to be passed on to the next generation together. Therefore, the "co-segregation" patterns of all markers can be used to reconstruct their order. (Gallavotti*et al* ,2015) With this in mind, the genotypes of each genetic marker are recorded for both parents and each individual in the following generations. The quality of the genetic maps is largely dependent upon these factors: the number of genetic markers on the map and the size of the mapping population. The two factors are interlinked, as a larger mapping population could increase the "resolution" of the map and prevent the map from being "saturated"

In gene mapping, any sequence feature that can be faithfully distinguished from the two parents can be used as a genetic marker. Genes, in this regard, are represented by "traits" that can be faithfully distinguished between two parents. Their linkage with other genetic markers is calculated in the same way as if they are common markers and the actual gene loci are then bracketed in a region between the two nearest neighbouring markers. The entire process is then repeated by looking at more markers that target that region to map the gene neighbourhood to a higher resolution until a specific causative locus can be identified. This process is often referred to as "positional cloning", and it is used extensively in the study of plant species. One plant species, in particular in which positional cloning is utilized is in maize. The great advantage of genetic mapping is that it can identify the relative position of genes based solely on their phenotypic effect.

Genetic mapping is a way to identify exactly which chromosome has which gene and exactly pinpointing where that gene lies on that particular chromosome. Mapping also acts as a method in determining which gene is most likely to recombine based on the distance between two genes. (Saygin*et al*,2017) The distance between two genes is measured in units known as centimorgan or map units, these terms are interchangeable. A centimorgan is a distance between genes for which one product of meiosis in one hundred is recombinant.The farther two genes are from each other, the more likely they are going to recombine. If it were closer, the opposite would occur.

Physical mapping:

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In physical mapping, there are no direct ways of marking up a specific gene since the mapping does not include any information that concerns traits and functions. Genetic markers can be linked to a physical map by processes like in situ hybridization. By this approach, physical map contigs can be "anchored" onto a genetic map. The clones used in the physical map contigs can then be sequenced on a local scale to help new genetic marker design and identification of the causative loci.Macro restriction is a type of physical mapping wherein the high molecular weight DNA is digested with a restriction enzyme having a low number of restriction sites.

Application of genomics:

The most obvious application of genomic technologies is the discovery of genes related to important traits. Availability of genome sequences for large number of plants, animals and microbes and their resequencing from different individuals are helping to isolate and clone important alleles. Due to characterization of genes in model organisms, its prediction in useful organisms is easy. Genomics is also important for developing markers on genomes and preparation of genetic maps. It is also supportive for marker assisted selection and QTL analysis.Gallavotti*et al*,2015)

Different applications of genomics are given below.

1. Genomics is useful for gene identification and cloning.
2. It can be applied for gene prediction and discovery of genes.
3. Genetic mapping and locating genes is possible through genomics.
4. Genomics is useful for genome manipulation as it is possible only after understanding genome thoroughly.
5. Molecular markers development and Marker Assisted Selection is easy by knowledge of genome structure.
6. QTLs analysis and fine mapping of genes is easy by generation of genome wide markers.
7. Comparative genomics helps to isolate genes using knowledge from model organisms.
8. Development of Gene banks and chromosome stocks can hasten.
9. Genomics improve understanding of expression profiles, responses and interactions within genome. (Morozova*et al* ,2008)

Conclusion:

Genomics refers to the study of structure and function of entire genome of a living organism. Mainly genomics is categorized into i) structural, ii) functional and iii) comparative genomics. Structural genomics deals with the study of the structure of entire genome through whole genome sequencing or EST sequencing.Genomics is useful in determination of genome size, number of genes per genome, mapping of important genes, construction of linkage maps, sequencing of genes and their alleles and cloning beneficial alleles, development of markers and their utilization for crop improvement through marker assisted selection, transgenic breeding, QTL mapping, gene therapy, metagenomics, tracking of evolutionary points, etc.

Even though it is a comparatively new branch of science, it has great impact on other biological sciences like human health, medicine, agriculture, environment, microbial and animal science. In future, genomics is going to play more robust role in our day-to-day life.

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CHAPTER- 16
ADVANCES IN VACCINE DEVELOPMENT, IMPROVING OUR ABILITY TO PREVENT INFECTIOUS DISEASES CAUSED BY PATHOGENIC MICROORGANISMS

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In recent years, advancements in vaccine development have significantly enhanced our ability to prevent infectious diseases caused by pathogenic microorganisms. These breakthroughs have revolutionized public health strategies, offering effective means to combat a wide array of diseases that pose significant threats to global populations.One notable advancement lies in the development of novel vaccine platforms, such as mRNA vaccines. These innovative vaccines, exemplified by the COVID-19 vaccines, utilize messenger RNA technology to instruct cells to produce a protein that triggers an immune response against specific pathogens. The speed with which mRNA vaccines were developed and their remarkable efficacy in preventing COVID-19 underscore the potential of this platform for rapidly responding to emerging infectious threats.Furthermore, advancements in vaccine adjuvants and delivery systems have bolstered vaccine effectiveness and safety. Adjuvants, substances added to vaccines to enhance the immune response, are continually being refined to optimize vaccine efficacy while minimizing adverse reactions. Likewise, novel delivery systems, including nanoparticles and microneedle patches, offer improved vaccine stability and targeted delivery, facilitating broader vaccine distribution and uptake.

Keywords: mRNA messenger RNA technology, covid 19, adjuvants, vaccines, novel delivery system, immune response

Introduction:

Absolutely, advances in vaccine development have been pivotal in our ability to combat infectious diseases caused by pathogenic microorganisms. Over the years, researchers have made significant strides in vaccine technology, leading to safer, more effective, and more accessible vaccines.(Koff, 2013)

Principle:

Advances in vaccine development have significantly enhanced our ability to prevent infectious diseases caused by pathogenic microorganisms. The principle behind vaccine development lies in training the immune system to recognize and combat specific pathogens without causing the disease itself.(França, 2013)

1.**Identification of Antigens:** Scientists identify the antigens (molecules that trigger an immune response) present on the surface of the pathogen. These antigens are often proteins or sugars unique to the pathogen.

2. **Vaccine Formulation:** Using various techniques, researchers create vaccines containing either weakened or killed forms of the pathogen, or specific antigenic components derived

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from it. These components are designed to stimulate the immune system without causing illness.(Nan, 2017)

3. **Immune Response:** When the vaccine is administered, the immune system recognizes the antigens present in the vaccine as foreign invaders. This triggers an immune response, including the production of antibodies and activation of specialized immune cells.

4. **Memory Cells Formation:** After the immune response, memory cells are produced. These cells "remember" the specific pathogen, enabling a quicker and more robust immune response upon subsequent exposure to the actual pathogen.

5. **Protection against Infection:** If the vaccinated individual is exposed to the actual pathogen in the future, their immune system recognizes it quickly and efficiently, neutralizing the pathogen before it can cause illness. This prevents or mitigates the severity of the infection.

Recent advances in vaccine development have introduced novel approaches such as mRNA vaccines, viral vector vaccines, and subunit vaccines. These technologies offer several advantages, including faster development timelines, increased safety profiles, and enhanced efficacy. Additionally, advancements in vaccine delivery systems and adjuvants have improved the immune response generated by vaccines, leading to better protection against infectious diseases.(Fries, 2021)

Types:

Certainly! Advances in vaccine development have significantly improved our ability to prevent infectious diseases caused by various types of pathogenic microorganisms. Here are some key advancements:(Wagner, 2020)

1. **Messenger RNA (mRNA) Vaccines:** mRNA vaccine technology has emerged as a groundbreaking approach. These vaccines work by introducing mRNA that encodes a viral protein into the body, prompting the immune system to recognize and mount a defense against the virus. The development and successful development of mRNA vaccines against diseases like COVID-19 have demonstrated the potential of this technology.

2. **Vector Vaccines:** Vector vaccines use a harmless virus or viral vector to deliver genetic material from a pathogen into the body, stimulating an immune response. For example, adenovirus vectors have been used in the development of vaccines against diseases such as Ebola and COVID-19.

3. **Subunit Vaccines:** Subunit vaccines contain purified protein antigens from a pathogen, rather than the whole pathogen. These vaccines are safer because they only contain specific parts of the pathogen that are necessary to stimulate an immune response. They have been developed for diseases such as hepatitis B and HPV.

4. **Nanotechnology:** Nanotechnology has enabled the development of novel vaccine delivery systems. Nanoparticles can encapsulate vaccine antigens, protecting them from degradation and improving their stability. Nanoparticle-based vaccines have shown promise in enhancing immune responses and enabling targeted delivery.

5. **Computational Biology and Bioinformatics:** Advances in computational biology and bioinformatics have revolutionized vaccine design and development. These tools allow researchers to analyze genomic data, predict antigen structures, and simulate immune responses, accelerating the identification of potential vaccine candidates and streamlining the vaccine development process.

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6. **Vaccine Platforms:** Innovative vaccine platforms, such as virus-like particles (VLPs), DNA vaccines, and live attenuated vaccines, continue to be explored and optimized for various infectious diseases. These platforms offer unique advantages in terms of safety, efficacy, and scalability.(Pollard, 2021)
7. **Adjuvants:** Adjuvants are substances added to vaccines to enhance the immune response. New adjuvants are being developed to improve vaccine efficacy, reduce the required vaccine doses, and enhance the duration of immunity. Advancements in understanding immune modulation have led to the discovery of novel adjuvants with improved safety profiles.(Wagner, 2020)

Overall, these advancements in vaccine development are not only improving our ability to prevent infectious diseases caused by pathogenic microorganisms but also offering hope for tackling emerging infectious threats more rapidly and effectively in the future.(Di Pasquale, 2015)

Advantages:

Advances in vaccine development offer numerous advantages in preventing infectious diseases caused by pathogenic microorganisms:(Rappuoli, 2014)

1. **Disease Prevention:** Vaccines help prevent the spread of infectious diseases by providing immunity to individuals, thereby reducing the overall incidence of the disease in the population.
2. **Eradication and Control:** Effective vaccines have the potential to eradicate certain diseases altogether, such as smallpox. For other diseases, vaccines can help control outbreaks and minimize their impact.
3. **Public Health Impact:** Vaccination programs contribute significantly to public health by reducing the burden of illness, hospitalizations, and deaths associated with infectious diseases.
4. **Herd Immunity:** Widespread vaccination creates herd immunity, which offers protection to individuals who cannot be vaccinated due to medical reasons or age, such as infants and immune compromised individuals.
5. **Cost-effectiveness:** Vaccines are often cost-effective compared to the long-term costs of treating infectious diseases and their complications. They reduce healthcare expenditures associated with illness, hospitalization, and disability.
6. **Global Health Security:** Vaccines play a crucial role in global health security by preventing the spread of infectious diseases across borders and reducing the risk of pandemics.
7. **Lifespan Extension:** Vaccines not only prevent acute illness but also contribute to extending lifespan by reducing the incidence of diseases that can lead to chronic health conditions or complications later in life.
8. **Research and Innovation:** Advances in vaccine development spur innovation in immunology, virology, and other related fields. This research can lead to the development of new and improved vaccines against existing and emerging infectious diseases.
9. **Health Equity:** Vaccination programs promote health equity by ensuring that vulnerable populations, including low-income individuals and those in underserved communities, have access to lifesaving vaccines.

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10. **Quality of Life Improvement:** By preventing infectious diseases, vaccines improve the overall quality of life for individuals and communities, allowing people to live healthier and more productive lives.

Applications:

Advances in vaccine development have significantly enhanced our ability to prevent infectious diseases caused by pathogenic microorganisms in various ways:

1. **Rapid Response to Emerging Diseases:** Modern vaccine development platforms, such as mRNA and viral vector vaccines, allow for the rapid design and production of vaccines in response to emerging infectious diseases. This was particularly evident during the COVID-19 pandemic, where vaccines were developed and deployed in record time.
2. **Improved Vaccine Efficacy:** Advances in vaccine technology have led to the development of more efficacious vaccines with higher levels of protection against targeted pathogens. New adjuvants, delivery systems, and antigen design techniques have contributed to enhancing the immune response and durability of vaccine-induced immunity.
3. **Broadening Vaccine Coverage:** Traditional vaccines often require multiple doses and booster shots to confer long-lasting immunity. However, newer vaccine technologies enable the development of single-dose or two-dose regimens that provide robust and durable protection. This simplifies vaccination schedules and increases vaccine coverage, especially in resource-limited settings.
4. **Targeting Previously Intractable Diseases:** Advances in vaccine research have enabled the development of vaccines for diseases that were previously considered difficult to prevent through vaccination. Examples include vaccines against malaria, tuberculosis, and HIV, where significant progress has been made in recent years, although challenges remain.
5. **Enhanced Safety Profiles:** Modern vaccine platforms often have improved safety profiles compared to traditional vaccines. For instance, mRNA vaccines do not contain live virus particles and cannot cause the diseases they protect against. This reduces the risk of adverse reactions and allows for broader vaccine administration, including in immunocompromised individuals.
6. **Customized Vaccines:** Advances in understanding host-pathogen interactions and immune responses have facilitated the development of personalized or customized vaccines. These vaccines can be tailored to individual genetic backgrounds or specific risk factors, potentially increasing their effectiveness and reducing adverse effects.
7. **Therapeutic Vaccines:** In addition to preventing infections, vaccines are being developed as therapeutic agents to treat existing infections or chronic diseases. Therapeutic vaccines aim to stimulate the immune system to target and eliminate pathogens or abnormal cells, offering new treatment options for conditions such as cancer and chronic viral infections.

Overall, advances in vaccine development have revolutionized our ability to prevent infectious diseases, offering hope for better control and management of both current and future outbreaks.(Lipsitch, 2016)

Conclusion:

Advances in vaccine development have significantly enhanced our ability to prevent infectious diseases caused by pathogenic microorganisms. These advancements have led to safer, more effective, and faster vaccine production processes, ultimately benefiting global

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public health. With innovations such as mRNA technology and viral vector platforms, we have seen remarkable progress in addressing previously challenging diseases like COVID-19. Moreover, ongoing research continues to broaden our understanding of immunology, allowing for the development of vaccines against a wider range of pathogens. This includes efforts to combat emerging infectious diseases and to improve vaccine accessibility and distribution, particularly in underserved communities.

In conclusion, the strides made in vaccine development offer hope for a healthier future, where the threat of infectious diseases can be mitigated through preventive measures. However, it's crucial to maintain momentum in research, funding, and collaboration to address current and future health challenges effectively.(Rosini, 2020)

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CHAPTER- 17

SEMIOCHEMICALSININSECTPESTSMANAGEMENT

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Abstract

Semiochemicals are informative molecules that are primarily utilized in plant-insect or insect-
insectinteractionsasalternativeorsupplementarycomponentstoinsecticideapproachesinvariousintegratedpestmanagementstrategies.Theirpurposeistomanipulateinsectbehaviorbyinfluencing the survival and/or reproduction of insect pests, thereby controlling their infestationson crops. This review provides a fundamental overview of the use of semiochemicals for themanagement of insect pests. The study explores two main topics. The first topic delves into adescription of semiochemicals and their different types, namely pheromones and allelochemicals.Pheromonesserveasameansofintraspecificcommunicationamongmembersofthesamespecies,while allelochemicals, produced by individuals of one species, alter the behavior of individualsbelonging to a different species, resulting in an interspecific effect. Allelochemicals encompass arange of informative molecules, including allomones, kairomones, synomones, anemones, andapneumones.Thesecondtopicfocusesonthepracticalapplicationofsemiochemicalsinintegratedpestmanagementprograms.Varioussemiochemicalsareincorporatedintotheseprogramsthrough differentapproaches,suchasmonitoring,masstrapping,attract-and-kill,push-pull,anddisruptionstrategies. Pheromones show promise and can be used either alone or in conjunction with othercontrol strategies for monitoring and managing insect pests in agricultural systems. For instance,sex pheromones have been successfully employed in mass trapping, disruption, and attract-and-killtactics withinintegratedpestmanagementprograms.

KeyWords: Semiochemicals,Insects,Pests,Management,Pheromones

Introduction:

Semiochemicals refertocompounds orcombinations of compounds thatare emittedby anorganism and elicit a behavioral or physiological reaction in individuals belonging to the same
ordifferentspecies(GhanyandNesreen,2019).ThetermsemiochemicalwasfirstproposedbyLawand Regnier in 1971 (Semeon-Marker or Signal). In simple term semiochemicals also called

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aschemicalsinvolvedincommunication.

Semiochemicals are informative molecules that are primarily utilized in plant-insect or insect-insectinteractionsasalternativeorsupplementarycomponentstoinsecticideapproachesinvariousintegratedpestmanagementstrategies.Theirpurposeistomanipulateinsectbehaviorbyinfluencing the survival and/or reproduction of insect pests, thereby controlling their infestationson crops. This chapter provides a fundamental overview of the use of semiochemicals for themanagement of insect pests. The study explores two main topics. The first topic delves into adescription of semiochemicals and their different types, namely pheromones and allelochemicals.Pheromonesserveasameansofintraspecificcommunicationamongmembersofthe samespecies,while allelochemicals, produced by individuals of one species, alter the behavior of individualsbelonging to a different species, resulting in an interspecific effect. Allelochemicals encompass arange of informative molecules, including allomones, kairomones, synomones, anemones, andapneumones.Thesecondtopicfocusesonthepracticalapplicationofsemiochemicalsinintegrated pestmanagementprograms.Varioussemiochemicalsareincorporatedintotheseprogramsthrough differentapproaches,suchasmonitoring,masstrapping,attract-and-kill,push-pull,anddisruptionstrategies. Pheromones show promise and can be used either alone or in conjunction with othercontrol strategies for monitoring and managing insect pests in agricultural systems. For instance,sex pheromones have been successfully employed in mass trapping, disruption, and attract-and-killtacticswithinintegratedpest managementprograms(VilelaandDellaLucia,2001).

1. OverviewofSemiochemicals:
   Semiochemicalscanbemainlyclassifiedaspheromonesandallelochemicals asabroadcategory.Semiochemicalsaredividedintointraspecificandinterspecificcommunicationc hemicals.

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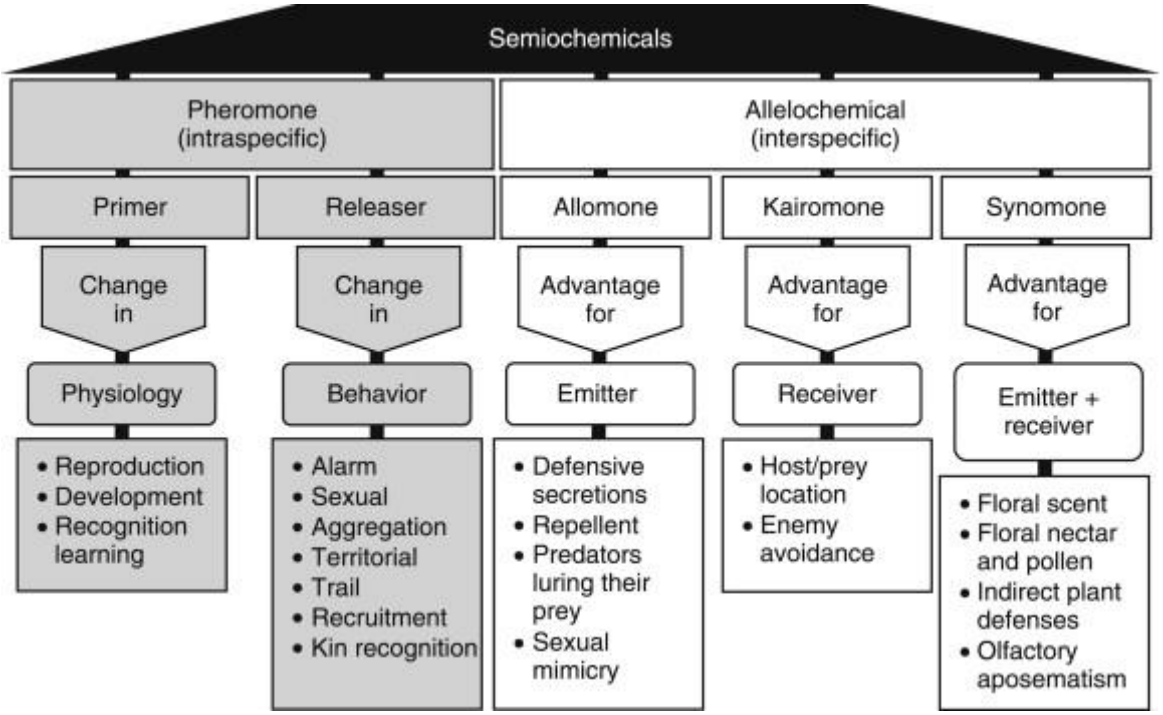

1.1. Pheromones:

The earliest record on the probable role of pheromones dates back to 1870 by French Naturalist,JeanHenriFabrewhoobservedthatfemalepeacockmoth*Saturniapyri*attractedmalemoths frommilesawayand laterJosefLinteragainin1870who noticedthat femalesofSpiceBushSilkmothattracted male moths. Pheromones can be defined as a chemical or a mixture of chemicals that isreleased to the exterior (outside environment) by an organism that causes one or more specificreactions in a receiving individual of the same species (Reddy, 2018). These pheromones wereearlier called as ectohormones in 1932 by Bethe to cover many chemical interactions, includingcommonattractiontoafoodsmell.Butin1959,Karlsonand Lusher proposed atermpheromone,a chemical used to communicate between individuals of the same species (pherein- to transfer,hormone-toexcite).

Pheromones are divided into two main categories i.e. releaser, which include an immediatebehavioralchange,andprimers,whichinitiatechangesindevelopment,suchassexualmaturation,physiologicalchangethatdonotresultinsuddenbehavioralchanges.Duetothisreasonthepri merpheromonesarelessexploitedintheagriculturalinsectpest'smanagementandareleastneglected
.

1.1.1. SexPheromones:

Sexpheromonesareamongthemostpowerfulofchemicalattractants.TheywerefirstdiscoveredbyButenandtin1959fromSilkwormmoth, *Bombyxmori* i.e.Bombykol.Thesearereleasedfromspecialized glands which open on the terminal segment of the female abdomen and are perceivedby chemosensillary of the male antenna. These chemicals have a great potential as pest controlagents. Atlow densities, these pheromones traps are a valuablemonitoring tool, providinginformation on the density and distribution of pest populations and at high densities, they can beusedformasstrapping sexuallyactiveadultsusuallymalesineffortstoreducepopulationdensityandlowera

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pest'sreproductivepotential.

Majority of sex pheromones are released by female species with few exceptions viz., Cottonbollweevil (*Anthonomus grandis*), Cabbage looper (*Trichoplusia ni*) and Mediterranean fruit fly(*Ceratitis capitata*) where male produce sex pheromones. Usually sex pheromones are packed inslowreleasedispenserslikerubbersepta,hollowfibersorropewicksthatareusedasluresintrapsofv arious designs.

Table1:ExamplesofSomeCommercially ExploitedSexPheromones

Insects	**LuresAvailable inMarket**
AmericanBollworm(*Helicoverpaarmigera*)	Helilure
Cottonbollweevil(*Anthonomusgrandis*)	Grandlure
Pinkbollworm(*Pectinophoragossypiella*)	Gossyplure(naturalsexpheromone),Pectinoloure, Hexalure(parapheromone)
Brinjalshoot andfruit borer	Leucinlure
DiamondBackmoth(*Plutellaxylostella*)	DBMLure
Tobaccocutworm(*Spodopteralitura*)	Spodolure
Spottedbollworm(*Eariasvitella*)	Ervit lure
Spinybollworm(*Eariasinsulana*)	Erinlure
Riceyellowstemborer(*Scirphphagaincertulas*)	Scirpolure
SugarcaneInternodeBorer(*Sacchariphagusindicus*)	INBlure
SugarcaneEarlyShootBorer(*Chilo infuscatellus*)	ESB lure
SugarcaneTopBorer(*Scirpophagaexcerptalis*)	STB lure

1.1.2. AlarmPheromone:

The substances produced by an insect to repel and disperse other insects in the area. ThesepheromonesarereportedinintheinsectOrder *viz.*,Isoptera,HomopteraandHymenopteraandarereleased by organs such as mandibular, anal, Dufour's and poison glands in ants, cephalic glandsin termites, sting and mandibular glands of worker bees and cornicles or siphunculi in aphids.These pheromones produce effects like dispersion, attraction or aggression. An individual alsoreleasesthemwhenanenemyattacks.Severalsignificantaphidspecieshavebeenfoundtopossess alarm pheromones consisting of various compounds. Notably, Sesquiterpene (E)-β-farnesene(EBF), Germacrene A, and α-Pinene have been identified as the primary constituents of thesepheromones(Vandermoten*etal.*, 2012).

1.1.3. AggregationPheromones:

The substances produced by one or both sexes that brings both sexes together for feeding andreproduction. These are the chemicals that allow insects to congregate for feeding and otheractivities.

Thesearereleasedbymembersofonesexonlybutelicitresponsesinmembersofbothsexesofaspecies

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.Examples:BarkandAmbrosiabeetlesoftheFamily:Scolytidae

Examplesofcommerciallyavailableaggregationpheromones:

1. Red palmweevil(*Rhynchophorusferrugineus*) - RPWlure
2. Rhinocerosbeetle(*Oryctesrhinoceros*) - RBlure

The research conducted by Bowers *et al*. (1991) confirmed that the hemiterpene 3-methylbut-3-en-1-ol serves as the aggregation pheromone for *Polygraphus rufipennis* Kirby and *Lasconotusintricatus* Kraus,twobeetlepests.

1.1.4. TrailMarkingPheromones:
Substancesoflowpersistencethosearereleasedandperceivedbyindividuals inatrail. Thesearefoundinsocialinsects ofHymenopteransandIsopterans respectively.
Examples: Formic acid: Ants used as a trial marker. They facilitate migration of colony to newsite in search of food. Recently, 6-*n*-pentyl-2-pyrone was shown to be the main trail pheromoneforthemyrmicineant,*Pristomyrmexpungens*Mayr(Hymenoptera:Formicidae)(McPheron*etal.*,1997)

1.1.5. Recruitmentpheromones:
These inducenestmatestoleavethenestandmigratetoaworksiteorviceversa.Exocrineglandsare responsible for emitting recruitment pheromones, and these glands are anatomical structuresthat are frequently specialized for the synthesis and release of these chemical signals (Meer andPreston, 2008). For example, terrestrial ants possess various glandular origins of recruitmentpheromones, includingDufour'sgland,thepygidialglands,poisonglands,sternalglands,hindgut,and rectal glands. Pheromones from these sources are often visible when deposited on a solidsurface.Animportantexamplethatillustratestherecruitmentprocessistheredfireant,*Solenopsi sinvicta* Buren (Hymenoptera: Formicidae). The process initiates when a foraging scout workerdiscovers a food source that is too large for it to transport back to the colony. The recruitmentmechanism encompasses several sub-categories: (i) initial trail marking by the scout ant, (ii)attraction of additional workers to the scout ant, (iii) encouragement of the workers to follow thetrail,and(iv)alignmentofthetrail.

1.1.6. EpideicticPheromones:
Thesearethecompoundsmainlyfunctionintheregulationofpopulationdensitybycontrollingthe dispersionofindividuals.
Example: Flieswillproduceepideicticpheromonewhenlayingeggstodeterotherfemalefliesfroml ayinglarvaeinthesamespot.

1.1.7. TerritorialPheromones:
Thesearethesubstancesreleasedbymalesofsomespeciesandattractbothmalesandfemales.
Example: MalesofBumblebeesandCarpenter Beesdemarcatetheterritoryfor forageactivity.

1.2. Allelochemicals:

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Chemicals involved in interspecific (in between different species) communication were called asallelochemicals.Allelochemicalsare primarily released by members of one species and arecomprehended by members of a distinct species. They have been categorized into five groups:allomones,kairomones,synomones,antimones,andapneumones(VilelaandDella,2001). The

wordallelochemicalwascoinedbyWhittakerin1970.Theseaffectsurvival,growthanddevelopmentofinsects aswellas theirnaturalenemies.

1.2.1. Allomones(fromGreekallos+hormone"=exciteothers):
Allomoneisasubstanceproducedoracquiredbyanorganismthat,whenit contactsanindividualof another species evokes in the receiver a behavioral or physiological reaction that is adaptivelyfavorable to the emitter but not the receiver. Granular trichomes which cover plant leaves andstems release herbivore-deterring allomones under stress conditions as a defense process. Theseallomonesaretoxicfortheherbivorousinsectpests,e.g.nicotinefromtobaccoplant.

1.2.2. Kairomones(fromGreekword"kairos"=opportunisticorexploitative):
A substance produced or acquired by an organism that, when it contacts an individual of anotherspecies evokes in the receiver a behavioral or physiological reaction that is adaptively favorableto the receiver but not the emitter. E.g. orientation of predaceous checkered beetles (Coleoptera:Cleridae)towardstheaggregationpheromoneoftheirprey;barkbeetle(Coleoptera:Curculionidae:Scolytinae)(PolandandBorden,1997).

1.2.3. Synomones:
A substance produced or acquired by an organism that, when it contacts an individual of anotherspecies evokes in the receiver a behavioral or physiological reaction that is adaptively favorabletothebothreceiverandemitter.E.g.Examplesincludescentsusedbyflowerstoattractpollinatinginsects.

1.2.3.4.Apneumones(fromGreekword"a-pneum"=breathlessorlifeless):
Asubstanceproducedbyanon-livingmaterialwhichevokesabehavioralorphysiologicalreactionthat is adaptively favorable to a receiving organism but detrimental to an organism of anotherspeciesthatisfoundinoronthenon-livingmaterial.E.g.hexanaland2-methyl-2-butanolreleasedfromrabbitstoolsattractsSandflyfemalesforoviposition(Dougherty*etal.*,1995).

1.2.3.4.5.Antimones:
When an organism generates oracquires certain substances,thesesubstances caninducearepellent reaction in another individual of a different species when encountered in their naturalsurroundings. This maladaptive trait affects both the emitter and receiver, causing an unfavorableresponseinthereceivingindividualtowardsbothpartiesinvolved.

2.1. Theresponseofinsectstosemiochemicalscanbeinfluencedbyvariousfactors.:
Before the utilization of semiochemicals for controlling insect pests, various factor we need tostudyandfactorsthoseaffectinsectresponsetowardsvarioussemichemicalsareasfollows.

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2.1.1. Semiochemicalsrelease rate:

The design of an efficient trap primarily hinges on identifying the most optimal approach toreleasingattractivechemicals.Incontrolstrategiesfortrapping,thereleaseratesareconsideredtob e of utmost importance. Interestingly, higher levels of semiochemicals do not necessarily resultincapturingagreaternumberofinsectscomparedtolowerlevels.Infact,releasinglargequantiti esofchemicalscanactuallyhavearepellenteffectintheimmediatevicinityofthetrap.Forexample,the redflourbeetle,*Triboliumcastaneum*(Herbst)(Coleoptera:Tenebrionidae),exhibitsdifferentrespo nses to pheromone lure formulations in laboratory settings. Surprisingly, high release ratesofpheromonesneitherattractnorrepelthebeetles,whileoldertrapsprovetobemoresuitablefort heiruse(Hussain*etal.*,1994;Phillips,1994).Hence,optimizingthereleaseratescouldsignificantlye nhance theperformance andeffectiveness ofpheromonetraps.

2.1.2. Trapdesign:

Various trapping strategies focus on enhancing the effectiveness of a particular trap rather thangathering data on host-plant volatiles or insect pheromones. Trap design encompasses severalelements that influence the trapping efficiency, such as shape, size, height, wind alignment,placement,andtiming.Sticky,water,andinvertedconetrapsarethemostcommonlyutiliz edtrapsforcapturinginsectsinthefield.Sticky trapshavebeenextensively developedforinsectexterminationandareemployedinmasstrappingorattract-killapproaches.Watertrapsstandoutforpreservinginsectspecimensin optimal condition,makingthem suitableformonitoringstrategiesaimedatidentifyingtheinsectpopulationinaspecificareaorestimat ingfluctuations in a target insect pest population (Abd El-Ghany *et al.*,2016). Additionally, the integration ofchemical and visual cues in trap design is deemed successful when it influences insect responsestothesameattractants (Singer,1986).

3. Devicesusedforapplication ofsemiochemicalsforcontrollinginsect pests:

3.1. Retrievabledispensers:

A−Passivedispensers(extrudedorreservoir):thesemiochemicalsubstancesarecontinuouslyemitt edintotheair.

B−Activedispensers:Thesemiochemicalsubstanceisreleaseddiscontinuouslyfromthedevice.(G hanyandNesreen,2019).

3.2. Passivenon-retrievableproducts:

A−Dispensers(biodegradabledispensers):thesemiochemicalisemittedcontinuouslyfromthedevi ce.

B−Dosablematrixdispensers:thesemiochemicalisenclosedinamatrix(e.g.stickypolymericmateri al)ontoplantsoranothersubstrateatthesite ofuse.

C−Capsulesuspension products: thesemiochemicalisformulatedasamicroencapsulation(commonlyusedforpheromoneapplicatio nthatallowseffectiveprolongationofreleasinglevels).

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D−Granularproducts (non-WDG):thesemiochemicalisformulatedinagranularform.(GhanyandNesreen,2019).

4. Semiochemicalsininsectpestmanagement:

The integration of semiochemical-based approaches has recently gained prominence in the realmofintegratedpestmanagement(IPM).Pheromonesandothersemiochemicalsarenotonlyeffective in controlling insect pests, but also play a crucial role in the conservation of rare andendangered insects. The efficacy of semiochemicals is primarily determined by their physicalproperties,whichincludemolecularstructure,volatility,solubility,andpersistenceintheenvironment. Temperature is a key factor influencing the stability of semiochemicals, as highertemperaturescanleadtoincreaseddiffusionofvolatilecompoundsandreducedmoleculelifetime.

The use of semiochemicals in IPM offers several advantages, such as long-distance diffusionfacilitated by high volatility, application in low concentrations, and rapid dissipation that helpsmitigate health and environmental risks associated with chemical pesticides. Consequently, theutilization of semiochemical substances holds significant promise for the advancement of IPMprograms(Larsson,2016).

4.1. Controlstrategiesutilizingsemiochemicals:

Semiochemicals are crucial components of integrated pest management (IPM) programs. Thesestrategies include monitoring, mass trapping, lure and kill, mating disruption, and the push-pullstrategy. Pheromones play a key role in controlling insectpests through indirectand directmethods.Indirectcontrolinvolvesmonitoringforquarantineandspraytiming,whiledirectcontrolincludesmasstrappingandarea-widedissemination.Area-widedissemination encompassesdisruption, attractant, and attract-and-kill strategies, which are commonly used in commercialsettings. Pheromone traps serve various purposes in pest management, such as attracticide andmating disruption techniques to hinder male reproduction. Furthermore, pheromone traps providevaluable information on population characteristics like sex ratio and mating status, aiding indetermining populationphasesthatundergocyclicalchangesindensity(Carter*et al.*2009).

4.1.1 InsectPestsMonitoring:

Monitoring helps us to detect population and level of infestation. Developing trap baited with sexpheromones on a large scale can do the monitoring of the insect pest. Semiochemical-baited trapsutilizingpheromonesorkairomonesarecost-effectiveandcommonlyutilizedtoolsformonitoringvariousinsectpests.Thesetrapsareefficientinidentifyingthepresenceofinsectpests,estimatingtheir population density, and tracking fluctuations to determine the initial peak flight activity(Weinzierl *et al.,* 2005). Pheromone traps offer a straightforward, effective, and highly sensitivemethodfordetectingdiverseinsects.Femaleinsectstypicallyemitsexpheromonestoattract malecounterparts for mating, while male insects release aggregation pheromones to attract both sexes,leading to mating and aggregation at a food source. Rhaindset *al.* (2016) employed a monitoringapproachtoassesstheabundanceofsprucebudwormmales*(Corihstoneurafumiferana* Clemens)inCanadabasedonpheromone-

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baitedtraps.Similarly,thismethodwasutilizedforthelesserdatemoth (*Batrachedraamydraula*Meyrick)(Lepidoptera: Batrachedridae)by Levi-Zada*etal.,(*2018).

DMBTRAP FRUITBORERTRAP FRUITFLYTRAP

FIG1:Different typesofTraps

4.1.2. MatingDispersion:

The effective use of sex pheromones in pests management is by confusing insect to find theirsexual counterparts for mating. The natural sex attractant emanating from female is masked,therebyconfusingmalesinlocating femaleswhichultimatelyresultsinlayingofunfertilizedeggsand subsequent suppression of pests population. The first successful practical demonstration ofmating disruption was with the Pink Bollworm, *Pectinophora gossypiella* using parapheromones“hexalure” which is more active than natural pheromone, ‘gossyplure’**.** Four mechanisms ofdisruptingmatingbehaviorareunderconsideration.Thesemechanismsinclude,

- Competitiveattraction
- Confusionofmales
- Sensorydesensitizationand
- Disguise.

Competitiveattractioninvolvessemiochemicalsubstancesdivertingmaleattentionawayfromwild females by leading them on a false trail. Confusion of males, on the other hand, resultsfromanenvironmentsaturatedwithsemiochemicalsubstances,leadingtorandom.

flight patterns and the inability to locate females for mating. Sensory desensitization occurswhen the male antennal receptor system or central nervous system becomes habituated tosemiochemicalsubstances,thusblockingmatingduetooverexposure.Lastly,disguise involvesmalesemigratingfrom an areadue toan excessof pheromones,renderingthemunavailableformatingwithvirginfemales(BarclayandJudd,1995;Mafra-Neto*etal.*,2014)

4.1.3. MassTrapping:

Alargenumberofpheromonetrapscanbeusedtocaptureadultmothsandthusreducethenumberof

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males for mating. In such cases, sufficient traps are to be used to capture as many insects aspossibleover alargeareatoavoidanyimmigrationofinsectsfromadjoiningareas.Thetrapscanbe impregnated with insecticides to kill insects falling in traps. This strategy involves a density-dependent approach that targets either males or females within the pest population, resulting in aslowdown of population growth. A lure is constructed using sleeves or rubber septa as a base forthe semiochemical substance, which is then placed on a sticky surface or in liquid containers.Regular maintenance of such traps is necessary as they become saturated with captured insects.Recently, this method has proven to be an efficient management strategy for managing
theJapanesebeetle,*Popilliajaponica*Newman(Coleoptera:Scarabaeidae)(PiñeroandDudenhoeffer,2018).

4.1.4. Push-pullStrategy:
Nowdays,thepush-p ı
iswidelyrecognizedasasignificantapproachinpestmanagement. Referred to as the "stimulo-deterrent diversion tactic," it combines two distinctstrategies: the push strategy, which involves deterring or repelling pests from crops, and the pullstrategy, which employs attractive stimuli to lure and control insect pests through trapping
orkillingmethods.Implementingthisstrategyeffectivelynecessitatesacomprehensiveunderstandingofinsectbiology,chemicalecology, andtheintricatedynamicsbetweenhostplantsand their natural enemies. Various studies have emphasized the importance of this knowledge insuccessfullyimplementingthe push-pullstrategy(Khan*etal.*,2010;França*etal.*,2013).

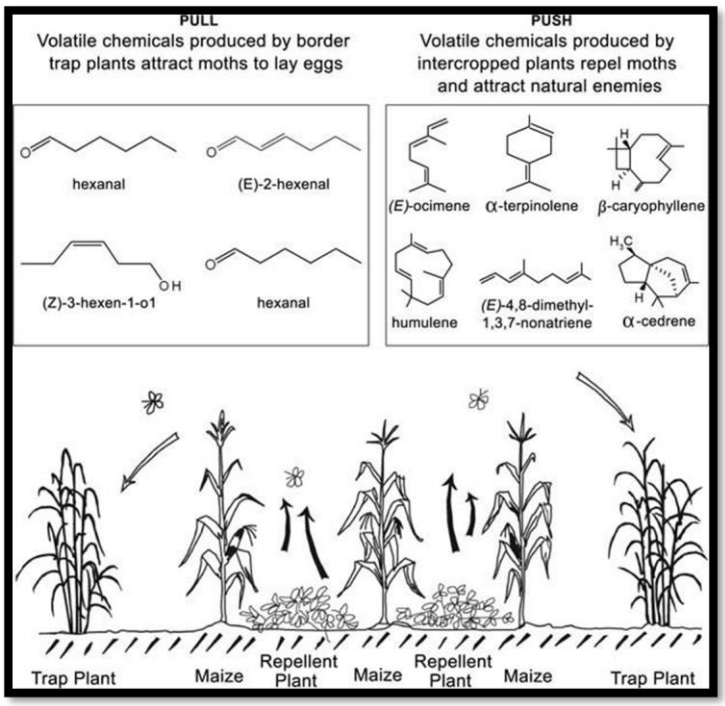

Fig.2.Push-pullstrategy forcontrollingcerealstemsborers(KhamandPickett,2004)

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CHAPTER- 18
SPIDER (ARANEAE) WEB TYPES AND SPIDER SILK – AN OVERVIEW

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Abstract

The common term for the arthropods in the Araneae order is "spider." They are a very important component of the earth's ecosystem's balance since, in addition to eating a lot of prey, primarily insects, they also provide food for many carnivorous creatures and seldom endanger people. Their biology, methods for capturing prey, methods for making silk, methods for building webs, and environment differed greatly throughout families. Because they are only predators, spiders perform a significant ecological function in preserving ecological equilibrium. Spider web organisation, construction and silks started to co-evolve a long million years ago. Spiders have modified and adapted their web construction to provide protection, guards, and an effective means of capturing prey in order to survive in a variety of environments. The existence of webs enables spiders to effectively carry out their predatory behaviour. There are many uses for spider silk, and these uses drive its evolution and diversification. They move, wrap prey, guard the egg sac, and weave webs, among other things. Different types of webs possess different function in biological control, medicinal value, prey – predation actions and many functions. An overview of spider webs and silk helps giving an good perspective for future research.

Keywords: Araneae, Spider Silk, Spider Web, Biological Control, Prey.

1. Arachnids and spiders:

Arthropods comprise more than “100,000” described insect species. The family of arthropods breathes air, and they typically have four pairs of legs, a cephalothorax formed by the fusion of their head and thorax, and no antennae, it appears. Arachnids can be either oviparous or viviparous. The young ones hatch from the egg. The arachnids, which belong to the arthropod -Chelicerata subphylum, share the anatomical features of chelate mouthparts and their complete absence of antennae. While many scorpions are extinct and eurypterids have complex eyes, adult arachnids possess four pairs of jointed legs and basic eyes with a single lens. The majority of spider orders have a cephalothorax, which combines the mouthparts, sensory receptor organs, and locomotory legs into a single tagma, and an abdomen, or opisthosoma, which houses the reproductive and digestive systems and may or may not show segmentation. The tagmatization of the various spider groups varies. The class Arachnida can be divided into the following orders of membership.

1. SCORPIONIDA
2. PEDIPALPIDA
3. MICROTHELYPHONIDA

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4. SOLPUGIDA
5. RLINCULEI
6. OPILIONES
7. ACARI
8. PSEUDOSCORPIONIDA
9. ARANEAE

Araneae are classified into three suborders: Mygalomorphae, Araneomorphae, and Mesothelae, which have segmentation on their abdomens but there is no indication of abdominal segmentation. Araneomorphae make up all aquatic and semiaquatic spider species. About 43,678 spiders belong to the order Araneae of class Arachnida (Platnick, 2013). Currently, more than 4000 genera of spiders and an excess of 50,000 species (WSC., 2023) are predicted to be living on the planet. It is estimated that there are around 1442 genuine species of spiders in India (Siliwal *et al*., 2005). Identified spider species from India are 1668, and a recent study has revealed that 350 genera of spiders are reported from India.

2. Morphology:

A slender pedicel connects the two separate cephalothorax and abdomen that make up a spider's body. A spider's body wall is composed of three layers: the cuticula, which is its outside protective layer; the hypodermis, which is its intermediate cellular layer; and the basement membrane, which is its inner, fragile membranous layer. The cuticula layer is made up of two layers: the deep, non-pigmented inner layer known as the secondary cuticula and the outer, pigmented layer termed the main cuticula.The presence of chitin accounts for some of the cuticula's hardness. A chitinized section of the body wall that can be identified from its surroundings is referred to as a sclerite, and the thin line that connects the two sclerites is known as a suture. Spiders typically have two types of respiratory organs: tubular trachea and book lungs. The air-filled respiratory sacs known as the book lungs open up at the base of the abdomen on the ventral side via a slit-like spiracle. Spiders' tubular tracheae open via a single spiracle that is often located in the center of the ventral side of the abdomen. This spiracle is frequently close to the midway length of the abdomen, although it is typically just a short distance in front of the spinnerets.

The carapace is a hard sclerotic covering the cephalothorax dorsally, and the sternum, covering it ventrally.Between the lateral borders of the carapace and the sternum, the legs are articulated in the pleural membrane. There are six to eight simple eyes on the cephalic area. There are two main types of eyes: white or nocturnal eyes and black or diurnal eyes, two rows at the front and the back. The four pairs of legs are numbered I, II, III, and IV, consecutively. Seven parts make up each leg – the coxa, trochanter, Jemur, patella, tibia, metatarsus, and tarsus. The legs are covered in a variety of hair kinds, bristles, spines, and spicules. Three distinct sections make up the alimentary canal, each with its origin. The proctodaeum is the source of the hindgut, the stomodaeum of the foregut, and the endoderm of the midgut. The mid-gut is composed with a layer of cells that makes up the gut epithelium, whereas the fore- and hind-guts are coated with a chitinous layer that is continuous with the cuticle.

The alimentary canal emerges from a very noticeable anal tubercle at the back of the abdomen. The most amazing features of a spider are located underneath the anal tubercle,

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spinnerets are used in employing in producing silk thread of spider. The spinnerets are shaped like fingers and typically counts six numbers; however, they can also be reduced to one pair or even two. The first or preceding pair, the subsequent or middle pair, and the last or posterior pair of spinnerets (Tikader, 1987).All arachnids have two different sexes, with the reproductive organs located in the abdomen and opening close to the base of each sex. Spiders have a rather straightforward reproductive system, with the exception of the male palpal organ. The vasa deferentia are two lengthy tubes that continue in front as two long, frequently highly coiled sperm ducts from the testis. The paired testis are located in the front region of the abdomen within the longitudinal ventral muscles and the body wall. The seminal vesicle is the common pouch that both vasa deferentia open into. The ovaries are situated underneath the intestine in the belly. The structures consist of two large tubes filled with many ovarian follicles that resemble a cluster of grapes. The degree to which the eggs have developed determines how big the ovaries are. Before the egg-laying phase, they enlarge significantly and take up a sizable portion of the abdomen. Every ovary opens via a small oviduct, which opens into the uterus, a shared pouch that leads to the vagina, which opens externally in the epigastric furrow in the centre of the body.

Although spiders generate venom, it is not harmful to humans for the following reasons:

1. There are no toxins in its venom that are safe for humans to consume.
2. The amount of venom injected is not adequate
3. They lack the strength of their chelicerae to pierce human flesh.
4. They are too shy to interact with people.
5. Because of their lifestyle, they are unable to communicate.

3. Diet of spider:

A spider's primary food sources include ants, mosquitoes, flies, moths, and occasionally even some other spiders. It has been observed that several large spiders consume tiny vertebrates, worms, and snails. Spiders use two methods to capture their prey: biting and injecting venom that causes paralysis, or wrapping and swathing in silk. Generally, web weavers have a narrower diet than hunting spiders. Certain spider species have evolved to capture certain kinds of prey,like the bolas and orb-weaving ladderspiderwebs are specifically designed to capture adult Species of Lepidoptera (Maloney *et al*., 2003).Web weavers such as Linyphiidae and Dictynidae mostly hunt soft-bodied insects such as aphids. A subfamily of cobweb weavers known as the Theridiidae became specialized in capturing fire ants. A few Salticidae species have also evolved their habit to include ant-eating (Nyffeler *et al*., 1994). Water spiders, or Argyronetidae, can hunt underwater and consume fly larvae, which include mosquitoes. Anteaters are the most specialized spiders. Certain spiders, including the Theridiidae family (Theridion), use silk strands to entice ants. Using an extremely quick assault-style, species ofgenus Zodarnion capture ants on the ground. Like roving spiders, some stalk their prey, others lie in wait for it, many use snares to capture it, and a small number coexist as commensals. The "scary spiders" Wandering spiders are often recognized as wolf-spiders (Lycosidae), leaping spiders(Salticiciae),and some crab-spiders (Thomisidae). These are always on the lookout for their victim and will seize the chance to attack. Ambush spiders: Certain crab spiders, such as Thomisus and Misumena, lurk among flowers and pounce on passing insects.Many burrowing spiders are grouped at the entrance of their tunnels, where they emerge to capture any approaching insects. The spiders that weave webs:

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The vast majority of stationary spiders create webs or snares to catch insects instead of foraging for prey. They then wait within or close to the webs so they may quickly attack the ensnared insects. The Eresidae family of commensal spiders constructs small nests on the leaves and branches of plants and trees, including several entrances and exits. A section of the nest has spread out like a wide sheet. Throughout our nation, acacia trees and other bushes are frequently home to these nests. The spiders kill the flying and leaping insects trapped in the sticky sheet on the snares and pull them into the nest. The number of people participating in a single hunt or sucking a single prey shows their social behavior.

4. Web and its types:

An animal invention with a recognized purpose, the spider's web enables comprehensive research on its ecology, biomechanics, behavior, and evolution. It started around 400 million years ago for silks and spider web arrangements to co-evolve.The one-line trap. The genus Miagrammopes, which includes stick spiders in the family Uloboridae, is native to the tropics and the Orient. The Miagrammopes snare is a single horizontal line that crosses open areas in the forest for about four feet and is fastened to branches on both ends. They have created an amazing trapping tool that uses an orb web with just one line to capture creatures.

The triangular web: Although it initially seems to be a piece of an orb-web, closer examination reveals that the web of limpiotes (Family Uloboridae) spiders is whole. It is composed of four simple lines that represent the radiating lines of an orb-web, and it is supported by a variable number of threads that resemble the spiral line of an orb-web in portions. A strong line that starts at the intersection of the radiating lines and goes to one of the supporting twigs.

Orb-webs: One of the greatest achievements of airborne spiders is the two-dimensional snare that is referred to as an orb-web. For the general public, the web represents a technical achievement – a fixed geometric entity that somewhat allays irrational fear of the creature while symbolizing spiders. An orb web's defining trait is its core section, which is the component that is enclosed by the supporting framework and is made up of several radiating lines made of dry, inelastic silk. The structure, form, and size of the many webs of this kind vary widely, based on the families and genera of the spiders.

Sheet-webs: The well-known illustration of a sheet-web is Linyphia. In this instance, the main component of the web is a sheet that is stretched in a plane and is composed of threads that are arranged in all directions within that plane but not in any apparent regular pattern. The sheet is roughly woven.

Funnel-Webs: A funnel web's main component has a structure like a sheet, but what sets these webs apart from actual sheet webs is a tube that extends from one side and either guides the spider's escape or acts as a retreat. Agelena's (Family Agelenidae) and Hippasa's (Family Lycosidae) common grassspider webs are excellent illustrations of this kind of funnel web.

Spiders have modified and adapted their web construction to provide protection, guards, andan effective means of capturing prey to survive in a variety of environments (Regassa *et al.*, 2021). The existence of webs enables spiders to effectively carry out their predatorybehavior. The spider's movement pattern when building its web resembles the finished structure, albeit it does not match exactly since the spider travels the paths it would

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later use to remove the threads (Zschokke & Vollrath, 2013). The four types of webs that are categorized are:

1. Orb web
2. CobWeb
3. Sheet web
4. Funnel web.

Of all of them, orb webs are themost common (Eisoldt *et al*., 2011). Due to its many uses, spider silk has undergone several evolutionary changes and has expanded in diversity. These include building webs, locomotion, encasing prey, and guarding the egg sac (Hsia *et al*., 2011).All these three steps depend on the prey flight behavior and mass. (Chacon and Eberhard, 1980, Nentwig, 1983; Rypstra, 1982; Denny, 1976; Nentwig, 1982). Cobwebs indicate the presence of web-building Theridiidae spiders, sometimes known as comb-footed spiders or cobweb weavers. The sheet-web weaver, family Linyphiidae, is a very widespread order Araneida spider with over 2,000 species worldwide. The majority are seldom observed and have a length of less than 6 mm (1/4 inch). Their webs are cup- or dome-shaped, and they are flat and sheet-like. The spider is often located between two layers of webbing on the underside of the web. Building a web essentially involved two alternating tasks: inserting support threads and finishing the page. It appears that the spider builds its web using both aciniform and ampullate strands (Rojas, A. 2011).

4.1 orb-weaving spiders and orb web:

Orb web weavers use the web to entangle their prey in a three-step process:

1. The interaction of the web with the prey.
2. The web absorbs the kinetic energy of the prey.
3. The adherence of the prey to the web's surface.

While some orb weavers create horizontal webs, most of them form webs in a vertical plane. To do this, first make a tiny sticky thread that will eventually drift across a gap on a little breeze. The spider detects a shift in vibration when it adheres to a surface at the further end. After carefully walking along the first strand, which it has reeled in and tightened, the spider adds another thread to reinforce it. This process is repeated until the thread has gained sufficient strength to support the rest of the web (Anotaux *et al*., 2012). The web's initial three radials are now built. As more radials are added, care must be taken to ensure that there is a sufficient space for each to cross one another.The non-adhesive spirals are eliminated as the sticky spirals develop. The spider bites off the first three center spiral threads after finishing its web, then sits and waits typically with its head pointing downward (Zschokke, S & Nakata, K. 2010). Stabilimentum is frequently added to the orb webs in the genera Argiope and Cyclosa. It is a structure that crosses the hub like a zigzag ribbon. It originates from the tiny spigots of the aciniform glands and is made up of a vast number of tiny threads that resemble a swathing band. The stabilimentum in *Argiope*'s webs is X-marked across the hub. The stabilimentum of *Cyclosa* orb-webs is typically tiny and situated somewhat above and below the hub.A significant development in the evolution of orb weaving spiders occurred when (Opell 1998) found that, on average, 95% of orb weaving species generate sticky threads. Compared to cribellar threads, adhesive threads use a higher substance to generate their stickiness. Measurements of cribellar threads generated by eight species of the

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Uloboridae family, sticky threads produced by four species of the Araneidae family, and one species of the Tetragnathidae family are included.

5. Spider silk:

A natural filamentous fibre protein which a spider produces is called "spider silk" (Saravanan, 2006). Their chemical structures and functions are remarkably diverse, ranging from the formation of orb webs to the creation of adhesives and cocoons (Kluge *et al*., 2008). The manufacturing of silk is influenced by both internal and external factors, and its chemical composition and mechanical characteristics are very varied (Vollrath, 1999). Proteins containing high concentrations of hydrophobic and nonpolar amino acids, like glycine and alanine, make up spider silk (Romer and Scheibel, 2008). The chemical makeup of silks varies according to the kinds of functions they must accomplish; fibroin, for example, is made up of about twenty alpha-amino acids. The majority of an orb web spider's energy is used to synthesize silk and a little bit of effort to find prey. The spinneret glands near the tip of an abdomen are where spiders make silk. The most intricate silk glands are found inside the abdomen of spiders and are known as silk organs. One may anticipate this condition given that a single species of spider spins many types of silk; an orb-weaving spider, for instance, produces five different types of silk. Given that spiders create several types of silk, it is evident that there are multiple types of silk glands, each with distinct functions. Silk glands are known to exist in seven distinct types. Their variations include form, quantity, colour, duct structure, and product characteristics. There isn't a single spider that possesses all seven types of silk glands; nonetheless, three types are shared by all spider species.

1. The berry-shaped glands known as acinforms 2. The pear-shaped, or pyriform, glands 3. Ampulate glands 4. Cylindrical glands 5. aggregate glands 6. Lobed glands 7. Cribellum glands

Every gland creates a specific type of thread, such as delicate silk for wrapping prey, and sticky silk for capturing it. Spiders use various gland types to produce unique silks; in the course of a lifetime, some species may synthesize up to eight different silks. They can synthesize seven distinct kinds of silk: minor, dragline, viscid, glue-like, wrapping, and attachment. Spider silk has demonstrated an amazing method of using water solvents to create filaments from the sensitive glands at extremely low temperatures (Saravanan, 2006). To create silk, spiders use their hind legs to pull thread out of the spinning warts, or their body weight or gravity if they are roping (Romer and Scheibel, 2008). Spider dragline silk possesses an exceptional blend of strength and flexibility, it is considered as the most durable biopolymer on Earth. Research on spider silk has been concentrated for over 20 years because of its exceptional mechanical and biophysical qualities (Tokareva *et al*., 2013). The tissues of plants such as potatoes and tobacco have been shown to contain spider silk proteins up to 100 KDa (Scheller *et al*., 2001). Its characteristics, including low density, high biocompatibility, biodegradability, and distinct processes, have led to its adoption as a natural biomaterial in several applications. The silk protein may now be produced in greater numbers and with more uniform quality. The majority of recombinant proteins are made using sequences from *Araneus diadematus* or *Nephila clavipes*(Salehi *et al*., 2020). Silk from the orb-web is the very best structural materials produced by nature among the other web spinners, The design of novel proteins is based on in-vitro and in-vivo results from silk research (Heim *et al*., 2009). In addition, spider silk's low immunogenicity, hydrophobicity,

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homogeneity, and adaptability have gained significant interest for medication and gene delivery applications (Bakhshandeh *et al*., 2022)

6. Economic importance:

As carnivorous arthropods, spiders don't harm plants and may eat a wide variety of prey (Rajeswaran *et al*., 2005). In 1958, spider mites were first discovered in Taiwan. With *Tetranychus kanzawai* at the highest rate, followed by *T. urticae*, *Panonychus citri*, *T. cinnabarinus*, *T. truncatus*, and *Oligonychus litchii*, it has developed into a significant pest of several crops. The primary abiotic factor limiting the proliferation of spider mites was precipitation (Ho, 2000). Additionally, spiders have been seen on grain sorghum, and they are a significant factor in pest control in this crop (Charles and Harvey, 1968). It may spread quickly in an appropriate habitat and consume vast quantities of insects. Spiders are thought to be a significant suppressor of several Dipterans, Lepidopterans, Coleopterans, and Orthopterans found in rice fields, including green leaf hoppers, brown planthoppers, and white-backed planthoppers. It can meet the needs of both pest stabilization and reduction. Spiders may dramatically lower prey numbers, as several studies have shown. Spiders may dramatically lower prey numbers, as several studies have shown. Spider control of insect populations can take the role of chemical control in epidemiological and agricultural contexts. Spiders have always been known as effective predators. Field tests revealed that spider populations have the ability to lower a mite population in specific crops, while laboratory investigations indicate that a range of mites present on cultivated crops are digested by spiders. Certain spiders are capable of recognising insect eggs as food, making them crucial predators. (Umarani and Umamaheshwari, 2013). Because of theirimpacts, their extended life cycle, spiders could be more susceptible to pesticide than insects. Some spiders may even be resistant to pesticides beyond tolerance. Insecticides based on neem, such as *P. pseudoannulata*, are very tolerable to some spider species, including wolf spiders. Because they have very long lives and are resistant to hunger and desiccation, they might be a viable biocontrol agent (Sarma *et al*., 2013).

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CHAPTER- 19
WASTE MANAGEMENT AND RECYCLING: COMPUTATIONAL TOOLS AND ANALYSIS

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Abstract

Waste management encompasses a multidisciplinary approach that integrates engineering principles, economics, urban and regional planning, management techniques, and social sciences. Its objective is to minimize the overall wastage within the system under consideration. A systematic waste management approach should address all types of resources at every stage. Notably, material constitutes a significant portion of the total production cost, making the management of wasted materials critically important. An efficient waste management system plays a crucial role in ensuring the smooth operation of various interconnected systems like computational tools & analysis. Computational Tools and Analysis of waste management & recycling by using different methods like Waste Sort recycle (WSR) in existing waste management systems in rivers. Many technical, climatic, environmental, biological, financial, educational and regulatory factors are involved in Solid Waste Management (SWM). Artificial Intelligence (AI) Techniques and Machine Learning (ML) lately gained attraction in providing alternative computational methods for resolving problems of solid waste management. The major AI technologies are recognized with the development of computer programs that can replicate human characteristics such as problem-solving understanding, interpretation, recognizing, justification. To maintain the clean and green environment we need a smart waste and management system with aligned classification. Effective implementation of a waste management system can lead to reduced costs in both the short and long term, provide protection for workers and local communities, and foster positive community relations. A Successful Waste Management System necessitates the establishment of procedures for monitoring performance and tracking progress towards clearly defined environmental objectives.

Key words:
Waste, Solid Waste Management, Waste Sort Recycle, AI Techniques, Machine Learning.

1.1. Introduction:

The growing consciousness about environmental issues, including the depletion of natural resources and the deterioration of the environment, has led to the emergence of the concept of sustainable development. The approach emphasizes governance as it focuses on the three fundamental aspects of development: economic, social, and environmental, for which waste management is very important. Waste management entails the organized management of waste materials, spanning from their creation to elimination. This comprehensive process encompasses the gathering, transportation, treatment, recycling, and disposal of waste, all

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conducted with a focus on environmental sustainability and efficiency. The overarching objective of waste management is to reduce the adverse effects of waste on both human health and the environment, concurrently optimizing the utilization of valuable resources by emphasizing recycling and recovery practices. Recycling involves the transformation of discarded materials into new products or substances and often includes the extraction of energy from waste materials. The viability of recycling a particular material hinge on its capacity to regain the properties it possessed in its original form. This approach serves as an alternative to traditional waste disposal methods, offering the potential to conserve resources, mitigate greenhouse gas emissions, and contribute to environmental sustainability. Recycling not only curtails the wasteful disposal of potentially valuable materials but also diminishes the demand for fresh raw materials, subsequently reducing energy consumption, mitigating air pollution resulting from incineration, and alleviating water pollution associated with landfilling**(Castilhos Junior and Rocha, 2014).**

Furthermore, recycling constitutes a pivotal element in contemporary waste management strategies, representing the third tier in the waste hierarchy of "Reduce, Reuse, and Recycle." **(Jekria and Daud, 2016).**This strategic placement underscores its significance in fostering environmental sustainability by diminishing the reliance on primary raw materials and redirecting waste within the economic system. To uphold and standardize recycling practices, several International Organization for Standardization (ISO) standards have been established, exemplified by ISO 15270:2008 for plastics waste and ISO 14001:2015 for the environmental management control of recycling processes. These standards serve as guidelines to ensure the effectiveness and environmental responsibility of recycling initiatives. Disposal and management of waste encompass solid, liquid, and gaseous forms, each requiring distinct methods. Waste management addresses various waste types, such as industrial, biological, household, municipal, organic, biomedical, and radioactive waste. Certain wastes may pose health threats, with associated issues spanning the entire waste management process. Health concerns may manifest directly, such as through solid waste handling, or indirectly, affecting water, soil, and food consumption. Human activities, like raw material extraction and processing, generate waste. The primary goal of waste management is to mitigate adverse impacts on human health, the environment, planetary resources, and aesthetics. Emphasis is placed on reducing hazardous effects on the environment and health, with a significant focus on municipal solid waste originating from industrial, commercial, and household activities **(Vyas et al., 2022).**The most useful tool for waste management is Computational tools and analysis , Computational tools in waste management involve the application of technology and data-driven methods to enhance efficiency and decision-making in waste-related processes. These tools include data analytics, modelling, simulation, and Geographic Information System (GIS) applications, providing valuable insights for optimizing waste collection, treatment, recycling, and disposal. By leveraging computational tools, waste management systems can improve resource allocation, reduce environmental impact, and promote sustainable practices.

1.2 Growing concern for environmental sustainability:-

Our Earth is not on a favourable trajectory, as seen by the rising global temperatures and our growing garbage output. Some experts even predict that we are approaching the point of no return. Severe weather occurrences and climatic calamities will become more frequent in the

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absence of environmental sustainability. Environmental sustainability has become a prominent global concern, drawing increasing attention in recent times**(Saridemir and Agbulut, 2019).** The escalating impacts of climate change, biodiversity loss, pollution, and resource depletion have prompted a collective realization of the urgent need for sustainable practices. Across individual households, multinational corporations, and governments, there is a growing acknowledgment that our current trajectory is unsustainable, necessitating immediate, concerted action. This essay explores the multifaceted dimensions of the expanding concern surrounding environmental sustainability, investigating the reasons behind the surge in awareness, the consequences of inaction, and the promising avenues for positive change.

The Surge in Environmental Awareness:

A primary catalyst for the heightened concern regarding environmental sustainability is the increased awareness among the general population. The advent of the internet and social media has made information on environmental issues more accessible and widespread. Disturbing images of oceans choked with plastic, deforested landscapes, and extreme weather events inundate social media platforms, eliciting public outcry and rallying support for environmental causes.Global initiatives and campaigns, such as the United Nations' Sustainable Development Goals (SDGs), have played a vital role in disseminating information and fostering a shared sense of responsibility. Expanded media coverage, documentaries, and scientific reports have further illuminated the intricate connections between human activities and environmental degradation, compelling individuals to reconsider their lifestyles and consumption patterns.

Economic Ramifications:

The growing apprehension about environmental sustainability is also fuelled by a deepening understanding of the economic consequences of environmental degradation. Particularly, climate change poses substantial risks to economies globally, as more frequent and severe natural disasters can disrupt supply chains, damage infrastructure, and lead to significant economic losses. Businesses are increasingly recognizing the importance of integrating sustainability into their operations. Informed consumers, armed with knowledge about the environmental impact of products and services, are demanding more eco-friendly alternatives. Consequently, companies are adjusting their practices to align with sustainability goals, not only to meet consumer expectations but also to mitigate risks associated with regulatory changes and market volatility.

Policy and Regulatory Framework:

Governments and international bodies are responding to the environmental crisis by implementing and strengthening regulations aimed at promoting sustainability. The Paris Agreement, for example, signifies a global commitment to limiting global warming and reducing greenhouse gas emissions. Countries worldwide are enacting laws to curb pollution, protect biodiversity, and transition towards renewable energy sources.

The regulatory landscape is evolving rapidly, with a focus on holding industries accountable for their environmental impact. Carbon pricing mechanisms, emissions trading systems, and

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eco-labelling are becoming more prevalent, incentivizing businesses to adopt sustainable practices. The growing alignment of policy frameworks with environmental goals reflects the understanding that sustainability is not merely a moral imperative but a crucial component of long-term socio-economic stability.

Interconnected Nature of Environmental Issues:

The mounting concern for environmental sustainability is heightened by the recognition that environmental issues are interconnected and often exacerbate each other. For instance, deforestation not only contributes to habitat loss but also intensifies climate change by reducing the number of trees that can absorb carbon dioxide. Similarly, pollution in water bodies not only harms aquatic ecosystems but also affects human health and agriculture.

This interconnectedness underscores the need for holistic and systemic solutions. Addressing one aspect of environmental degradation without considering its broader implications is unlikely to yield sustainable outcomes. As such, the acknowledgment of these interconnections has fuelled a comprehensive approach to environmental management, emphasizing the significance of a circular economy, conservation efforts, and sustainable resource management.

Technological Innovations and Sustainable Approaches:

Another facet of the growing concern for environmental sustainability is the rapid progress of technology and its potential to drive positive change. Innovations in renewable energy, waste management, and sustainable agriculture are providing viable alternatives to traditional, resource-intensive practices. The development of electric vehicles, the utilization of solar and wind power, and the emergence of circular economy models illustrate the transformative power of technology in mitigating environmental impact.

Moreover, sustainable practices extend beyond large-scale industrial solutions. Individuals and communities are adopting eco-friendly habits, such as reducing plastic use, embracing renewable energy at the household level, and supporting local and sustainable products. The integration of technology with sustainable lifestyles is fostering a synergy that holds the promise of achieving meaningful and widespread environmental change.

1.3 Analysis of Computational Tools:-

The purpose of knowing the role of computational tools and analysis in improving waste management and recycling processes,The increasing global waste crisis has necessitated innovative solutions to improve waste management and recycling practices. In recent times, computational tools and sophisticated analyses have emerged as transformative elements, providing unprecedented opportunities to optimize and revolutionize waste-related processes. This essay explores the pivotal role of computational tools in waste management, examining their applications, advantages, and the potential to usher in a new era of sustainability.

I. Present Challenges in Waste Management:

Before delving into the role of computational tools, it is essential to grasp the current challenges in waste management. Accelerated urbanization, population growth, and shifts in consumption patterns have led to a substantial rise in waste generation. Landfills are nearing capacity, and improper disposal practices contribute to environmental degradation. Traditional waste management approaches are strained, necessitating a shift towards more efficient and sustainable solutions.

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II. Computational Tools in Waste Characterization:

A crucial area where computational tools play a significant role is in waste characterization. Utilizing advanced sensors, data analytics, and machine learning algorithms enables the precise identification and categorization of various types of waste. This precision is vital for effective waste management, allowing municipalities and recycling facilities to tailor their strategies based on the composition of the waste stream. Automated sorting systems, driven by artificial intelligence, can notably enhance the efficiency of recycling plants by accurately segregating materials for recycling.

III. Optimization of Collection Routes:

Another domain where computational tools prove their efficacy is in optimizing waste collection routes. Smart waste management systems leverage real-time data, GPS technology, and predictive algorithms to optimize collection routes, thereby reducing fuel consumption, emissions, and operational costs. By dynamically adjusting collection schedules based on the actual fill levels of waste bins, municipalities can enhance efficiency, improve service quality, and reduce the environmental impact of waste collection operations.

IV. Predictive Analytics for Waste Generation:

Predictive analytics, powered by computational tools, provide the ability to forecast future waste generation patterns. By analysing historical data, considering seasonal variations, and factoring in socio-economic elements, municipalities can anticipate peak waste periods and allocate resources accordingly. This proactive approach enhances overall waste management efficiency, enabling timely adjustments to collection frequencies, resource allocation, and planning for recycling infrastructure.

V. Life Cycle Assessment (LCA) and Circular Economy Modelling:

Computational tools play a crucial role in conducting comprehensive Life Cycle Assessments (LCAs) of waste management processes. LCAs analyse the environmental impact of products and services throughout their entire life cycle, from raw material extraction to disposal. This holistic approach aids in identifying areas for improvement and facilitates informed decision-making. Furthermore, computational models assist in designing and analysing circular economy initiatives, promoting the reuse and recycling of materials to minimize waste generation.

VI. Enhancing Recycling Processes:

In the realm of recycling, computational tools contribute to process optimization. Advanced robotics and artificial intelligence are employed to enhance sorting capabilities, ensuring a higher degree of accuracy in separating recyclable materials from the waste stream. Additionally, simulations and modelling assist in refining recycling techniques, improving the recovery rate of valuable materials, and reducing contamination.

VII. Data-Driven Decision-Making:

The integration of computational tools enables data-driven decision-making in waste management. Municipalities and waste management entities can leverage real-time data to monitor and assess the performance of various processes. This data-driven approach

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facilitates continuous improvement, allowing for the implementation of more effective and sustainable waste management strategies.

VIII. Challenges and Considerations:

While computational tools offer tremendous potential, their implementation is not without challenges. Initial investment costs, the need for skilled personnel, and concerns related to data privacy and security must be addressed. Additionally, the digital divide may limit the adoption of these tools in certain regions or communities. Overcoming these challenges requires collaborative efforts between governments, private sectors, and communities to ensure equitable access and effective implementation.

IX. Future Prospects and Sustainability Impact:

Looking forward, the prospects for computational tools in waste management are promising. Continued advancements in sensor technologies, artificial intelligence, and data analytics will further enhance the capabilities of these tools. The integration of the Internet of Things (IoT) and blockchain technologies may provide new avenues for transparent and traceable waste management systems.Regarding sustainability impact, the application of computational tools can significantly contribute to achieving environmental goals. By optimizing waste management processes, reducing resource consumption, and enhancing recycling rates, these tools play a crucial role in fostering a more sustainable and circular economy.

1.4 Computational Tools in Waste Management:

Computational tools and analysis play a crucial role in modern waste management strategies, providing inventive solutions to streamline processes and augment overall effectiveness. These tools capitalize on cutting-edge technologies and data-driven methodologies to tackle the intricacies associated with waste collection, disposal, and recycling. The following outlines how computational tools and analysis are revolutionizing waste management:

1.4.1. Data Analytics:

Utilizing data analytics to scrutinize extensive datasets concerning waste generation, composition, and disposal patterns.Extracting insights from analytics aids in optimizing collection routes, foreseeing waste generation trends, and pinpointing areas for improvement.Data analytics plays a crucial role in transforming waste management methods, offering valuable insights and streamlining processes to tackle the increasing challenges associated with waste generation, disposal, and recycling. Within the sphere of waste management, the utilization of data analytics involves a thorough examination of extensive and diverse datasets related to various aspects of the waste management lifecycle.

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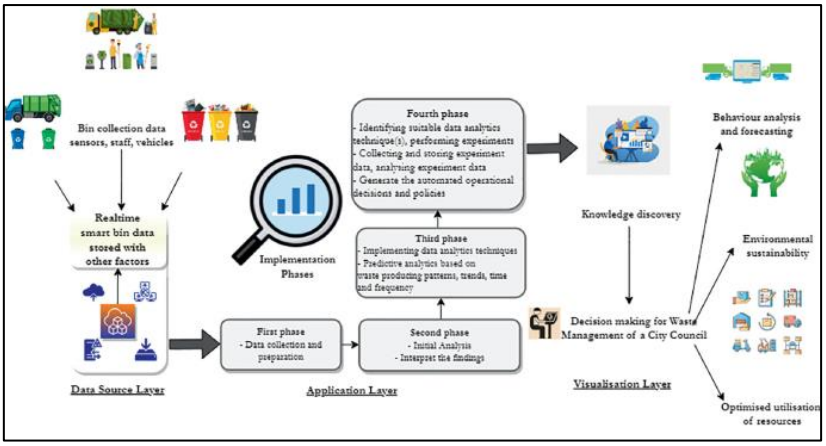

Fig 1.4.1 Showing the Data Analytics *(Sabber et. al, 2023*)
https://www.google.com/url?sa=i&url=https%3A%2F%2Flink.springer.com%2Fchapter%2F10.1007%2F978-3-031-39821-6_11&psig=AOvVaw2mBn6VokprScLfR0x-A4ct&ust=1709745382264000&source=images&cd=vfe&opi=89978449&ved=0CBMQjRxqFwoTCOCM24nQ3YQDFQAAAAAdAAAAABAE

One primary application of data analytics in waste management involves optimizing collection routes. Through the analysis of historical data on waste generation patterns, collection points, and transportation logistics, data analytics facilitates the creation of efficient routes for waste collection vehicles. This optimization not only diminishes fuel consumption and operational expenses but also reduces the environmental impact of transportation, contributing to a more sustainable waste management system**(Sabber et. al., 2023).**

Predictive analytics stands out as another influential tool in waste management. By analyzing historical data, machine learning algorithms have the capability to forecast future waste generation trends. This forecasting ability empowers waste management authorities to anticipate peak periods of waste generation, allowing for proactive planning and resource allocation. The ability to predict spikes in waste generation ensures that sufficient collection and processing facilities are in place during specific times, preventing disruptions and overflows.Data analytics also contributes to identifying areas for improvement in waste management processes. Through a detailed examination of data on recycling rates, contamination levels, and public engagement in waste separation programs, decision-makers can pinpoint areas requiring interventions. For instance, in neighbourhoods consistently exhibiting low recycling rates, targeted awareness campaigns can be implemented to educate residents and promote sustainable waste practices,data analytics revolutionizes the waste management sector by leveraging data to inform decision-making, enhance operational efficiency, and contribute to environmental sustainability. Whether optimizing collection routes, predicting waste generation trends, or evaluating the environmental impact of waste management practices, data analytics plays a central role in the transition towards more intelligent, efficient, and environmentally conscious waste management systems. As technology progresses, the ongoing refinement and application of data analytics hold the promise of further advancements in waste management practices.

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1.4.2. Simulation Models:

Employing simulation models to construct virtual representations of waste management systems for assessment and optimization.These models facilitate scenario planning, enabling decision-makers to simulate the repercussions of changes before implementing them in reality.Simulation models serve as potent instruments in waste management, creating virtual representations of intricate systems to conduct in-depth analyses of their performance and explore possible enhancements. These models employ mathematical algorithms and computer-based simulations to replicate the dynamic interactions and processes inherent in the waste management lifecycle. One notable application of simulation models lies in the optimization of waste vehicle collection routes. Through the input of data on waste generation patterns, collection points, and logistical constraints, these models can generate and assess diverse scenarios, identifying the most efficient routes that minimize fuel consumption and operational costs. Moreover, simulation models play a pivotal role in forecasting and preparing for peak periods of waste generation. By scrutinizing historical data, these models utilize forecasting algorithms to predict future trends, enabling waste management authorities to foresee and plan for heightened waste loads during specific periods. This proactive approach ensures that the essential infrastructure and resources are in place to manage increased demand, preventing disruptions and overflow situations.

Simulation models also contribute significantly to understanding the impacts of various interventions and alterations within waste management systems. Decision-makers can employ these models to simulate the effects of changes in waste separation programs, recycling initiatives, or the introduction of new technologies. This allows for a thorough evaluation ofpotential improvements before implementing changes in the real world, minimizing risks and optimizing resource allocation.

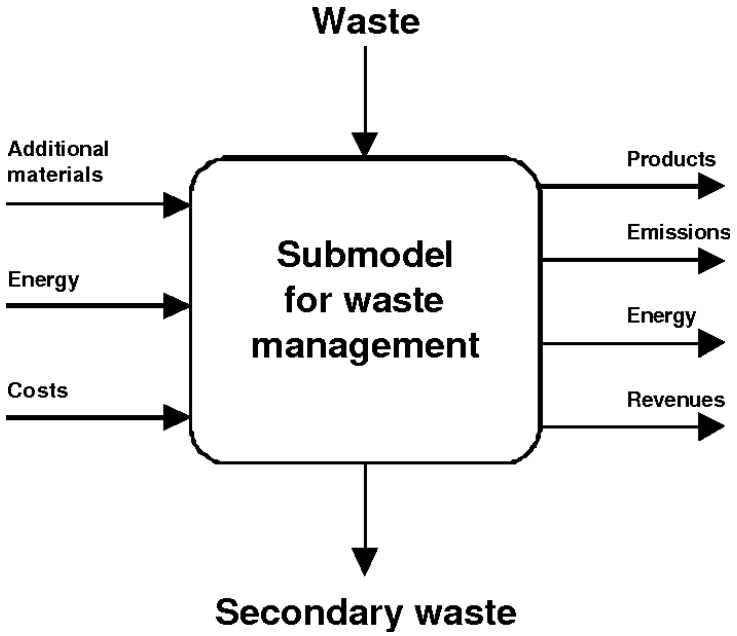

Fig1.4.2 Stimulation model in waste management ***(O. Eriksson et. al., 2002)***
https://www.semanticscholar.org/paper/ORWARE-a-simulation-tool-for-waste-management.-Eriksson-Frostell/974f18444a818b95a6a91443d91824b2d9e5b0d1/figure/0

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Furthermore, simulation models enhance the overall efficiency of waste processing and disposal facilities. By modelling the material flow through these facilities, decision-makers can identify bottlenecks, inefficiencies, and areas for improvement. This valuable insight aids in streamlining operations, reducing processing times, and optimizing resource utilization, ultimately fostering a more effective waste management system.**(O. Erikssonet.al., 2002)**

1.4.3. Geographic Information System (GIS) Applications:

Employing GIS for mapping and analysing spatial data related to waste generation and management infrastructure.GIS optimizes the positioning of waste collection points, identifies suitable locations for recycling facilities, and enhances overall logistics.Geographic Information System (GIS) applications have become essential tools in waste management, offering a comprehensive and spatially informed approach to addressing various aspects of the waste lifecycle. GIS technology encompasses the mapping and analysis of geographic data, furnishing decision-makers with valuable insights into the spatial relationships inherent in waste management systems. A critical application of GIS in waste management involves optimizing the placement of waste collection points. By integrating data on population density, waste generation patterns, and transportation infrastructure, GIS aids in identifying strategic locations for collection bins, ensuring effective and equitable waste disposal coverage.

S.No	Authors	Techniques	Area of Application	Study Location
1	Sudhir et al. (1996)	Multi-objective decision making approach	Waste collection system	Tamil Nadu (Chennai)
2	Natesan and Suresh (2002)	GIS based decision support system, MCE models: AHP, FIC, ANN and Delphi	Landfill site selection	Tamil Nadu (Chennai)
3	Reddy (2005)	ArcGIS platform	Data Management, Validation and Analysis module. Through these modules transfer station capacity, site sensitive index (SSI) and suitable site for landfill were selected.	Medak (Maharashtra)
4	Ghosh et al. (2006)	Rout planning using GIS	Collection and Transport Route Planning.	Asansol (West Bengal)

Table 1.4.1 Showing the Chronology of GIS in Waste management (Meena et. al., 2014)

Moreover, GIS plays a crucial role in pinpointing suitable sites for recycling facilities and waste treatment plants, considering environmental factors and proximity to waste sources. The spatial analysis capabilities of GIS contribute to the optimization of routes for waste collection vehicles, minimizing travel distances and reducing fuel consumption. Additionally, this technology facilitates the monitoring of illegal dumping hotspots through spatial tracking, empowering authorities to implement targeted enforcement actions. In essence, GIS applications are pivotal in enhancing the spatial intelligence of waste management, fostering more informed decision-making, efficient resource allocation, and sustainable practices within the waste management **domain(M Deswal, JS Laura, 2014).**

1.4.4. Routing and Scheduling Algorithms:

Applying computational algorithms to optimize the routing and scheduling of waste collection vehicles.This optimization results in more efficient and cost-effective waste collection, minimizing fuel consumption and reducing the environmental impact. Algorithms for scheduling and routing serve as the strategic planners for garbage collection operations,

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making them essential instruments in waste management. Imagine a city with a fleet of trash trucks and many locations for collecting rubbish. Similar to a navigator, the routing algorithm chooses the best routes for every vehicle to take in order to collect garbage from different sites. It takes into account variables including collection priority, traffic conditions, and distance. Scheduling algorithms are used to determine the best time for each collection stop after the routes have been set. This guarantees that garbage is collected in an organized and prompt way, avoiding delays and maximizing the collection process's overall effectiveness. These algorithms are essential for cutting down on travel time and fuel use, which eventually helps to a waste management system that is better structured and sustainable. Algorithms for scheduling and routing essentially serve as the logistical brains, coordinating the movement of garbage collection trucks to maximize effectiveness and minimize environmental damage.

1.4.5. Smart Bin Technology:

Implementing smart bins with sensors and communication technology to provide real-time data on fill levels.This data facilitates dynamic route adjustments, prevents overflows, and optimizes collection routes for enhanced efficiency.

Fig 1.4.3 Showing the Smart Bin (Alexander et. Al., 2017)
https://africanian.com/tech/how-a-trash-can-became-an-expert-in-sorting-out-your-waste/

Advantages of Smart Bin:-

I. The “smart bin” communicates information on fill levels and ensures collection only when the bin is full.

II. Fewer collection visits reduce congestion and traffic interruption, resulting also in cleaner and safer streets.

III. Traffic reduction due to fewer collection visits helps reduce carbon dioxide and other emissions.

IV. The “smart bins” are standardized so that they can be emptied with existing equipment.

1.4.6. Life Cycle Assessment (LCA):

Engaging in LCA to assess the environmental impact of a product or system throughout its life cycle.In the realm of waste management, LCA aids in evaluating the overall environmental footprint, guiding decisions towards more sustainable practices.

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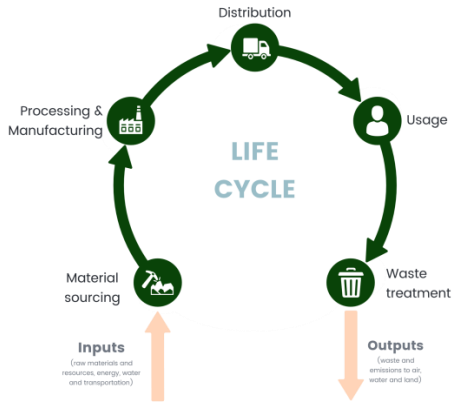

Fig 1.4.4 Showing the Comparative LCA (Christina et. al., 2017)
https://root-sustainability.com/wp-content/uploads/2022/07/Life-Cycle.svg

A product, process, or service's environmental effects are assessed over the course of its whole life cycle using a thorough method called life cycle assessment, or LCA. The extraction of raw materials, manufacturing, shipping, usage, and disposal at the end of the product's life are all taken into account in this evaluation**(Chod et al., 2020)**. An integrated picture of the environmental impact connected to a specific product or activity is what life cycle assessment (LCA)aimstodeliver. LCA can be used in waste management to evaluate the environmental effects of various waste treatment and disposal techniques. Making educated decisions to reduce environmental impact and advance sustainability can be achieved by examining variables like energy usage, greenhouse gas emissions, and resource depletion at every stage of the waste management process. LCA facilitates the identification of waste management improvement potential.

1.4.7. Blockchain for Traceability:

Utilizing blockchain technology to improve traceability and transparency in waste management supply chains.This ensures accountability in waste disposal and recycling processes, mitigating the risk of illegal dumping and promoting responsible practices**(Gomez-Sanabria et. al., 2022).**

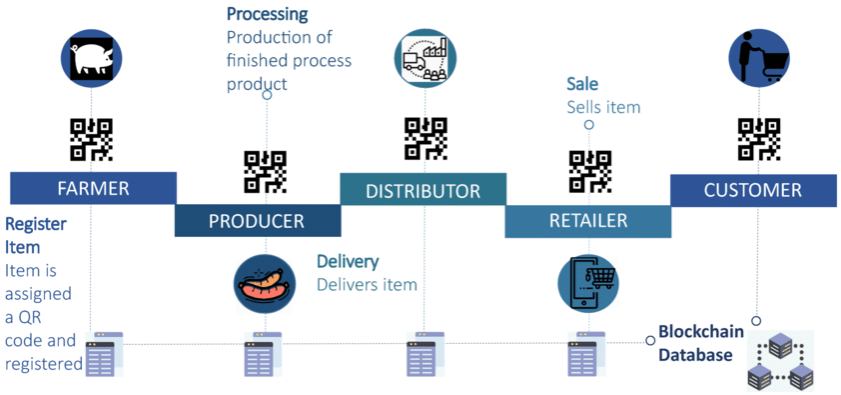

Fig 1.4.5 Showing the Blockchain Traceability System (Murthy, 2019)
https://www.researchgate.net/publication/335010631_Market_Drivers_and_Discovering_Technologies_in_Meat_Species_Identification

Amongst emerging technologies, blockchain, a technology that integrates smart contracts, distributed ledgers, IoT, and big data can be used to build smart waste management systems

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(Fatimah et al., 2020). The whole process data of the waste management system handled by blockchain technology cannot be falsified, since the information is open and transparent, and each stage of the business/management process is easier to monitor **(Berdik et al., 2021).** Combined with other digital technologies, blockchain could solve the drawbacks of traditional waste management systems and has the potential to open up new business models that could benefit society and the environment **(Lamichhane, 2017).**

Since its inception in 2008 **(Nakamoto, 2008),** blockchain technology has seen an exponential growth in theoretical research **(Zheng et al., 2018)** and industrial applications **(Zhao, 2019)** across a wide range of fields, including the circular economy **(Upadhyay et al., 2021),** smart governance **(Lumineau et al., 2021),** information sharing and research & development (R&D) **(Vander Waal et al., 2020),** smart city and society (Mora et al., 2021), and industrial applications such as supply chain monitoring mechanisms **(Bai and Sarkis, 2020),** supply chain transparency **(Chod et al., 2020),** supply chain sustainability management in Industry 4.0 **(Esmaeilian et al., 2020),** smart grid **(Mollah et al., 2020),** energy dispatch and trading **(Chen et al., 2022)** and smart energy systems **(Zhao et al., 2023).** Most review/survey papers on the integration of blockchain technology and waste management focused on small segments of the waste management industry, such as plastic waste **(Steenmans et al., 2021),** hospital waste **(Bamakan et al., 2022),** textile waste **(Alves et al., 2022),** and healthcare waste **(Ranjbari et al., 2022).** However, no systematic or comprehensive evaluations of the broader waste management business have been published, with a focus on trends, challenges, and possibilities. A comprehensive analysis of the integration of blockchain technology with waste management, including theory, methodologies, technology implementation, applications, and implications, is urgently required.

1.4.8. Optical Sorting Systems:

Implementing optical sorting systems using sensors and imaging technology to identify and separate recyclable materials.These systems enhance the efficiency of recycling facilities, improving the quality of recovered materials and reducing contamination.

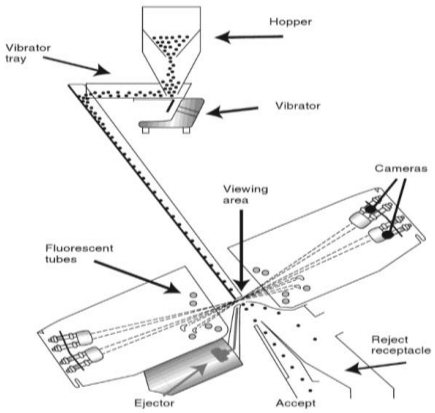

Fig1.4.6 Showing the Optical sorter (Bee et. al., 2007)
https://www.researchgate.net/figure/1-Schematic-diagram-of-an-optical-sorting-machine_fig1_31182865

According to Manouchehri optical sorting is one of the most efficient and cost-effective procedures for treating, separating, and recycling secondary materials**(Manouchehri 2003).** This will reduce investment costs, mitigate the environmental impact of mining waste, and

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increase ore quality. The optical separator is mostly made up of the following components **(Suhasaria and Pathak, 2012):**

Feeding: The crushed feed material is moved to the location where the optical data is taken.

Collecting Optical Data: The ore must pass via an optical sensor system (ColourCamera), where the reflected light from each particle is detected and analysed.

An electrical processor performs optical data processing, which involves transmitting appropriate signals to a mechanical mechanism for material separation. According to the processor's instructions, the mechanical separation system includes a valve or a compressed air rejecter.

1.5 Challenges:

1.5.1. Increased Waste Generation

Rapid urbanization and population increase cause a rise in waste production, which overwhelms existing waste management facilities. Computational technologies can help optimize collection and disposal strategies to deal with this increase.

1.5.2. Inefficient Infrastructure

Many locations, particularly in developing countries, lack adequate waste management infrastructure, which leads to ineffective collection and disposal. Computational analysis can help to develop efficient systems and optimize resource allocation.

1.5.3. Lack of Recycling Infrastructure

Inadequate recycling facilities impede efforts to divert garbage away from landfills. Computational methods can help to optimize the location of recycling facilities and improve recycling operations.

1.6 Future Directions:

1.6.1. Advanced Data Analytics

Using Advanced Data Analytics can provide deeper insights into waste generation patterns, allowing you to forecast trends and plan for future waste management requirements.

1.6.2. Machine Learning for Predictive Modelling

Machine learning algorithms can be used to predict waste generation and composition, allowing for more proactive planning and resource allocation.

1.6.3. Blockchain for Transparency

Blockchain technology can improve transparency and traceability in waste management supply chains, lowering the danger of unlawful dumping and encouraging responsible waste disposal practices.

1.7. Conclusion:

In Conclusion, including Computational Tools and Analysis into waste management and recycling processes is a critical step toward developing more efficient, sustainable, and adaptive systems. The constraints faced by rising trash quantities, inadequate infrastructure, and environmental concerns need novel solutions, and computational methods offer a promising route for tackling these issues. Waste management may become more sophisticated and responsive by leveraging advanced data analytics, machine learning, and

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technologies like GIS and Blockchain. These techniques can optimize collection routes, predict trash generation patterns, and improve transparency in the waste supply chain. Furthermore, the adoption of smart technology and the progress of circular economy principles help to reduce waste and promote responsible resource management. As we navigate the intricacies of waste management and recycling, computational tools help us analyse, strategize, and execute sustainable approaches. Future developments in these tools have the potential to further transform the waste management landscape by supporting a circular economy, reducing environmental impact, and encouraging a more ethical approach to trash disposal.

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CHAPTER -20
HISTORICAL BACKGROUND AND DEVELOPMENT OF PHARMACY

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Abstract

Pharmaceutics is the overall process of developing a new chemical entity into an approved therapy that is safe and effective intreating or preventing disease. It is a complex process requiring multiple scientific, medical, legal, commercial and regulatory expertise. Multidisciplinary research is that team members have access to a broader range of data at no additional cost. Technical issues are almost always encountered in empirical research, particularly in studies involving stimulations.

Keywords: Pharmaceutics, Drugs, Medical, Research, Disease.

Introduction:

Drugs are chemicals or substances that change the way our bodies work. Some are medicines that help people when doctors prescribe them. Many have no medical use or benefits. When taken usally by swallowing, inhaling, or injecting, abused drugs find their way into the bloodstream.

Defination:

WHO defines drug as any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the receipt.

Examples: Tablet, Caspsules, Patches, Emulsion, and Suspensions etc.

Pharmacy:

Pharmacy is the science and practice of discovering, producing, preparing, dispensing, reviewing and monitoring medications, aiming to ensure the safe, effective, and affordable use of medicines. It is a miscellaneous science as it links health sciences with pharmaceutical sciences and natural sciences.

Fig: Drugs

Mechanism of action:

In medicine, a term used to describe how a drug or other substance produce an effect in the body.

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For example, a drugs mechanism of action could be how it affects a specific target in a cell, such as cell growth. Knowing the mechanism of action of a drug may help provide information about the safety of the drug and how it affects the body. It may also help identify the right dose of a drug and which patients are most likely to respond to treatment also called MOA.

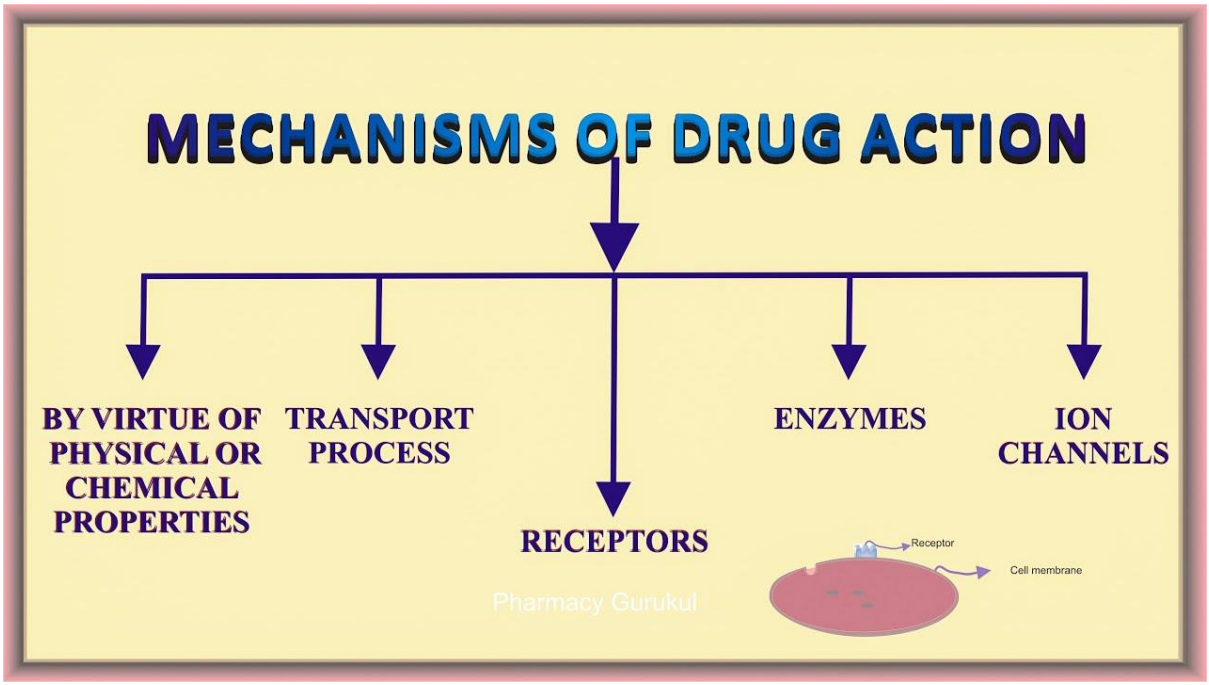

Fig: Mechanism of drug action.

History of the profession of Pharmacy in India:

The history of pharmacy as a modern and independent science dates back to the first third of the 19th century. Before then, pharmacy evolved from antiquity as part of medicine. The history of pharmacy coincides well with the history of medicine, but it's important that there is a distinction between the two topics. Pharmaceuticals is one of the most-researched fields in the academic industry, but the history surrounding that particular topic is sparse compared to the impact its made world-wide. Before the advent of pharmacists, there existed apothecaries that worked alongside priests and physicians in regard to patient care.

Ancient Era:

Sumerian cuneiform tablets record prescriptions for medicine. Ancient Egyptian pharmacological knowledge was recorded in various papyri, such as the Ebers Papyrus of 1550 BC and the Edwin Smith Papyrus of the 16th century BC.

The very beginnings of pharmaceutical texts were written on clay tablets by Mesopotamians. Some texts included formulas, instructions via pulverization, infusion, boiling, filtering and spreading.

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history of pharmacy coincides well with the history of medicine, but it's important that there is a distinction between the two topics.

Prof MahadevaLalSchroff is credited as the Father of Pharmacy Education in India because he provided the right direction to the field of pharmacy and inspired many generations of pharmacists. Father of pharmacy Education in India, passed away on August 25,1971 and he surely remains an inspiration to all pharmacist working in this nation, regardless of their branches of responsibilities. Prof. Schroff, who was not qualifies as a pharmacist, guided not just pharmaceutical education but also the Indian industry with his aptitude, comprehension, talent, and broad vision.

Relation to Pharmacy Education:

In 1948, the Pharmacy Act[5] was enacted as the nation's first minimum standard of educational qualification for pharmacy practice to regulate the practice, education, and profession of pharmacy. Currently, one needs at least a diploma in pharmacy to practice as a pharmacist.

Pharmacy education in India was certified level offered in Goa by the Portuguese in 1842 and the university level education started in 1937 at Banaras Hindu University (BHU). Since then, several universities, and colleges are offering courses across the country.

Pharmacy education equips them with the communication skills needed to explain complex medical information in an understandable manner. This is vital for promoting medication adherence and patient safety. Medication Management: Pharmacists are responsible for medication management in various healthcare settings.

A pharmacist is a health care professional specializing in the usage and administration of medication. They dispense prescriptions to patients upon receiving a physician's orders. Pharmacists are experts on how drugs work and interact with the body so that patients who take them achieve the best possible results.

Industry Organization of Pharmacy:

Industry Organization of Pharmacy is the organization whose pharmacist members are universally recognized within the pharmaceutical industry as being the most professionally equipped to contribute to the development, commercialization, promotion, and optimal use of medicines.

Pharmaceutical Industry:

The pharmaceutical industry is an industry in medicine that discovers, develops, produces, and markets pharmaceutical drugs for use as medications to be administered to patients (or self-administered), with the aim to cure and prevent diseases, or alleviate symptoms.Pharmaceutical companies may deal in generic or brand medications and medical devices. They are subject to a variety of laws and regulations that govern the patenting, testing, safety, efficacy using drug testing and marketing of drugs.

Pharmacyas a Career:

Pharma program opens up a wealth of career opportunities in the pharmaceutical field. Students can pursue jobs in either the public or private sector or abroad. Options include working in government hospitals, private medical shops, and private hospitals/clinics.You could also start your medical practice or store.

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Scope of Pharmacy Profession:

Pharmacy graduates can work in several different industries such as sales, marketing, research and development, manufacturing, quality check, education, etc.

Pharmacopeia

A pharmacopoeia, or pharmacopoea (from the obsolete typography pharmacopœia, meaning "drug-making"), in its modern technical sense, is a book containing directions for the identification of compound medicines, and published by the authority of a government or a medical or pharmaceutical society.

A book describing chemicals, drugs, and other substances and how they are used as medicines.

Introduction:

Pharmacopoeia, book published by a government, or otherwise under official sanction, to provide standards of strength and purity for therapeutic drugs. The primary function of a pharmacopoeia is to describe the formulation of each drug on the selected list.

Indian Pharmacopeia:

Indian Pharmacopeia contains a collection of authoritative procedures of analysis and specifications of drugs for their identity, purity and strength. The standards of the IP are authoritative in nature and are enforced by the regulatory authorities for ensuring the quality of drugs in India.

First edition IP- In 1955 indian pharmacopeia commitee under chairship of Dr.B.N. Gosh published first edition in 1955

Second Edition IP – In 1965

Third edition IP- In 1985 third edition IP was published in 1985 with two volume and nine appendices.

Fourth Edition IP- In 1996 under the chairmanship of Dr. Nityanand.

Fifth Edition IP- In 2007 and ammendum to this edition was published in 2008.

Sixth Edition IP – is published in 2010. The Indian pharmacopeia is represent in three volumes.

Seventh Edition IP- The seventh edition of the Indian Pharmacopeia in 2014. The Indian pharmacopeia presented in 2014 in four volume.

Eight Edition IP- The eighth edition of the Indian Pharmacopoeia (IP 2018) is published by the Indian Pharmacopoeia Commission (IPC) in accordance with a designed plan by the Scientific Body of IPC, in fulfilment of the requirements of the Drugs and Cosmetics Act 1940 and Rules.

Nineth Edition IP-The Union Health Ministry has recently released the 9th edition of Indian Pharmacopoeia (IP) 7 december 2022 containing 92 new monographs for drugs, 12 new general chapters, 1245 monographs for formulations, 930 monographs for active pharmaceutical ingredients (APIs) as well as dissolution specifications for all prolonged release.

- **British Pharmacopeia:**

The British Pharmacopoeia (BP) is the official collection of standards for UK medicinal products and pharmaceutical substances. It is an essential reference for all individuals and

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organizations working within pharmaceutical research and development, manufacture and testing across the globe.

The BP has been providing official standards for medicines since 1864, and provides the only comprehensive collection of authoritative official standards for UK pharmaceutical substances and medicinal products, including all the monographs and texts of the European Pharmacopoeia (Ph. Eur.).

The first edition of what is now known as the British Pharmacopoeia was published in 1864, and was one of the first attempts to harmonise pharmaceutical standards, through the merger of the London, Edinburgh and Dublin Pharmacopoeias.

Second edition of BP was published in 1867. Third edition of BP was published in 1885. Fourth and fifth editions of BP were published in 1898 and 1914. During 1953, the eight edition of BP was published and edition titles of drug & preparation were changed in English instead of Latin and matric system.

- **United State Pharmacopeia:**

USP is an independent, scientific nonprofit organization focused on building trust in the supply of safe, quality medicines. We are working to strengthen the global supply chain so that the medicines people rely on for health are available when needed and work as expected.

The United States Pharmacopeia (USP) describes quality standards for medicines and is continuously updated as USP-NF, a combination of the United States Pharmacopeia and the National Formulary (NF).

- **Extra Pharmacopeia**

The Extra Pharmacopoeia, originally produced by William Martindale in 1883 and now published by the Pharmaceutical Society of Great Britain, contains information on the drugs presently used in Great Britain.

Dosage forms:

Dosage forms (also called **unit doses**) are pharmaceutical drug products in the form in which they are marketed for use, with a specific mixture of active ingredients and inactive components (excipients), in a particular configuration (such as a capsule shell, for example), and apportioned into a particular dose. For example, two products may both be amoxicillin, but one is in 500 mg capsules and another is in 250 mg chewable tablets. The term **unit dose** can also sometimes encompass non-reusable packaging as well (especially when each drug product is individually packaged, although the FDA distinguishes that by unit-dose "packaging" or "dispensing". Depending on the context, multiple unit dose can refer to distinct drug products packaged together, or to a single drug product containing multiple drugs and/or doses. The term **dosage form** can also sometimes refer only to the pharmaceutical formulation of a drug product's constituent drug substances and any blends involved, without considering matters beyond that (like how it is ultimately configured as a consumable product such as a capsule, patch, etc.

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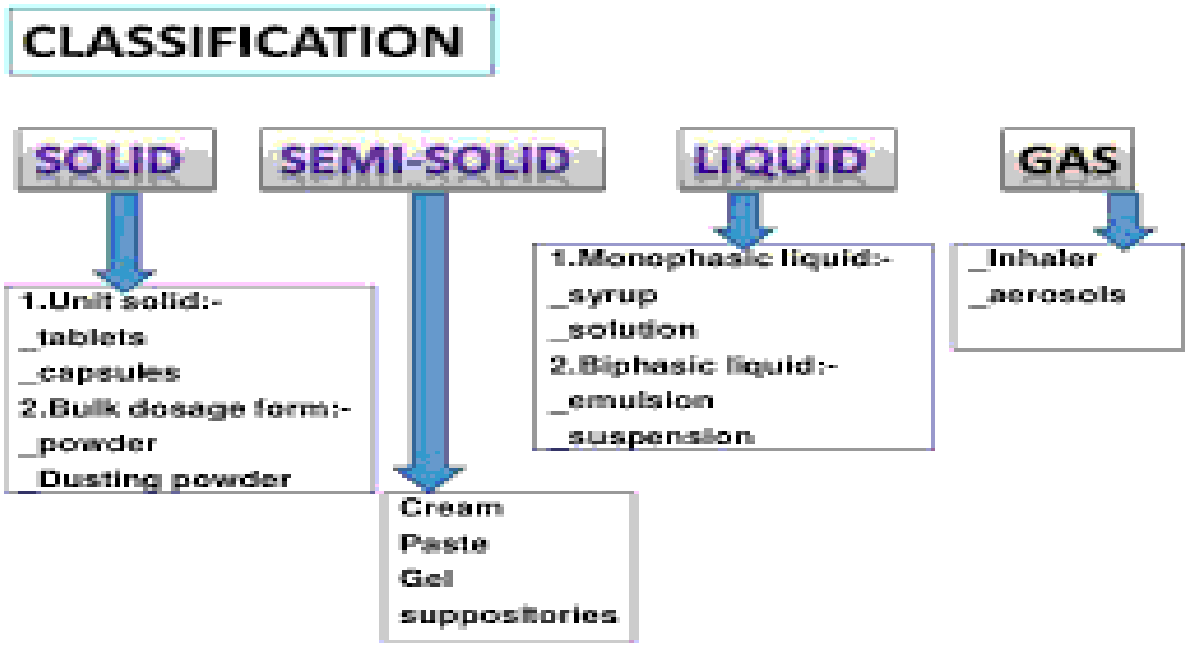

Fig.- Classification Dosage form

- **Solid Dosage Form**

  The solid dosage form are most commonly used dosage form because of the stability and easy of mass production.
  Tablet,Capsule,Powder, Granules.

Tablet:

A tablet (also known as a pill) is a pharmaceutical oral dosage form (oral solid dosage, or OSD) or solid unit dosage form. Tablets may be defined as the solid unit dosage form of medication with suitable excipients. It comprises a mixture of active substances and excipients, usually in powder form, that are pressed or compacted into a solid dose. The main advantages of tablets are that they ensure a consistent dose of medicine that is easy to consume.

Fig: Tablet

Capsule:

In medicine, a sac of tissue and blood vessels that surrounds an organ, joint, or tumor. A capsule is also a form used for medicine that is taken by mouth. It usually has a shell made of gelatin with the medicine inside.

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Fig.- Capsule

Capsule are two parts:

The capsules are manufactured in two halves – a body and cap – to assist with filling. Gelatin has been the material of choice for capsules because of the ability of a solution to gel and form a solid at a temperature just above room temperature.

Powder:

Drug composed of a solid dry substance in the form of finely divided particles used for external and internal use in the form of dosage is termed a pharmaceutical powder. These powders are used in doses and can be obtained through the processes like. crushing and grinding.

Fig. – Powder

Granules:

Drug composed of a solid dry substance in the form of finely divided particles used for external and internal use in the form of dosage is termed a pharmaceutical powder. These powders are used in doses and can be obtained through the processes like. crushing and grinding.

Fig.- Granules

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Semi solid Dosage form:

Semi solid dosage form are the topical dosage form used for the therapeutic protective or cosmetic production.

1. Cream,
2. Paste ,
3. Gel ,
4. Suppositories

- **Cream**

  Creams are semisolid dosage forms containing more than 20% water or volatile components and typically less than 50% hydrocarbons, waxes, or polyols as vehicles. They may also contain one or more drug substances dissolved or dispersed in a suitable cream base.

- **Paste**

  Paste is a basic pharmaceutical form. It consists of a fatty base (e.g., petroleum jelly) and at least 25% of a solid substance (e.g., zinc oxide). Pharmaceutical pastes are typically intended for external application to the skin. They are usually thick and do not melt at physiologic temperatures.

Fig: Paste

- **Gel**

  A gel refers to the semi- solid, 3-dimensional matrix formed from an interspersed system of colloidal particles or the permeation of a solvent into an entwined polymer chain network.

- **Suppositories**

  A suppository is another way to deliver a drug. It's a small, round or cone-shaped object that you put in your body, often into your bottom. Once it's inside, it melts or dissolves and releases its medication. Suppositories may not be the most pleasant product you'll ever use.

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Fig. – Suppositories

- **Liquid dosage form**

  Dosage forms are essentially **pharmaceutical products in the form which involves a mixture of active drug components and** nondrug components (excipients).

  - **Syrup**
  - **Solution**
  - **Emulsion**
  - **Suspension**

  - **Syrup**

    Syrups is a concentrated solution of sugar in water or other aqueous solutions. Medicinal syrup is a nearly saturated aqueous solution in which the medicinal substance or drug is dissolved. Basically, it is an oral suspension in liquid form. Medical syrup or medicinal syrup is actually used as a carrier for drugs.

  - **Solution**

    Solutions are liquid preparations containing one or more drug substances molecularly dispersed in a suitable solvent or a mixture of mutually miscible solvents. Oral liquids contain one or more substances with or without flavoring, sweetening, or coloring agents dissolved in water or cosolvent–water mixtures.

  - **Emulsion**

    An emulsion is a. thermodynamically unstable system consisting of at least two immiscible liquid phases one of which is dispersed as globules in the other liquid phase stabilized by a third substance called emulsifying agent.

    Emulsion is Four types

    - **O/W Emulsion**
    - **W/O Emulsion**
    - **Microemulsion**
    - **Multiple Emulsion**

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- **Suspension**
  A pharmaceutical suspension may be defined as a coarse dispersion containing finely divided insoluble material suspended in a liquid medium.

Prescription:
A prescription is an order for medicine which a doctor writes, and which is given to a pharmacist to prepare and administer the medicine. The new drug does not require a physician's prescription - it can be bought over the counter. The pharmacist cannot dispense certain medicines without a doctor's prescription.
Defination: A written direction or order for the preparation and use of a medicine.

Parts of Prescription:
Preparation of generally written of typical formate which is usally kept as pads.
Date
Name, age,sex and address of the patient
Superscription
Inscription
Subscription
Signature
Renewal Instruction
Signature, address, and registration number of prescriber.
Date
It helps a pharmacist to find out the date of prescribing and date of presentation for filling the prescription. The prescription for filling the prescription.The prescription which prescribe narcotic. On other habit farming drugs must be prescription. It is present by the patient a number of time for dispensing.
Name, Age, Gender and Address of the patient
It must be written in the prescription because it serves to identify the preparation the same way includes by the pharmacist after proper enquiry from the patient. Age and gender of the patient, specially in the case of the check the prescribe close of medication. In some cases, the wt. Of the patient also be required in order to calculate the appropriate dose.

Superscription
It is represented by the symbol Rx which is written before writing the prescriotion. Rx is an abbreviation of latinword recepe incoming you tube in ..... days the be aunginated from the sign of Jupiter (God of healing).
This symbol was complayed by the ancient in requesting and for thr quick recovery of the patient.
Inscription
This is the main part of the prescription order contain the name and quality of the prescribed indegrids are generally
Written in English and latin language the name of each ingredient is written on a separate time along with its quality. In complex prescription containing several ingredients.

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Base

The active medicament which are intended to produce active therapeutics effect.

Adjuvant

It is included weither the action of medicament or to improve the preparation of patient.

Vehicle

It is included in prescription either in dissolve the solid ingredients or to increase the volume of prescription.

Subscription

This compreses direction to the pharmacist for preparing the prescription and in of to doses to be dispense. These days the prescription are a meeting the specific instruction to the pharmacist because the majority of the prescriotion are not compounding and dispense.

Signature

This is consists of the direction to be given to the patient regarding the administration of the drug it is usally written as sig on the prescription.

The instruction may include the following it:-

- The quality to be taken.
- The mode of administration
- The frequency of administration
- The special instruction such as dilution direction.

Renewal Instruction

The prescribe indicate every prescription order rather it may be renewal and it so how many times. It is very important particularly in the prescription containing and other habit forming drugs to prevent its misuse.

Signature , address and Registration number of the prescribe

The prescribe must bear the signature of the prescriber along with its registration number and address. It is very important particularly containing the narcotic and other habit forming drug to prevent its misuse.

Primo mane - early morning
Mane - morning
Omni mane- every morning
Inter nacti - every night
Nocte - at night
Omni nara - in every hour
Ominiquatahara - every four hour.

Error of Prescription

Errors in prescribing can occur when an incorrect drug or dose is selected, or when a regimen is too complex. When prescriptions are transmitted orally, sound-alike names may cause error. Similarly, drugs with similar-looking names can be incorrectly dispensed when prescriptions are handwritten.

Posology

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Posology: Derived from the greek◊Posos-how much, and logos- science is the branch of pharmacology dealing with doses. Dose: Is the quantitative amount administered or taken by a patient for the intended medicinal effect.

So posology is a branch of medical science which deals with dose & quantity of drugs which can be administered to a patient to get a desired action. In this, there are many factors which influence the doses. The pharmacokinetics of many drugs changes with age.

- **Clark**'s **rule**

  Clark's rule equation is defined as the weight of the patient in pounds divided by the average standard weight of 150 pounds (68 kg) multiplied by the adult dose of a drug to obtain the pediatric medication dose, as is demonstrated below:

  (Weight× 150 lbs.) x Adult Dose = Pediatric Dosage.

- **Fried's rule**

Fried's rule is a method of estimating the dose of medication for a child by dividing the child's age in months by 150 and multiplying the result by the adult dose.

Infant age in Month/ 150×Adult Dose

Young's Rule

Young's rule for calculating the dose of medicine correct for a child by adding twelve to the child's age, dividing the sum by the child's age, then dividing the adult dose by the figure obtained, as it expressed beneath:

(age in years / age (years) + 12) × adult dose

Cowling Rule

A method for calculation of pediatric drug dosages in which the age of the child at the next birthday is divided by 24. However, the most safe and accurate methods of pediatric dosage calculation include the weight and body surface area or both of the patient.

Age at next birth/24×Adult Dose= Dose for child

Factor affecting Posology

Different factor affecting posology are as follow:

- Age
- Sex
- Body weight
- Time of administration
- Body surface area
- Route of administration
- Emotional factor
- Accumulation
- Environmental factor
- Presence of disease
- Additive effect
- Idosyncrasy
- Synergism
- Antagonism.

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Body weight and Body surface:

The body surface area (BSA) of any adult, when derived from the arithmetic mean of the different values calculated from four independent accepted formulae, can be expressed accurately in System International 'Unites (SI) units by the simple equation

BSA = 1/6 (WH) 0.5

where W is body weight in kg, H is body height

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CHAPTER- 21
APPLICATION OF RT-PCR IN COVID-19 PANDEMIC

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Abstract
The COVID-19 pandemic has brought into sharp focus the critical importance of accurate and efficient diagnostic testing. Qualitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) has emerged as the cornerstone of COVID-19 diagnosis due to its ability to amplify viral RNA for identification. However, the test is not without its limitations. Challenges include the potential for sample contamination, variability in viral load, and the need for specialized equipment and trained personnel, which can impact accessibility and turnaround time. Despite these hurdles, optimizing the RT-PCR protocol through simplification of procedures, repeated testing with different specimen types, and integration with other diagnostic modalities such as clinical observations and immunodiagnostic tests can enhance its sensitivity, reliability, and applicability. As the pandemic continues to evolve, staying at the forefront of technical advancements in RT-PCR and other testing platforms will be crucial for maximizing diagnostic precision and informing effective public health interventions. This chapter provides a comprehensive exploration of the role and application of RT-PCR in the COVID-19 pandemic response, highlighting both the opportunities and challenges of this critical diagnostic tool.

Keywords: COVID-19, pandemic, RT-PCR, immunodiagnostic, public health.

Introduction :
The worldwide COVID-19 pandemic was first identified in December 2019 when SARS-CoV-2 first appeared in China. The virus is known for its fast spread and variety of clinical manifestations (Muralidar et al., 2020). While some carriers of the virus stay asymptomatic and can unintentionally transfer it to others, others have severe symptoms that need prompt medical treatment. A thorough strategy to disease control is required because of the difficulties in early identification and contact tracing that arise from this diversity in symptomatology. The alarming global mortality toll of almost 3 million underscores the critical need for effective diagnostic techniques to precisely identify and isolate individuals in order to prevent further spread (Juneau et al., 2023).
Qualitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) testing, which amplifies viral RNA for identification, is the cornerstone of modern COVID-19 diagnosis. Even though it's the gold standard, sample contamination or variations in viral load make it difficult to distinguish between true positive and true negative findings. A multimodal diagnostic approach that incorporates both clinical observations and molecular data is recommended in order to solve these diagnostic difficulties. Testing repeatedly over a

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longer period of time and with different kinds of specimens can improve sensitivity and reliability and reduce the possibility of inaccurate results (Dutta et al., 2022).

The RT-PCR testing procedure must be made as simple as possible in order to be widely used, provide results quickly, reduce outbreaks, and make contact tracing more effective. Testing protocols may be made more efficient to minimize the gap between sample collection and reporting, allowing for rapid interventions and more testing as necessary. Additionally, simplifying procedures may lower test costs, improving accessibility and affordability for larger-scale population screening initiatives (Tsang et al., 2021).

This thorough analysis explores the subtleties of RT-PCR testing's diagnosis, examines the consequences of diagnostic errors, and suggests fresh approaches to maximize the test's usefulness in COVID-19 case discovery. The research highlights the favorable correlation between RT-PCR testing and immunodiagnostic tests, and emphasizes the significance of utilizing a combined testing method to reduce the likelihood of both false-positive and false-negative outcomes. In the ongoing fight against the pandemic, this research seeks to improve the precision and efficacy of COVID-19 diagnostic techniques by investigating state-of-the-art technical developments in test design and implementation(Teymouri et al., 2021a).

Principal of PCR:

DNA polymerase catalyzes the addition of deoxynucleotides to expand and create new strands complementary to each of the target sequence strands after the target sequence of nucleic acid is denatured to single strands and primers specific for each target strand sequence are added (cycle 1). After cycle 1's double-stranded products are denatured in cycle 2, DNA polymerase uses them as targets for further primer annealing and extension. Using this heat cycling technique, at least 107 copies of the target DNA may be generated after 25 to 30 cycles (Aryal, 2022).

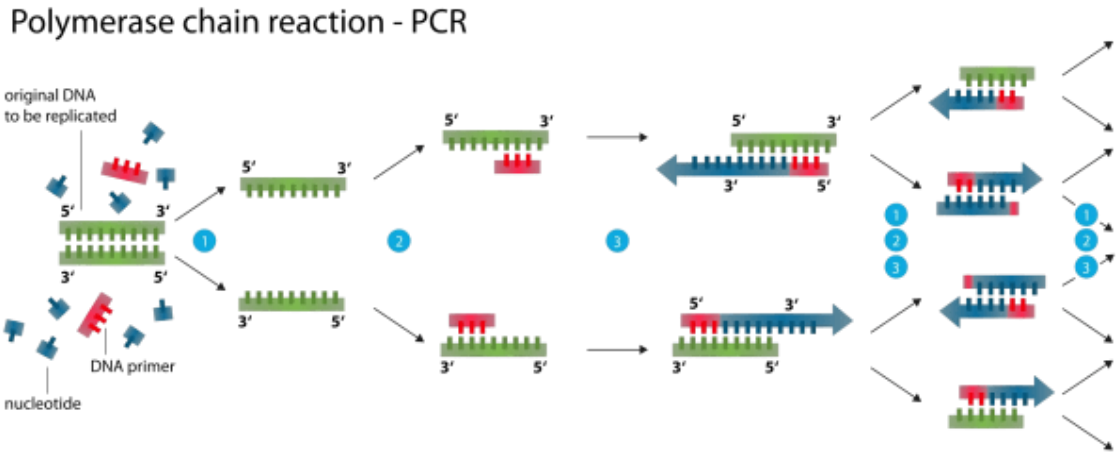

Fig.1 polymerase chain reaction – PCR

Components:

- In a PCR procedure, the target double-stranded DNA, two primers that hybridize to flanking sequences on the target's opposing strands, all four deoxyribonucleoside triphosphates, buffer, enzyme cofactors, and water are added.

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- Heat-stable DNA polymerase is required for PCR because the process occasionally reaches high temperatures.
- A wide range of these heat-stable enzymes derived from thermophilic bacteria—bacteria that thrive in environments with high temperatures—are currently offered for sale.
- The Taq polymerase derived from the thermophilic bacteria Thermus aquaticus is the earliest and most widely utilized.

Steps of PCR :

The extracted material, which contains the target DNA template, is put to a tube containing primers, Taq polymerase, and free nucleotides (dNTPs) to perform PCR. In a PCR machine, the PCR mixture is put. The PCR machine produces exponentially more copies of the target sequence by automatically and systematically adjusting the temperature of the PCR mixture (Aryal, 2022).

Polymerase Chain Reaction (PCR) has three major steps.

1. **Denaturation (strand separation)**: heating (from 94°C to 96°C) to separate the two hydrogen-bonded complementary chains of DNA into a pair of single-stranded polynucleotide molecules
2. **Annealing (primer binding):** In order for the primers to bind to the single-stranded DNA strands, the temperature is decreased to 45–60 °C.
3. **Extension (synthesis of new DNA):** It moves along the 72°C DNA strand, beginning at the annealed primer.

When the first round of denaturation, annealing, and extension is finished, the procedure is repeated by cycling back to the initial reaction temperature (an automated operation in thermocycler). The target gene sequence amplifies exponentially as a result of repeating this three-step temperature cycle around thirty times.

RT-PCR :

a) **Principle**:

Reverse transcription is combined with a traditional PCR procedure to create RT-PCR. The reverse transcriptase enzyme initially transforms the sample RNA into complementary DNA, or double-stranded DNA, during the reverse transcription process. Once the cDNA has been thermally degraded, two single-stranded DNA templates may be produced. The nucleic acid hybridization concept allows primers to anneal to their corresponding sequences in these ssDNA templates. After that, DNA polymerase lengthens the primer by successively appending nucleotides to the 3' end, producing dsDNA in accordance with the DNA replication principle. Millions of copies of the cDNA are produced by the cyclic repetition of these three steps, which also control the reaction temperature: denaturation, annealing, and elongation (Santos et al., 2004).

b) **Components**

1. Nucleic Acid Sample (Sample RNA)

RT-PCR use RNA as the sample, as opposed to conventional PCR methods that use DNA. mRNA is often utilized as the sample. Prior to amplification, the RNA will be changed into cDNA.

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2. Reverse Transcriptase Enzyme

It is an enzyme that helps the RNA strand split into complementary DNA (cDNA) strands. It is also known as the RNA-dependent DNA polymerase enzyme and is the cause of the reverse central dogma. It is the main part of RT-PCR as it amplifies sample RNA by converting it to cDNA.

3. DNA Polymerase Enzyme

DNA polymerases are enzymes that assemble nucleotides in a sequential manner in accordance with the template strand to catalyze the synthesis of complementary DNA strands. The most extensively used DNA polymerase is Taq DNA polymerase, which is an enzyme that was isolated from the bacterium Thermus aquaticus. Taq DNA polymerase is thermally stable and retains its activity even after repeated cycles of heating and cooling.

4. Primers (Oligo (dT) primers, random primers, and sequence-specific primers)

RT-PCR uses three different kinds of primers;

1. **Random Primers**

These short, single-stranded sequences, ranging in length from six to eight nucleotides, bind to the complementary site of RNAs, whether or not they include poly(A), to facilitate the production of cDNA by reverse transcriptase.

2. **Oligo (dT) Primers**

These are oligonucleotides, with the majority having between 12 and 18 nucleotides. One of their segments, repeating deoxythymidine (dT), attaches to the polyA tail of mRNA.

3. **Sequence-specific Primers**

These are the brief nucleotide single-stranded sequences that attach to the particular location of interest in the sample RNA. Primarily, one-step RT-PCR uses it.

5. Deoxynucleotide Triphosphates

During amplification, deoxynucleotide triphosphates (dNTPs), which are synthetic nucleotides, serve as building blocks for the synthesis of cDNA and new strands of cDNA. Deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP), and deoxycytidine triphosphate (dCTP) are the four distinct dNTPs that are employed.

6. PCR Buffers and Other Chemicals

7. Thermocycler (PCR Machine)

Steps involved in polymerase chain reaction:

Reverse transcription and amplification are the two main stages of the process. Additionally, the process differs for one-step and two-step RT-PCR. However, the main procedures for both kinds are the same and may be distilled into four phases: the amplification stage, the

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reverse transcription stage, the preparatory stage, and the product analysis stage, that is (Montarras et al., 1994):

1. Preparatory Stage:

In this first step, all of the reaction mixture is created and RNA extraction is completed. Prior to extracting or bringing the sample from storage, all supplies must be organized, safety precautions must be followed, the PCR reaction preparation space must be cleaned, and all reagents must be brought to working temperature.

In the one-step RT-PCR, A single reaction tube contains the following ingredients: sample RNA, reverse transcriptase enzyme, RNase H, primers, DNA polymerase, dNTPs, buffers, and all other ingredients added in a predetermined and specified amount. After that, the tube is put into a thermocycler to be processed further.

In the two-step, RT-PCR, To facilitate reverse transcription, a tube is filled with sample RNA, reverse transcriptase, RNase H, primers, dNTPs, and other buffers and reagents. After that, cDNAs are created in the tube by heating it to a certain temperature in a thermocycler.

2. Reverse Transcription:

The RNA is first transformed into cDNA in this stage, after which amplification takes place. The entire reaction mixture—which includes DNA polymerase and other amplification components for the two-step RT-PCR and reverse transcriptase, RNase H, dNTPs mixture, primers, nuclease-free water, reverse transcription buffer, and other components for the one-step RT-PCR—is added to a tube and heated to 40 to 50°C for 10 to 30 minutes in a thermocycler. The reverse transcriptase enzyme will create cDNA by adding the free dNTPs once the primer binds to the appropriate location of the RNA sample at this temperature.

3. Amplification:

This stage is comparable to the amplification procedure used in other PCR methods for amplifying DNA. The same reaction mixture is put through an amplification procedure in a one-step RT-PCR. In the two-step RT-PCR, primers, PCR buffer, dNTPs, and other reagents are added to the extracted cDNA in a separate tube along with DNA polymerase. After that, the tube is amplified by placing it in a thermocycler. The amplification process consists of cyclically executing denaturation, annealing, and elongation for a predetermined number of user-programmed cycles.

4. Product Analysis Stage

In order to verify that the intended amplification was accomplished, the reaction mixture that was exposed to PCR is examined in the last step. The majority of applications for gel electrophoresis are in product analysis. This extra step is not necessary with real-time RT-PCR.

1. covid -19 pandemic

- **About covid -19**

The coronavirus family of viruses is responsible for illnesses including the common cold, Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome (SARS) (Amanat & Krammer, 2020). A novel coronavirus illness 2019 (COVID-19) was discovered

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in China in 2019. The COVID-19 was classified as a pandemic disease by the World Health Organization on March 11, 2020. The COVID-19 is spreading quickly over 194 nations in Latin America, Europe, Asia, the Middle East, Africa, and North America. More than 620,000 individuals in the United States have contracted the virus, which has led to 27,000 fatalities as of April 15, 2020. The condition has not yet been lessened by currently available medicationsFever, cough, sore throat, runny nose, and breathing difficulties are the disease's symptoms. For elderly patients, the China International Exchange and Promotive Association for Medical and Health Care (CPAM) advises employing lopinavir and ritonavir capsules' anti-SARS characteristics. Those with COVID-19 who have severe symptoms and the underlying illness should utilize these capsules as an emergency (Boopathi et al., 2020).

- **Virus structure:**

Since coronavirus is one of the viruses that cause the common cold, humans have been infected with it for a very long time. Because it is a contagious viral illness, the main ways to contract it are via coughing, sneezing, or touching an infected surface. Viral droplets can also be inhaled or consumed. The genome of the coronavirus has around 30,000 nucleotides. In addition to a number of non-structural proteins (nsp), it encodes four structural proteins: the nucleocapsid (N) protein, membrane (M) protein, spike (S) protein, and envelope (E) protein (Figure 1). The nuclear capsid, also known as N-protein, is found inside the capsid, the protein shell that surrounds the virus. It is attached to the virus's single positive strand RNA, which enables the virus to infect and multiply in human cells.The viral RNA genome, which the N protein covers, is essential to both transcription and replication. The N protein's N-terminal is responsible for processing viral transcription and replication by binding to genomic and subgenomic RNAs in MHV and IBV virions. One of the key unsolved research issues is the creation of a medication that blocks the interaction between the N-terminal of the N-protein and a single positive RNA strand, which can halt the transcription and replication of viruses. According to Sarma et al. (2020), two significant classes of chemicals, theophylline and pyrimidone medicines, may act as RNA binding inhibitors to the N terminal domain of the coronavirus N protein, providing new opportunities for in vitro validations (Kumar et al., 2021).

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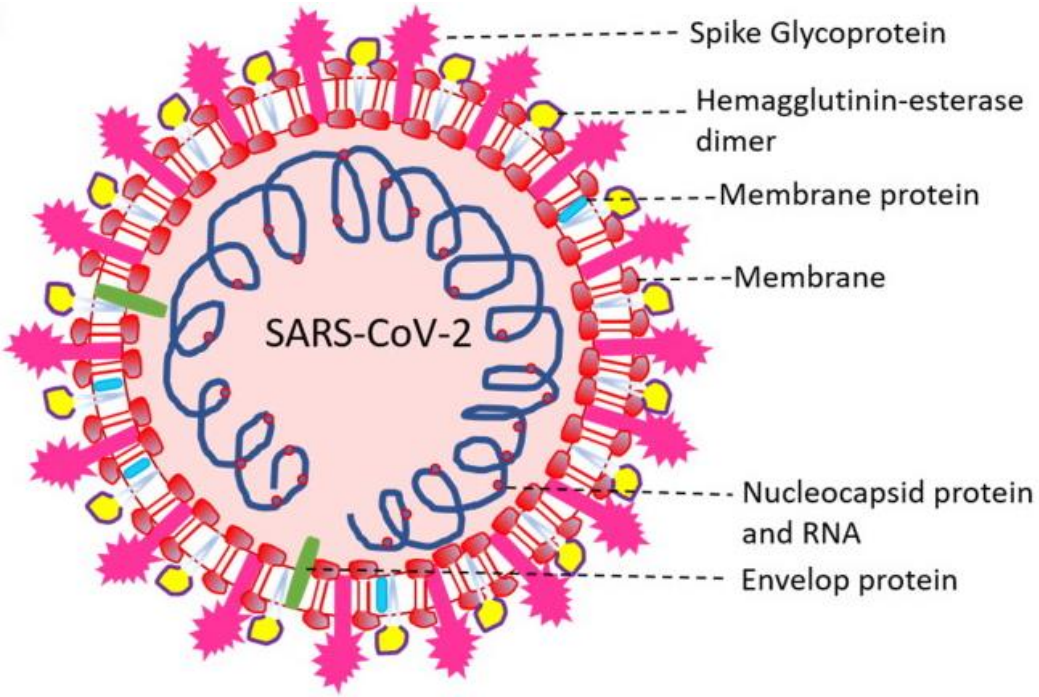

Fig.2 structure of corona virus

- **Use of RT-PCR in detection of corona virus :**

A new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing coronavirus disease 19 (COVID-19) cases to spread quickly. As a result, timely and accurate detection of the virus and/or disease is becoming more and more important in order to control the sources of infection and assist patients in halting the progression of their illness. The use of the nucleic acid test or the clinical features of infected patients as the reference standard to establish a conclusive diagnosis of COVID-19 patients has been fraught with difficulties since December 2019. Clinical features alone cannot diagnose COVID-19 as early detection is essential for pandemic prevention and control, particularly for individuals with early start of symptoms(Dhama et al., 2020).

As medical diagnosis has progressed, nucleic acid detection-based methods have emerged as a dependable and quick method for viral identification. The polymerase chain reaction (PCR) test, which is distinguished by quick detection, high sensitivity, and specificity, is regarded as the "gold standard" among nucleic acid assays for the identification of some viruses. Because of this, real-time reverse transcriptase-PCR (RT-PCR) is a very desirable and straightforward qualitative technique for the detection of SARS-CoV-2. Furthermore, real-time RT-PCR provides sufficient sensitivity to greatly aid in the early diagnosis of infection. Thus, it is possible to see the "criterion-referenced" real-time RT-PCR test as a primary technique for identifying the SARS-CoV-2 causal agent of COVID-19(Tahamtan & Ardebili, 2020).

False-positive and false-negative findings are a significant problem with the real-time RT-PCR test. Numerous "suspected" cases with similar particular computed tomography (CT) pictures and typical clinical features of COVID-19 are said to have remained undiagnosed. Therefore, a negative result does not rule out the potential of COVID-19 infection and should not be the only factor taken into account when making decisions about patient care or therapy. It appears that managing the SARS-CoV-2 epidemic is made easier by the

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combination of real-time RT-PCR and clinical characteristics. A number of issues have been suggested as being connected to real-time RT-PCR's inconsistent results. We aim to address a number of issues pertaining to real-time RT-PCR SARS-CoV-2 detection in the following. It is anticipated that this might offer helpful information for understanding the limitations of the results obtained and to enhance methods for illness diagnosis and management(Teymouri et al., 2021b).

It is commonly recognized that variations in viral RNA sequences can have an impact on real-time RT-PCR results employing primers in various genes. Numerous investigations have noted this new coronavirus's fast development and genetic diversity. Mutations in the SARS-CoV-2 genome's primer and probe target areas may result in false-negative findings. Despite the best efforts to precisely design the real-time RT-PCR assay using the conserved regions of the viral genomes, assay performance may be reduced and false-negative results may result from variability that causes mismatches between the primers and probes and the target sequences. To prevent inaccurate results in this regard, multiple target gene amplification might be employed. Rapid development and approval of many SARS-CoV-2 real-time RT-PCR kit types have resulted in varying degrees of quality. It's important to note that the real-time RT-PCR test's sensitivity and specificity are not 100%. Some of the false-negative findings can be explained by the personnel's proficiency with pertinent technical and safety procedures and the laboratory practice standard(Torretta et al., 2021).

The natural history of the COVID-19 and the viral load kinetics in the patients' various anatomic locations indicate that sampling practices are a major cause of false-negative outcomes. It is yet unknown what sample types and times work best for peak virus loads during SARS-CoV-2 infections. According to a research, sputum and nose swabs are the most reliable samples for laboratory identification of COVID-19; throat swabs are not advised for this diagnosis. Additionally, they recommended that, in situations of severe infection, viral RNAs be found in bronchoalveolar lavage fluid (BALF) for the purpose of diagnosis and virus surveillance. However, collecting BALF is not only uncomfortable for the patients but also requires a suction tool and a skilled operator. While collecting BALF samples is impractical for regular laboratory diagnosis and illness monitoring, other sample types, including as sputum, nose swabs, and throat swabs, may be done quickly, easily, and safely. It would be preferable to utilize multiple specimen types (blood and stool) in addition to respiratory specimens at different phases to prevent conflicting findings. It is important to remember that samples must be collected using dacron or polyester flocked swabs and must arrive at the laboratory as soon as feasible. Amplification inhibitors in the sample or inadequate organisms resulting from improper handling, shipping, or collecting practices might cause false-negative findings(Shenoy, 2021).

Two individuals in Korea have had their SARS-CoV-2 infection characterized, and the viral load dynamics of these cases differ from those of other coronavirus infections that have been previously documented. Days two and three after the beginning of symptoms, respectively, were when the virus was found in upper respiratory tract (URT) and lower respiratory tract (LRT) specimens from the first patient. In the LRT samples, the viral load had risen from day 3 to day 5. However, in both URT and LRT specimens, the virus loads started to decline around day 7. Up to days 13 and 14 (for the LRT and URT specimens, respectively), real-time RT-PCR remained positive at a low level. At last, the test lost its ability to be detected

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for two days in a row starting on day 14 (LRT specimen) and day 15 (URT specimen), respectively. On day 14 of the second patient's symptom start, SARS-CoV-2 was found in both URT and LRT specimens. Nevertheless, compared to patient 1, whose test was conducted on the second day of symptom onset, the initial virus loads were comparatively lower. Real-time RT-PCR was not detected for two days in a row starting on day 18 (URT specimen) and day 20 (LRT specimen), respectively. Day 25's URT sample once more tested positive for the RdRp and E genes. However, because of the high Ct value of the RdRp gene (36.69), it was regarded as negative. These data show that the SARS-coV-2 viral load kinetics vary between individuals, indicating that the moment of sampling and the stage at which the illness develops influence the real-time RT-PCR results(Kim et al., 2020).
In conclusion, a SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel has been developed by the Centers for Disease Control and Prevention (CDC) in an effort to reduce the possibility of false-positive findings. Accordingly, the fluorescence growth curves of the negative template control (NTC) sample should not pass the threshold line and should be negative. Sample contamination is indicated by the development of false positives with one or more of the primer and probe NTC reactions. Crucially, the internal control must be present in order to identify specimens that include materials that could obstruct the PCR amplification and nucleic acid extraction. All clinical laboratories that utilize this test must adhere to the normal requirements for confirmatory testing and reporting, as set out by their appropriate public health authorities, due to the several hazards that might arise for patients in the case of a false-positive result (Anahtar et al., 2021).

Conclusion:

Like other screening techniques, the RT-PCR approach has limitations when used to clinical samples. Based on viral load, there is a limited window of time for high sensitivity. There are fewer false negative results during the first three days of symptoms, but there is also a high test failure rate. Test sensitivity varies between patients and samples. Inadequate management may lead to viral RNA breakdown and test failure. It is advised to repeat the test in order to improve the chances of discovery. The positive rate is increased by repeating on various samples and over time. Sample pooling allows for sensitivity maintenance and repetition reduction. Alternative tests that are easier to perform and more straightforward are being researched.Widespread use of RT-PCR is hampered by the requirement for complex equipment, several processes, skilled personnel, and long wait periods for results. For the exam to be widely used, it must be made simpler, more learnable, and speedier. Improvements have been made to RT-PCR testing through the use of various techniques. A thermal cycler is not required when using the RT-LAMP approach. Reduced viral loads may now be detected with increased sensitivity. Without the need for pricey equipment, methods like Penn-LAMP and DETECTOR provide noticeable effects.

The total sensitivity is increased when immunodiagnostic tests are combined with RT-PCR. Over the duration of infection, RT-PCR and serologic ELISA tests together increase sensitivity. Although there is doubtful association between antibody response and severity, tracking a patient's antibody response might shed light on the course of their illness. For

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diagnosing SARS-CoV-2, the RT-PCR test is still the most effective option overall, although there is room for development to overcome its shortcomings.

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CHAPTER- 22
NEURODEGENERATIVE DISORDERS: ITS CONSEQUENCES, PATHOPHYSIOLOGY AND INTERVENTIONS

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Abstract

Chronic illnesses known as neurodegenerative disorders cause gradual harm and eventual death to many nervous system components, most notably in the brain. Despite being permanent and incurable, many of these illnesses can now be treated because of advancements in medicine. The current focus is on treating the symptoms and, when feasible, delaying the progression of these illnesses, depending on which parts of the brain are impacted, it can subsequently result in a variety of symptoms. These encompass a wide range of illnesses, including Lewy body dementia for e.g; Alzheimer's disease, front temporal dementia, and chronic traumatic encephalopathy (CTE). In Parkinson's disease are characterised by tremors, slowness of movement, balance issues, stumbling steps, and a slumped over posture.In motor neuronal diseases ,these impact the brain and nervous system areas in charge of controlling your muscles. Persons become less able to regulate muscles as those areas' neurons die. Weakness and ultimately paralysis result from that.

Keywords: Neurodegenerative disorders,Parkinson's disease ,Motor Neuron,Tremor,Dementia,Cognitivedisorders,Lewy Bodies

Introduction:

Neurodegerative Disorders ,Characterised by Progressive loss of Neurons of Central Nervous System(CNS),accompanied by FibrillaryAstrocytosis.Etiologically it is associated with Certain kind of Metabolic disorders ,Various Vascular Diseases ,Nutritional Deficiencies as well or Certain Infections can involve to arise degeneration of Nerves.

A Considerable Proportion of Degenerative disorders are genetic to some extentEither dominant or recessive inheritance traits,can be occurs as Sporadically Family History can also induce to this.

This Neural Lesions characterized by bilaterally Symmetric DistributionOr happened by disorders associated with anatomical as well as Physiological system disorders may be Selectively Partially affected ,rest of the Unaffected portions are intact structurally along with functionally.Common Neurodegenerative disorders are **A) Parkinson's Disease B)Alzheimer's Disease**

Some of Common Degenerative Disorders are listed below table:

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Region Affected	Diseases	Main Features	Predominant Pathology
1)Cerebral Cortex	i)Alzheimer's disease	Progressive Senile Dementia	Cortical atrophy,Senile plaques (Neurities),Neurofibrillary tangles,amyloidangiopathy.
	ii)Pick's disease	Pre-Senile Dementia	Lobar cortical atrophy,ballooning degeneration of neurons(pick's Cells)
2)Basal Ganglia And brainstem	i)Hungtington's Disease	Progressive dementia with Choreiform movements	Atrophy of frontal lobes,fibrillaryastrocytosis
	i)Parkinson's Diseases	Abnormalities of posture and Movement	Aggregates of melanin containing nerve cells in brainstem,intracytoplasmic neuronal inclusions(Lewy Bodies)
3)Spinal Cord and Cerebellum	Cerebellar Cortical degeneration Olivoponto Cerebellar atrophy SpinoCerebellar atrophy (Friedreich's ataxia)	Progressive Cerebellar ataxia Cerebellar ataxia Gait ataxia, dysarthria	Loss of Purkinje cells in Cerebellar Cortex Combination of atrophy of Cerebellarcortex,inferiorOlivary nuclei and pontine nuclei Degeneration of Spinocerebellar tracts ,Peripheral Axons and myelin Sheaths
4)Motor neurons(UMN and LMN)	Motor neuron disease (Amyotrophic lateral Sclerosis)	Syndromes of Muscular Weakness and wasting Without Sensory loss	Progressive loss motor neurons,both in the Cerebellar Cortex (UMN),and in the anterior horn of Spinal cord (LMN)(Fig 1)

Table 1; Common Degenerative Disorders

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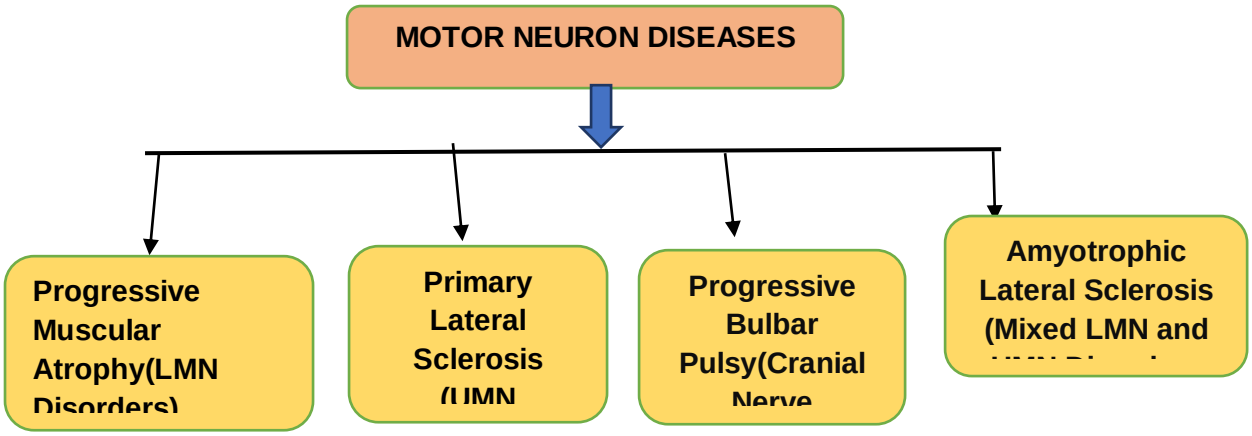

Fig 1: Examples of LMN and UMN disorders

Parkinson's Disease (Dysfunctions Of Basal Ganglia)

Parkinson's Disease results From Degenaration of nigrostriataldopaminergic neurons.

With age,there is loss of Dopaminergic neurons with Dopamine receptor in Basal Ganglia insufficiency leads to Parkinsonism.

When this Processes is accentuated ,ParkinsonismResults in.

It can be forms as Cross Sheets Structure ofAmyloid , Abnormalities appears with Alpha-Synucleininduced mutated expression of SNCA Gene ,inhibits to release Dopamine Neurotransmitter ,which leads to movement disorders,motor disabilities as well as Postural instability of Parkinsonism.

Pathophysiology/Itiology:

1) Idiopathic Causes

A)Cerebrovascular diseases

2) Drugs and toxins

- Antipsychotic Drugs
- Metaclopramide,Prochloperazine
- Tetrabenazine
- Sodium Valproate
- Lithium
- Manganese
- MPTP(Methyl Phenyl Tetrahydropyridine)
- Phenothiazine
- D_2 receptors Blockers
- Clinical Manifestations:

Physical Signs

a) General
   - Expressionless Face (Hypomimia)
   - Flexed(Posture)
   - Impaired Postural Reflexes

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b) Gait

- Slow to start Walking
- Reduction of Arm Swing
- Impaired Balance On turning
- Rapid ,Short stride length,tendency to Shorten

C)Tremor

- Resting (3-4 HZ)
- Asymmetric ,Usually first in arm/hand
- May affect legs,jaw and Chin but not head
- Intermittent ,present at rest,often briefly abolished by movement of limb
- Postural
- Present immediately on stretching out arms

Hypokinetic Movements

1.Akinesia: Akinesia is defined as difficulty in initiating movements and decreased Spontaneous Movements

2.Bradykinesia : Bradykinesia is Slowness of Movement.

Hyperkinetic Movements:

1)Rigidity: Rigidity of Parkinsonism ,arises with UMN (Upper Motor Neuron) Paralysis .In Rigidity , as neuronal discharge increases in both agonists and antagonists .

Sometimes the limbs shows resistance to passive bending throughout the movement(Lead Pipe rigidity).

Sometimes Cogwheel Rigidity will appears with Jerky movements of limbs

However the clasp-knife spasticity (Sudden loss of resistance while moving a rigid limb) of UMN paralysis is never see

2)Tremor: Tremor occurs due to regular alternating contractions of antagonist muscles,Once patient initiates the movement,tremordisappears,known as resting tremor.

Diagnosis:

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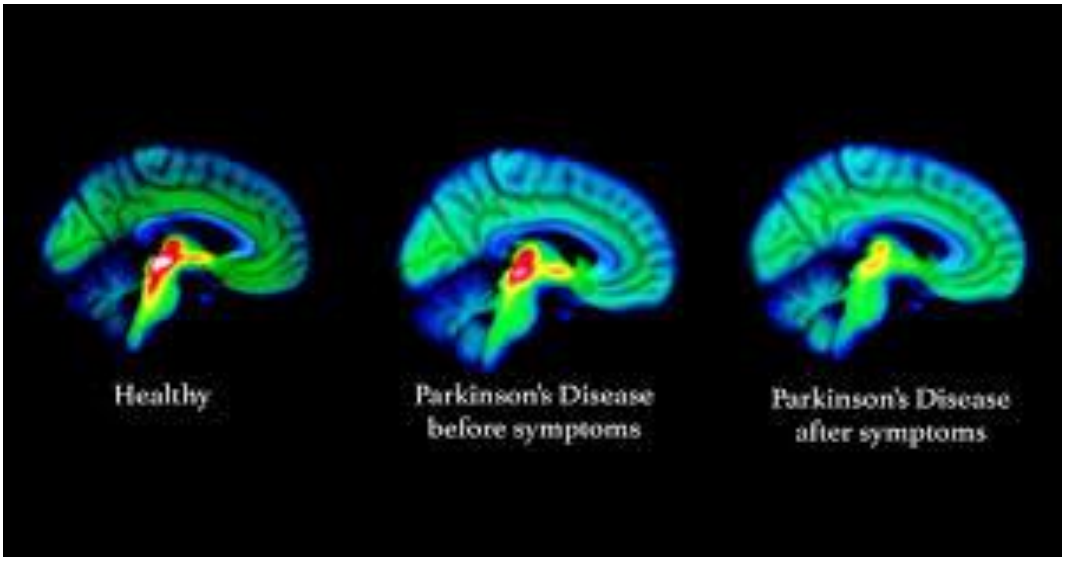
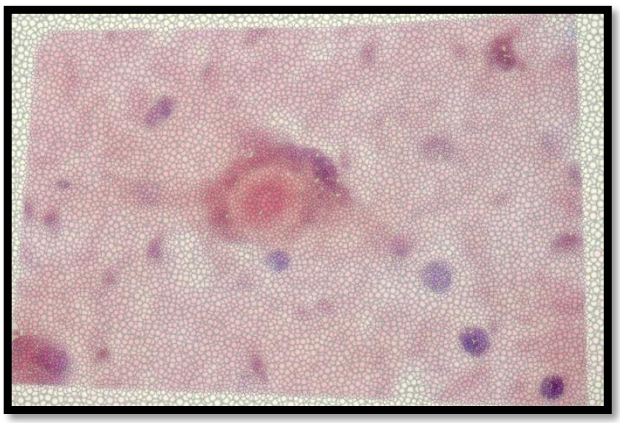


Fig 1.1:(PET Scan Brain Imaging) ;Development of **alpha-synuclein(SNCA)** Aggregates ,Appears as Lewy Bodies Inclusions,Gradually Spreads throughout the brain,affects dopaminergic and non-dopaminergic neuron.

Grossly,the brain is atropic or may be normal externally.

Microscopic Evaluation :Depigmentation of SubstantiaNigra and Locus Ceruleus Occurs due to loss of Neuromelanin Pigment from neurons and Accumulated this Neuromelanin Pigment in the Glial Cells,Some of the residual neurons in this area contain intracytoplasmic ,Eosinophilic ,elongated inclusions called ***Lewy Bodies.(fig* 1.2)**



Fig;1.2 ;Parkinson's Disease (High power(400*) view of Substantianigra of patient with Parkinson's disease Showing Classical Lewy body(Haematoxylin and Eosin stained)

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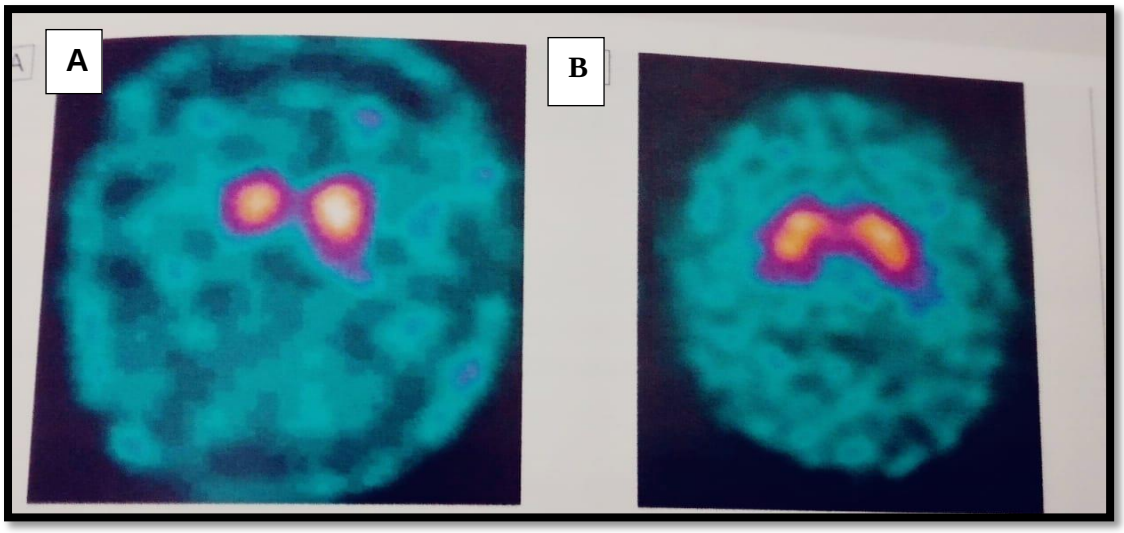

Fig 1.3; Neuroimaging in Parkinson's Disease A)Single Photon Emission Computed Tomography(SPECT)Showing reduced dopamine activity in the basal ganglia B) Normal

Treatment:

1.Replacement of Dopamine: Dopamine cannot cross Blood-Brain Barrier.
L-Dopa ,a Precursor of Dopamine Neurotransmitter ,that easily
Blood Brain Barrier ,Works on Parkinsonism .
Dopamine Agonists like bromocriptine are also used.

2.AntiCholinergics: Due to Dopamine deficiency in the striatum ,the alteration in the ratio of dopamine to acetylcholine plays a important role Thus injection of Anticholinergics ,Decreases acetylcholine Concentration in the basal ganglia ,replenish Acetylcholine dopamine ratio,improves the symptoms.

3.Deprenyl :Deprenyl act as Monoamine Oxidase B inhibitors ,prevents the formation of Methyl-Phenyl-pyridinium(MPP),Methyl-Phenyltetrahydropyridine(MPTP)

4.COMTInhibitors:Catechol-O-methyltransferase (Along with Dopamine decarboxylase)is involved in peripheral breakdown of Levodopa Three inhibitors are available :Entacapone,Opicapone,Tolcapone (inhibits central COMT)

5.Amantidine

6.Transplantation of Adrenal Gland: Transplantation of adrenal medulla from one of the adrenal gland of the patient into his basal ganglia helps to regenerate Dopaminergic Neurons.

Huntington's Disease

This is a genetic defect of autosomal dominal type that Occurs due to defective gene on the Chromosome 4.The gene codes for Huntingtin ,an Abnormal mutated gene causes the Disease.

Itiology:

This disease occurs due to degeneration of Gabaergic neurons associated with Striatonigral Pathway .GABAergic and Cholinergic neurons are lost in the striatum .

Clinical Manifestations:

Disease appears at the age between 30-40 Years and progresses uniformly till death occurs within 10-15 years

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Clinically appears as features of Chorea,Dementia and Slurred Speech .

A)Huntington Chorea: Chorea is defined as rapid involuntary and dancing movements .The chorea is called Huntington's Chorea.It is believed that loss of GABA-ergic neurons in the striatum removes its Inhibitory Influences on the Globus Pallidus,Which reduces the activities in the thalamic nucleusthat results in chorea.

B)Dementia: Dementia Occurs due to Simultaneous and Progressive loss of Cholinergic neurons in the Cerebral Cortex.

Slurring Speech: Gradually ,Speech is Slurred in Huntington disease.

Alzheimer's Diseases:

Alzheimer's is a Progessive disorder charecterized by dementia ,leads to memory loss can be occurs severelysometimes.Thus brain has around 100 billion nerve cells(Neurons) ,which Communicates as networking manner ,generate and supplies energy,construct equipment and get rid of waste .

From recent research findings ,Patients with Alzheimer's disease prevents this actions ,neuronal communications will get disrupted due to cellular abnormalities of neuronal tissues .So cells will loses their ability to do their activities,due to cellular damage or cells are eventually die,causing irreversible changes in the brain.Senile Plaque will forms and intracytoplasmicneurofibrillary tangles will Appears that can destroying memory as well as other important neurological functions.

Clinical Manifestations:

In Alzheimer's disease causes brain to shrink clinically associated with ;

a) Gradual Decline of Memory leads to Dementia
b) Loss of Thinking and reasoning ability
c) Changes of Behaviour
d) Changes of Social Skills.
e) Depression
f) Anger or Aggression
g) Delusions
h) Loss of Cognitive ability

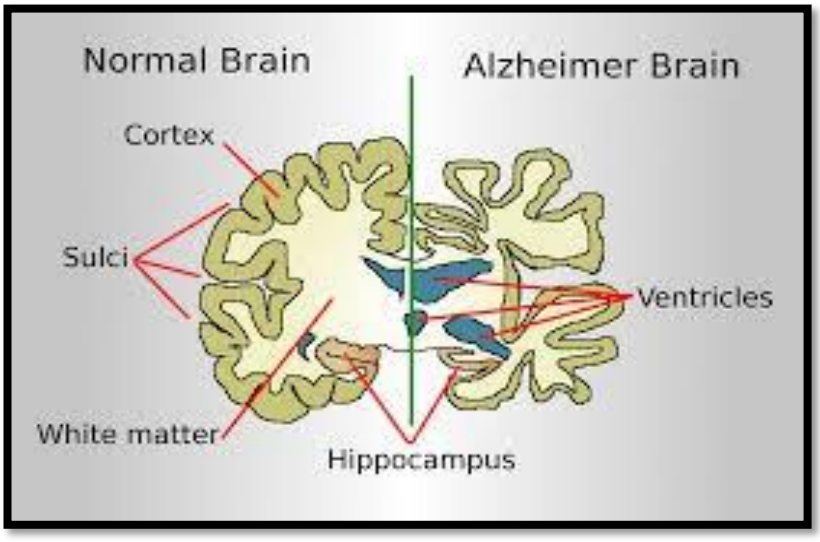

Fig 2.1: Neuronal Disruption of Brain due to Alzheimer's Disease

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Fig 2.2: Healthy Brain Vs Severe Alzheimer's Brain

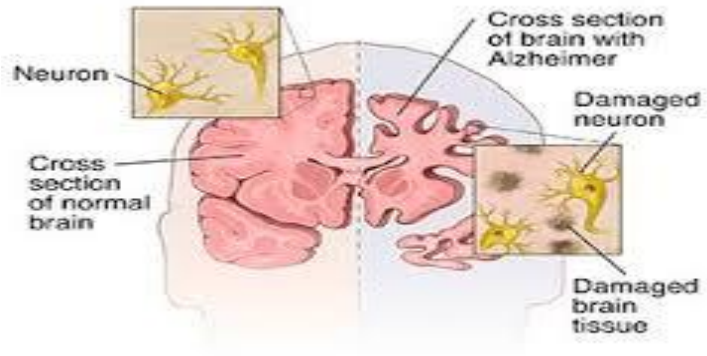

Fig 2.3: Cross Sectional View of damaged neuron with Alzheimer's Disease

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CHAPTER- 23
Mutagenesisand DNARepairMechanisms:TowardAMultitargetApproachCancer

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All cells have multiple repair system: -

During 24-hour period, the genome of a classical mammalian cell piles up lots of almost thousands of lesions. DNA is relatively stable and sturdy molecule, nonetheless in the absence of repair mechanisms, the progressive effect of many infrequent though damaging reactions would make life impossible. So, for the existence of healthy life repair systems are utmost crucial. The diversity and number of DNA repair systems reflects two things: Firstly, the importance of DNA damage. Secondly, the diverse sources of DNA damage. Pyrimidine dimers, common type of lesion can be repaired by various distinct systems. Even a lot of DNA repair processes are seemed to be an extraordinary inefficient energetically – an exception to the pattern which is observed in the vast majority of metabolic pathways; pathways where every ATP is generally accounted for and used optimally. The amount of chemical energy invested for a repair process seems almost immaterial, at the time when the unification of the genetic information is at stake. As DNA consists of two strands DNA repair is possible. DNA lesion that causes distortion in one strand can be removed from one strand and is then accurately replaced by using complementary strand as a template. The principal types of repair systems are methylation and mismatch, base pair excision and nucleotide excision repair.

Methylation And Mismatch Repair: -

To reflect the information in template strand of DNA, mismatches are needed to be corrected. To do so, repair system by some means distinguish between the template and the newly synthesized strand. The cell accomplishesto distinguish newly synthesized strand bytagging the template DNA strand with methyl groups. The strand discrimination mechanism is well understood in *E. coli* and some of the very closely related bacterial species. Strand discrimination is done based on action of enzyme named **Dam methylase** – deoxy- adenine methylase. This enzyme will methylate DNA at N^6 position of all adenine within (5') GATC sequences. Thus, after passageof the replication fork in the course of replication, there is a short few seconds orminutes periods where only template strand is methylated and not the newly synthesized strand (Fig1). This way the newly synthesized strand is unmethylated and can be distinguished from template strand. However, this stageis transient and we get hemi-methylated GATC sequences. In the vicinity of a hemi methylated GATC sequence replication mismatches are repaired as per the information in the template strand i.e., methylatedparent strand (Fig1).

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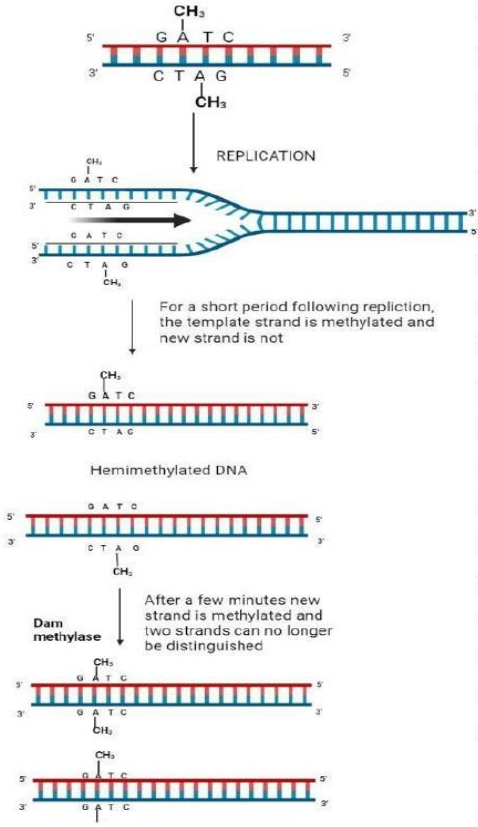

FIG 1: - **Methylation (to distinguish template and newly synthesized strand) andmismatch.**

After replication, methylation is done to the template strand of DNA and not to the newlysynthesized strand. This way both the strands can be differentiated with the help of methylation.

MutS protein recognizes the damage site and binds to that region, further MutL protein will form a complex with MutS protein. Thus, MutS- MutL complex will bind to all mismatched basepair except C-C. Hence, GATC sequence is encountered by MutL – MutS complex.

Simultaneously MutH proteins binds to this complex. Now, the mismatch DNA sequence on both sides is threaded by the MutT-MutS complex which creates a DNA loop (Fig2).

Both the legs of loop simultaneously move through the complex which is exactly like the complex is moving in both directions along the DNA sequence all at once. Until the complex come across a hemimethylated GATC sequence, MutH is in indolent or in inactive form. Mut Hhas endonuclease activity that is site specific and is activated only when complex encounters GATC sequence of hemimethylated DNA sequence. Mut H gets activated and catalyzes the cleavage of unmethylated strand on 5' side of the G in GATC (Fig2). This will mark the strandfor repair.

On the basis of location of mismatch with respect to cleavage site, further steps of pathway occur. From the cleavage site through the mismatch the unmethylated strand is undamaged as well as disintegrated in 3'- 5' direction, at the time when the mismatch ison the 5' side of the cleavage site, further the mismatched portion of the cleavage is restored with new DNA. Enzymes used in this process are DNA helicase II, SSB, Exonuclease I or Exonuclease X, DNA ligase, Polymerase III. Exonuclease II and Exonuclease X both degrade DNA strand in 3' – 5' direction only. Combined action of all these enzymes is required for completion of this process (Fig 3 – left portion). Similarly, when the mismatched is on 3' side of cleavage site, pathway is almost exact, except that exonuclease is either Rec I nuclease, which degrades or devalue single stranded DNA in the 5' – 3' direction, or additionally exonuclease VII is there, which degrades

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ssDNA in 5' –3' or 3' – 5' direction. (Fig3 – right portion).

Protein / Enzyme	Function
➢ MutS (as dimer $MutS_2$)	Recognizes single base mismatches
➢ MutL (as dimer $MutL_2$)	Binds MutS and coordinates repair
➢ MutH	Cleaves hemi methylated DNA
➢ Helicase II	Unwinds DNA
➢ SSB	Binds unwound ssDNA
➢ Exonuclease I	3' → 5' exonuclease
➢ Exonuclease X	3' → 5' exonuclease
➢ Exonuclease VII	5'→ 3' exonuclease
➢ Rec J	5'→ 3' exonuclease
➢ DNA ligase	Seals DNA
➢ Pol III holoenzyme	Fills in gap

Table 1: - Proteins / Enzymes involved in methylation and mismatch repair system. It includes: - MutS, MutL, MutH, Helicase II, Exonuclease I, Exonuclease X, Exonuclease VII, RecJ, DNA ligase, Pol holoenzyme.

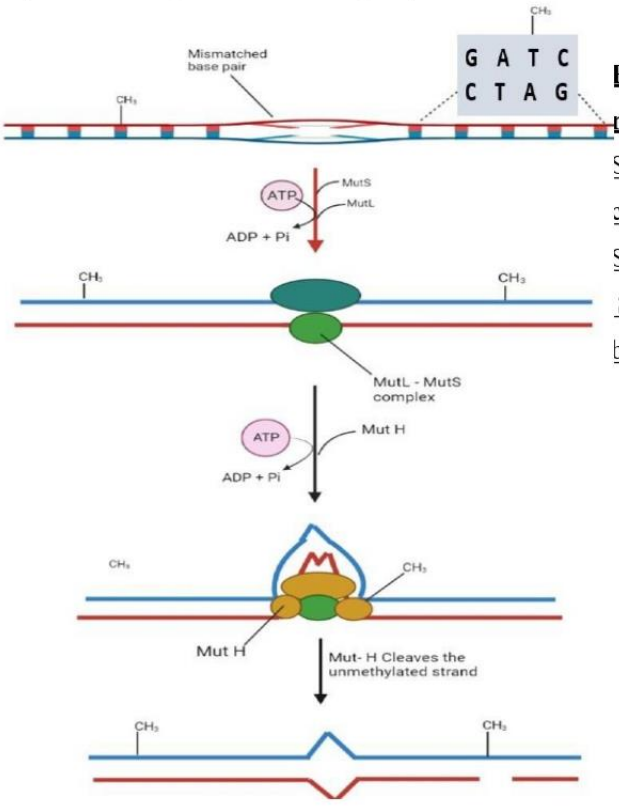

FIG2: - A model depicting early steps of methyl mismatchrepair system.

Step 1; - binding of MutS and MutL complex.

Step 2: - binding of MutH.

Step3: - cleavage of unmethylated strand by MutH.

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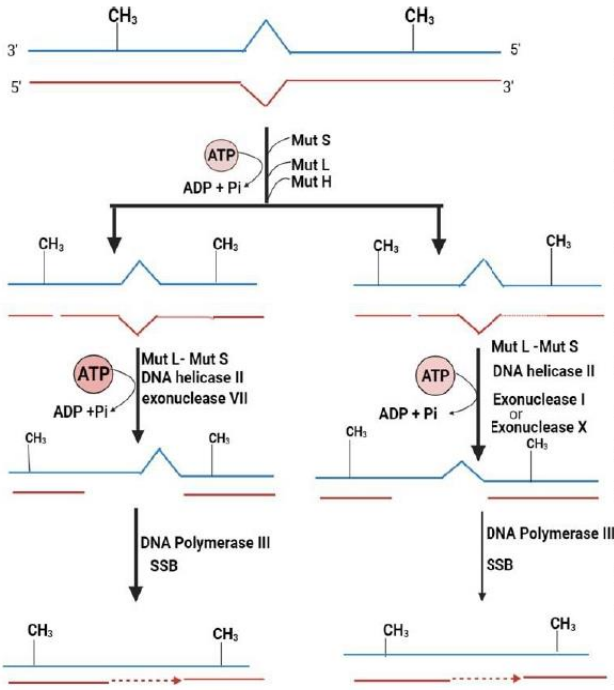

Mismatch excision repair in human cells: -

To determine which strand is normal and which one is mutant DNA strand is the conceptual problem in mismatch excision repair. Exactly how this happens in human cells is no yet known with certainty, but is assumed that the proteins that binds to mismatch segment of DNA distinguish the daughter and template strand. Thus, the one with the replication error, that is the mis-paired of DNA strand is excised and simultaneously repaired to produce an exact complementary strand of template strand. Mismatch excision repair occur after DNA replication.Thus, the errors made during replication are corrected by mismatch excision repair.

FIG3: - Finalized methyl- directed mismatch repair. The combined action of DNA helicase II and SSB, also one of the four different exonucleases detaches a segment between a point just beyond mismatch and the MutH cleavage site. DNA polymerase fills the gap andDNA ligase will seal the nick.

MSH2 / MSH6 proteins are similar to bacterial Mut S homologs 1 and 6. MSH2 and MSH6 together forms a complex that binds to a mismatch segment of DNA in such a way that itdistinguishes between the newly synthesized daughter strand and template strand (Fig4). This binding of MSH2 and MSH6 complex triggers the binding of MLH1 and PMS2 (Table 2). MLH1 and PMS2 both are homologous to bacterial MutL. The resulting DNA – protein complexbinds to and hold together with exonuclease. DNA exonuclease will cut the newly synthesized daughter strand, then comes DNA helicase which will unwinds the helix and exonuclease removes several nucleotides from the cut end of the daughter strand including mismatch base. Gap filling is done by DNA polymerase (Pol δ) and then the strand is sealed by DNA ligase (Fig4).

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Protein/ enzyme	Function
MSH2 / MSH6	Repairs single base mismatches, small loops
MSH2 / MSH3	Repairs larger loops; functions with MSH2 / MSH6
MLH1 / PMS2	Functions with MSH2 / MSH6 and MSH2 / MSH3
DNA endonuclease	Cuts the newly synthesized daughter strand
DNA helicase	Unwinds DNA strands
DNA polymerase (Pol δ)	Fill in gaps
DNA ligase	Seals DNA

Table 2: - Proteins and enzymes used in mismatch repair system in case of humans. It includes MSH2/MSH6, MSH2/MSH3, MLH1/PMS2, DNA endonuclease, DNA helicase, DNA polymerase, DNA ligase.

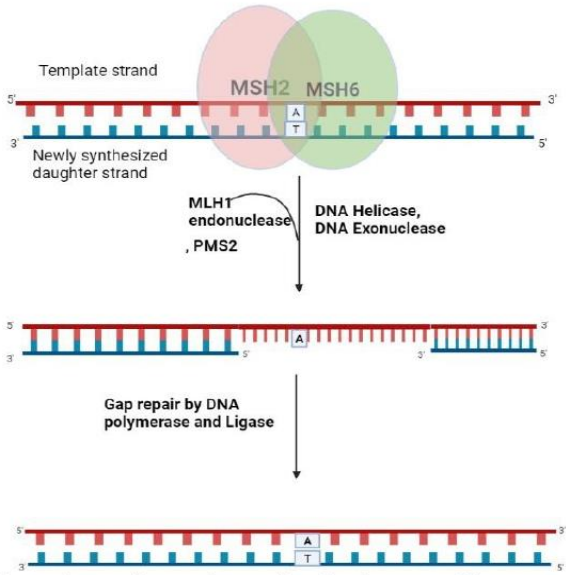

Fig4: - Steps depicting mismatch repair mechanismin case of humans. Step 1: MSH2 and MSH6 together forms a complex that binds to a mismatch segment of DNA. Step 2: DNA exonuclease will cut the newly synthesized daughter strand, then comes DNA helicase which will unwinds the helix. Step3: Gap filling is done by DNA polymerase (Pol δ) and then the strand is sealed by DNA ligase.

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Base Excision Repair: -

DNA glycosylates are the class of enzymes present in every cell. Common DNA lesions such as products of adenine as well as cytosine deamination can be identified by glycosylates. DNA glycosylates are specific to particular lesions. After recognition of DNA wound, they discard the affected base by splitting the N-glycosyl bond. Due to this cleavage, an apurinic or apyrimidinic site in DNA is formed and is generally referred to as an AP site or abasic site (Fig5).

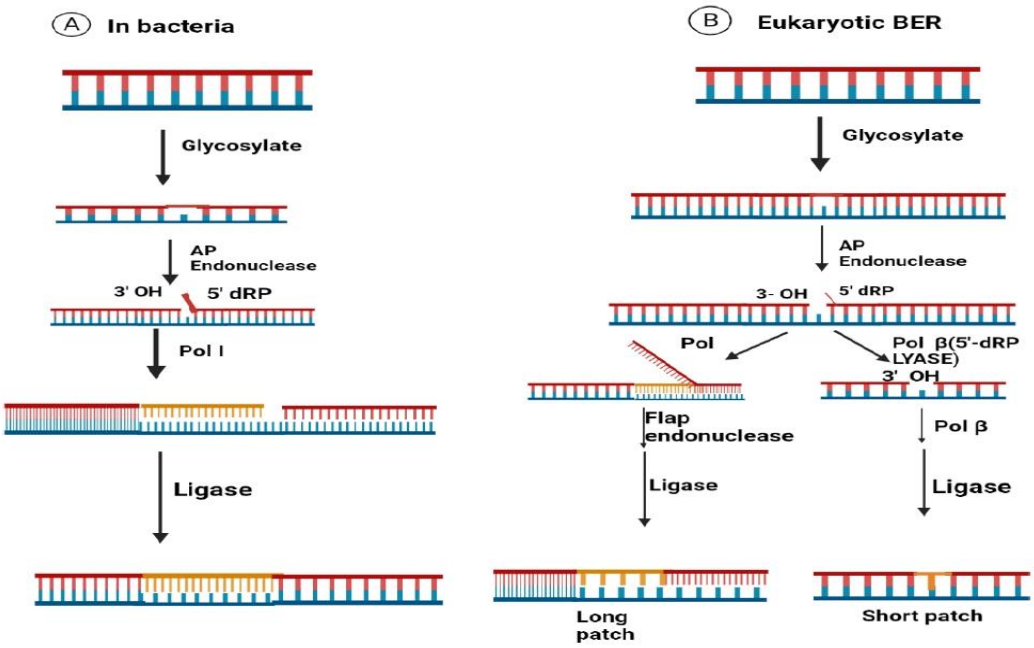

FIG5: -Base Excision repair mechanism (on left side- E. coli and on right side- human). Cleavage between base and deoxyribose by DNA glycosylate. AP endonuclease cleaves phosphodiester bond, DNA polymerase will start the repair synthesis, further DNA polymerase will fill the gap and strand is sealed by DNA ligase.

Slow spontaneous hydrolysis of *N*- glycosyl bonds in DNA creates AP sites (an apurinic or an apyrimidinic site) also referred as abasic site. As soon as AP site is formed by DNA glycosylate another type of enzyme should repair it. Repair is not simply made by inserting a new base and reforming the *N*- glycosyl bonds (Fig 5). Instead, the deoxyribose5' phosphate leftbehind is first removed and then replaced with a new nucleotide. To begin this process, we will need AP endonucleases enzymes, that will cut the DNA strands containing AP site. So, the segment of DNA containing AP endonucleases is removed. Thereafter, DNA polymerase I and DNA ligase play their role. DNA polymerase will replace DNA and ligase will seal the remaining nicks (Fig 5)

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Nucleotide excision repair in *E. coli*: -

DNA lesions causing huge amount of distortion in helical structure of DNA are casually repaired by the repair mechanism named nucleotide excision repair. For the survival of all free- living organisms this pathway is very critical. In nucleotide excision repair mechanism, two phosphodiester bonds are hydrolyzed by multi-subunit enzyme (excinuclease). Excinuclease willhydrolyze one on each side of the falsification caused by the damage. Excinuclease enzyme system will hydrolyzes the eighth phosphodiester bond on the 5' side, in case of *E. coli* and otherbacterial species, and fifth phosphodiester bond on the 3' side of the DNA strand where deformation has occurred. This will create a fragment of around 27 – 29 nucleotides (Fig6).

Succeeding the duplex incision, the excised oligonucleotides are allowed to leave from the duplex. The resulting gap is filled by DNA polymerase ε in case of humans and in case of*E.coli* it is filled with the help of DNA polymerase I. Afterwards, DNA ligase will seal thenicks.

The key enzymatic complex of *E. coli* is the ABC excinuclease. It is named as excinuclease because it narrates the inimitable capacity of this enzyme to catalyze two distinct endonucleolytic cleavages. This quality distinguishes excinuclease from other standard endonucleases. ABC excinuclease consists of three subunits, UvrA (*Mr* 104,00), UvrB (*Mr* 78,000), UvrC (*Mr* 68,000). A complex of UvrA (*Mr* 104,00) plus UvrB (*Mr* 78,000) (A_2B) scans the DNA and will bind to the damage site (Fig6). Simultaneously, UvrA dimer will dissociates, leaving UvrB on the lesion site. Though this UvrB- DNA complex is quite tightly bounded. UvrC protein comes and binds along with UvrB. UvrB will make incision on the 3' side of the lesion and particularly at the fifth phosphodiester bond. This is then succeeded by theincision formed by the UvrC, particularly at the eighth phosphodiester bond on the 5' side of thelesion (Fig6). This is how excinulcease will hydrolyze one on each side of the damage caused bythe lesion. The derived nucleotide of 12 – 13 fragments is detached with the help of UvrD helicase. Then, DNA polymerase I and DNA ligase will fill the short gap. For damages like 6-4 photoproducts, cyclobutane pyrimidine dimers, and for various other types of bases adducts inclusive of benzo[α]pyrene-guanine, which is generated in DNA by exposure to cigarette smoke; for all such lesions nucleotide excision pathway is a primary sole route. In the sense that two cuts are made in the DNA, the nucleotide activity is novel.

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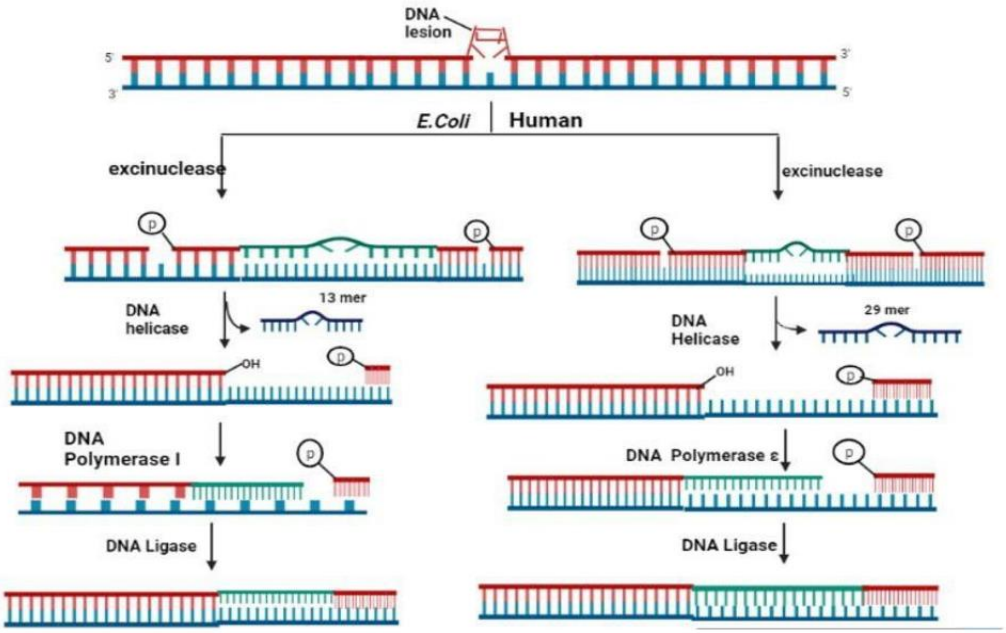

FIG6: - Nucleotide Excision Repair Mechanism in E. coli. Step1: - binding of an excinuclease and cleavage of damage DNA strand on each side of lesion. Step2: - 13 nucleotide (13 mer) and 29 nucleotide (29 mer) are removed with the help of DNA helicase. Step3: - Gap filling is done by DNA polymerase (Pol δ) and then the strand is sealed by DNA ligase.

About this Book :

This book is the compilation of esteemed chapter of acknowledged experts in the fields of basic and applied Life Science. This book is published in the hopes of sharing the excitement found in the study of Life Science. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

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