EXPLORE

DEPARTMENTAL JOURNAL

DEPARTMENT OF BOTANY

SHRI SHIKSHAYATAN COLLEGE

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PREFACE

This edition of EXPLORE, is the 1st online version of the departmental journal of Botany. 2020 has been a year of turmoil, distress and uncertainty. The global pandemic shuddered the world and left all awestruck. Amidst chaos new rays of hope are unfolding and people all over are fighting with a strong mindset and hard work to restore normal life. Our students who represent youth of today have ventured their creative mind into different areas of biological science as young researchers. They have represented this effort in form of experimental science articles which has been included in this edition. This journal has been categorized into three chapters: the first includes articles by current students of Botany Department; the second science articles by alumni of the department and third include articles by Faculty members.

Some articles been peer reviewed by eminent Academic personalities.

On behalf of the Department of Botany, I wholeheartedly thank our College Management for their consent and cooperation for this online version publication. We sincerely thank our College Principal, Dr. Aditi Dey for extending her support, encouragement and good wishes.

We take this opportunity to thank all connected with this effort and look forward to continue this academic journey in future.

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CONTENT

CHAPTER 1

This section includes science articles by the current students of Botany Department. The content of each article reflects their innovative mind and interest areas in applied plant science. Any update or enhancement is welcome from the readers.

Article 1

Bees Battling a Novel Pandemic

Shramona Chaudhuri[1], Shourjya Ghosh[1] and Rupsha Dasgupta[1]

SEMESTER 3 BOTANY (HONOURS)

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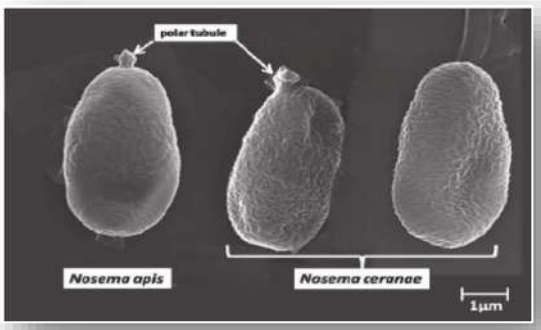
Introduction

Around the world, domesticated and native bee populations have been declining in the recent past due to colony collapse disorder. Investigations on the European honey bee, *Apis mellifera*, into the core of this issue revealed evidence of a pandemic of bees, culminating due to the spread of a novel species of the fungal genus *Nosema*, different strains of which are causing perennial nosemosis infections in new regions where previously the bees were seasonally unsusceptible.

***Apis mellifera* worker**

Source:
https://en.wikipedia.org/wiki/Western_honey_bee



***N, ceranae* and *N. apis* spores; they are almost indistinguishable from each other, rendering the diagnosis a difficult feat**

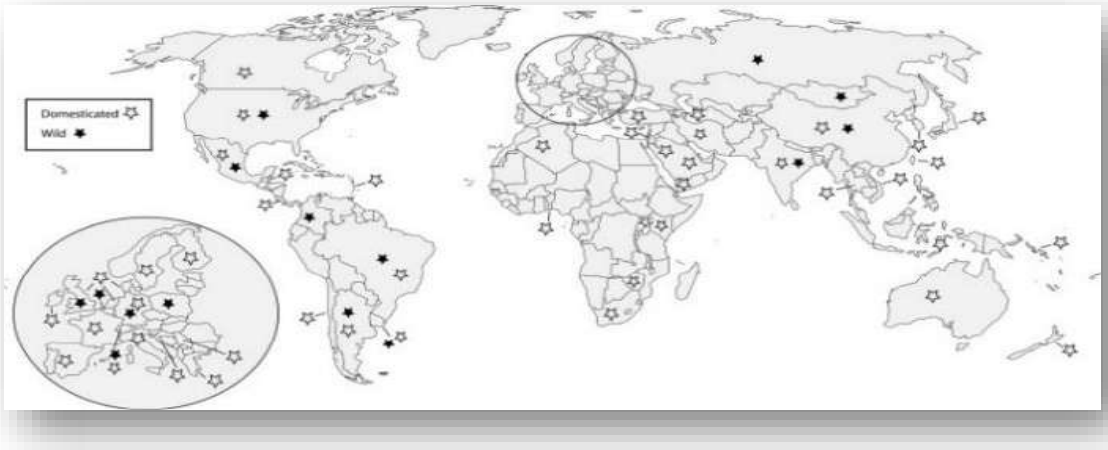
Source:
https://www.researchgate.net/figure/Nosema-apis-and-N-ceranae-spores-observed-under-SEM-Arrows-indicate-the-start-of_fig1_233808987

This novel form of the disease causes a severe even fatal form of Bee Dysentery leading to progressive depopulation of the hive but is exceptionally difficult to detect due to lack of noticeable symptoms. While it's been chronicled across Europe, Canada and Kenya, it has almost exclusively been recorded in the European honeybee, the chief commercial pollinator in these

regions as honeybees are not native to these regions. Scientists fear for the future of bees, commercial or native, if the propagation of the disease goes unchecked.

The novel coronavirus pandemic is sweeping across the world and human beings are struggling with all that it entails. But recent researches suggests that we are not the only ones facing a pandemic; honeybees are facing similar challenges. In the recent past, population of bees, both domesticated and native, are declining due to colony collapse. Recent studies revealed that one of the major contributing factors behind this, especially for the European honeybee *Apis mellifera*, might be the nosemosis pandemic which is growing and is the result of the spread of a novel species of the fungal pathogen in the genus *Nosema*.

The infectious spores of *Nosema ceranae* belonging to Class Microsporidia, first isolated



Worldwide distribution of *Nosema* species infecting bee

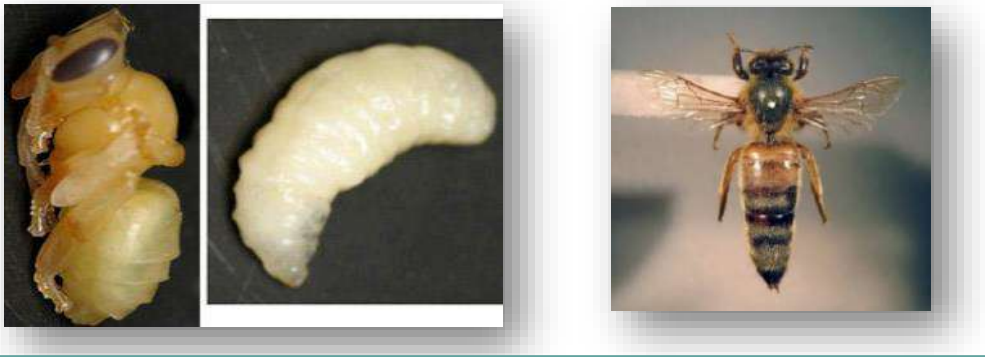
Source: https://doi.org/10.1371/journal.ppat.1008580.g001

in 1996 by Fries in Southeast Asian honeybee species *Apis cerana* is the main troublemaker. Only in 2006 was it first found in *Apis mellifera* by Higes. *N. apis*, another species included in this genus, was the dominant strain causing dysentery in the European Honeybees up until now but only during winter and spring. Historically, these species were found in distinct geographical locations: *N. apis* in Europe and North America and *N. ceranae* in South East Asia. (4)

Of the total number of *Nosema* infections, evidence of a significant increase in *N. ceranae* nosemosis to has been found to the extent that, in *A. mellifera*, *N. ceranae* has now replaced *N. apis* as the prime *Nosema* pathogen. Although both species induce two different forms of the same disease, the temperature-resistant spores of *N. ceranae* and its perennial

infection cycles cause a swifter depopulation of the *A. mellifera* hives. Generally, the worker bees are the main casualties as the larvae and queen do not contract the disease. (1)(7)(8)

Pathogenesis: The infection occurs on oral ingestion of the spores. These enter the cells of the mid-gut, where new spores are formed within 48-60 hours, destroying the cells and causing severe diarrhoea which can be fatal. These spores can infect other cells in the bee's body while passing



Larval stage and queen of *A. mellifera*; these two generally remain uninfected in case the hive suffers from *Nosema* infection

Source: http://entnemdept.ufl.edu/creatures/misc/BEES/euro_honey_bee.htm

through the digestive tract, sickening the bee and contaminating flowers, pollen,

and hives along the way. Other bees are at risk of infection via faecal–oral transmission. If excreted at a floral resource, the fungus can spread to new habitats and hosts that are exposed to contact with that flower due to the bee foraging coverage ranges. (1)

Symptoms*: N. ceranae* nosemosis produces distinct clinical signs from the classical nosemosis, such as: (2)(6)

- severe injuries on the body of the bee
- the absence of diarrhoea as a conventional symptom

- the absence of dead bees at the bottom of the hive. Evidently, they go to die away from the hive, causing a progressive depopulation of the colonies that it is difficult to detect leading to total loss of the family.
- perennial Infection cycles

Diagnosis and Cures: Unfortunately, the prognosis for *N. ceranae* nosemosis is usually serious because its onset almost always goes unnoticed due to the absence of diarrhoea and dead bees in the hive. The only obvious symptom is depopulation that occurs at an advanced stage and without laboratory inspection, the disease is nearly impossible to determine. (1)(2)

Previously, Fumagillin had been used against nosemosis, but due its carcinogenic properties, toxicity to both humans and bees and uncertainty in its efficacy against *N. ceranae*, many countries have banned its use in agriculture. In recent times, molecular, phytotherapeutic, and supplement-based scientific advances are being conducted to control the disease. Chemical alternatives include a combination of aqueous extracts of *Artemisia dubia* and *Aster scaber* that prevents *N. ceranae* spore proliferation. (1)(3)(12)

There has been some success in breeding for *Nosema*-resistant lines of honeybees. This prospect is disputed by experts who view a genetically engineered bee as a direct threat to smaller, struggling bee species. These resistant species would not only dominate and potentially wipe out conventional bee strains but could alter the gene pool of traditional strains like *Apis mellifera*. Such genetically modified species may even introduce new allergens in humans. (1)(11)

Pollinator Pathways: The US Fish & Wildlife Service working alongside private and public landowners are attempting to create **pollinator pathways**, i.e., pesticide-free corridors of native plants that ensure nutrition and shelter for pollinators and help them find new habitats as a solution to climate change. Pollinator pathways help restore and diversify natural wildflower-rich habitats. Construction of these pathways requires coordination and forethought, but come with ample

benefits the prime one being the boost in the pollinator population, their foraging behavior and services. (13)

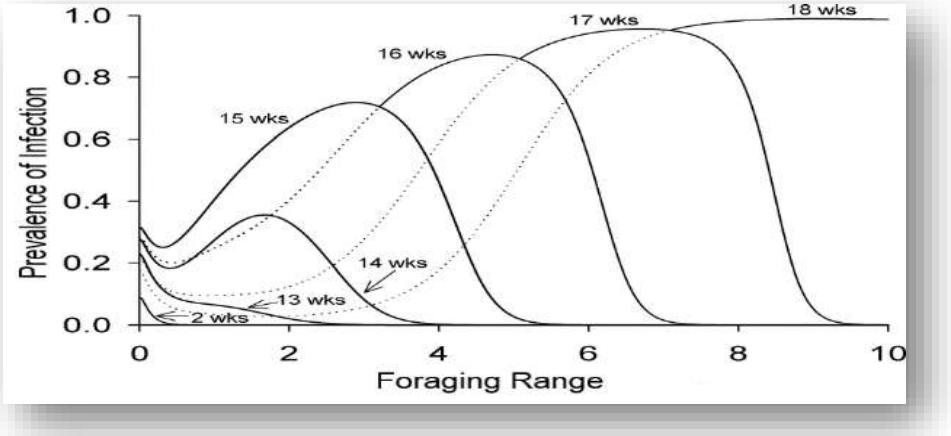
The Compounding Issue: Native bees comprise the majority of the roughly 20,000 bee species on the planet. Of the 800 wild bee species are found in Europe, International Union for Conservation of Nature (IUCN) has listed 7 as critically endangered, 46 endangered, 24 vulnerable and 101 near threatened. While extinction of all the native species is quite unlikely, a deterioration in their numbers would still impact pollination around the globe heavily, potentially wiping out plant species including commercial crops. Diseases and foraging ecology are intricately connected in multiple ways and they can impair or alter foraging behavior, thereby affecting pollination services. (5)(10)(14)

Pathogen Spill-over: An infected bee, on visiting a floral resource and defecating there can contaminate it and leading to pathogen spill-over which is defined as the relay of diseases from domesticated organism to their wild counterparts living in the vicinity. Any bee that subsequently visits the flower, native or domesticated, is susceptible to infection and further

spread of the infection throughout its foraging range. Imbalance in pathogen-prey balance due to a hike in pathogen burden can severely reduce pollinator efficacy. Moreover, pathogen spill-over can accelerate the extinction of smaller populations vulnerable to the novel pathogen, reverse spill-over back to the original populace and evolution of novel strains. (1)(9)

Wild bumblebees, for example, are susceptible to contract *Nosema ceranae*

infections with higher virulence from honey bees. (14)



Predicted long-term dynamics of pathogen spillover into nearby wild bee populations
Source:
https://www.researchgate.net/publication/51422333_Does_Pathogen_Spillover_from_Commercially_Reared_Bumble_Bees_Threaten_Wild_Pollinators

Effect of Agrochemicals: Ever since the inception of chemical insecticide industry, it had been palpable that these are perilous for bees as they are, in fact, insects and, therefore, vulnerable to pesticides designed to kill insect pests. Despite all regulations put in place to prevent any harm to bees, *Apis mellifera* colonies in USA declined drastically in numbers between 1947-2010 due to DDT usage in agriculture. Prolonged usage of herbicides can reduce diversity of flowering plants and hence, adversely affect bee species in the vicinity. Clothianidin, a pesticide proven to be 10,000 times more potent than DDT, is responsible for mass poisoning of bees in Germany. It disrupts the bees' super-acute sense of direction and feeding habits and induces sterility in the males and the queen. Combinations of certain fungicides and insecticides used in agriculture and an indiscriminate administration of acaricides in apiaries are fatal to honeybees with many experts holding pesticides as much responsible for colony collapse disorder as any pest or disease. (11)(16)

Status of Bees (honeybees and native) in India: Status of *Nosema* infections in Indian bees at present is minor. As of 2004, *A. mellifera* apiculture has been largely free of any major predators- *Tropilaelaps clareae* mite and wax moth were the only pests of the bees. But, the 2004 *Varroa* epidemic of the Korean haplotype of the ectoparasitic mite paved the way for

radical changes in the beekeeping industry in terms of production cost and process, degree of monitoring and handling and disease management. (15)

Documentation of the prevalence and distribution of *Nosema* species in bees of commercial interest has been extensively done due of their economic importance, but hardly any information exists for the same in native bees. Even though, as of now, the pandemic has been restricted to European honeybees of the native variety, there is no saying that it will not spread all over the globe. The loss incurred if these critical pollinators are wiped out will be heavy; diminished pollination can have immense impact on plant community fitness and the ecosystem on the whole. The delicate balance of nature will be sent teetering over the edge.

This research is based on secondary data gathered through various sources, like, Plos Pathogen journal and the Food and Agriculture Organization of the United Nations website. This article is a review of the study conducted by a research team headed by Arthur Grupe II, a postdoctoral researcher in the Department of Ecology and Evolutionary Biology, University of Colorado, Boulder. We researched the information through journals, magazines and other websites as well to ensure the comprehensiveness and effective analysis of the topic.

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Article 2

ECO-FRIENDLY TEXTILES

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ABSTRACT

The evolution of clothing from its fibre stage to fabric requires a lot of processes which are harmful to our environment. So it is very important to make textile industry more sustainable. The textile industry has been condemned as being one of the most chemically intensive industries on earth, and the no.1 polluter of clean water for using as many as 2,000 different chemicals in the textile industry from dyes to transfer agents. For these kinds of ecological hazards, nowadays the use of eco-friendly textile material is increasing significantly. Which types of textile fibres are very friendly with environment and no hazardous impact on ecology those are called **Eco Textiles**. Sustainable fashion, also called eco fashion or green fashion, is a part of the growing design philosophy and trend of sustainability. So, there is a need to produce the textile materials which are eco-friendly through using different processes like enzyme technology, plasma technology, super critical carbon dioxide dyeing or foam technology etc. This review presents an overview of the textile industry highlighting eco-friendly fibres, techniques and innovations that are developed to make textile industry more sustainable.

Keywords: Eco-friendly Fibres, Sustainability, Textiles, Green fashion.

INTRODUCTION

Sustainable apparel can be seen as a contradictory concept because the apparel industry, which heavily reliant on the concept of fashion, is guided by constant change and replacement of old styles with new ones (Farley and Hill, 2015). This creates novel challenges to apparel designers and product developers; however it has not stopped them from adopting and looking for sustainable and environmentally responsible practices. The textile industry is a major contributor to environmental pollution and depletion of resources due to two main reasons:

1. **Contamination of wastewater** by chemically intensive processes like dyeing, finishing, slashing etc. Textile wastes are a major contributor to landfills and most scrap materials are not biodegradable because of the synthetic components present in them.

2. **Cotton is not an eco-friendly crop**. This is because it requires very large amounts of water and pesticides during its production. The production of 1 kg of cotton fibre can require more than 20,000 litres of water. The chemical fertilizers and pesticides are also a major pollutant of fresh water sources.

Although two thirds of the earth's surface is covered with water, almost 97.4% is salty sea water, 2.05% is frozen water and 0.65% is fresh water which is suitable for human, animal and plant consumption. Out of this fresh water, only 0.3% is renewable (Krenkel and Novotny, 1980). Considering the fact that cotton cultivation is responsible for destruction of ecosystems on a global scale, it is necessary to find alternative fibres which are more eco friendly and sustainable in nature. The main characteristics for such a fibre should be reduced water requirement during production and processing, and low dependence on fertilizer and pesticides.

DISCUSSION

Eco-friendly, products ensure safety from all dangerous chemicals, and allows families to avoid risky additives that can cause any of these issues. Using eco- friendly products improves quality of life in terms of mortality, age, diseases, and illnesses. They ensure the safety of families and the planet.

Bamboo has emerged as an ultimate green material (Netravali, 2005). It is a renewable and sustainable raw material and does not adversely affect the environment. It is one of the fastest growing plants and can also be used as animal feed. **Bamboo viscose fibre** is regenerated from bamboo plant which requires no irrigation and can be grown in natural conditions with no need for pesticides. **Organic Crop Improvement Association** has certified bamboo viscose fibre as an organic fibre and it can degrade under the influence of microorganisms and sunlight (Rana et al., 2014). Bamboo fibre is breathable, cool, soft and has high moisture absorption capacity. It also possesses anti-bacterial and anti-UV properties. The cross section of bamboo fibre contains microgaps and microholes which contributes to enhanced moisture absorption and ventilation (Erdumlu and Ozipek, 2008; Filiz, 2011; Majumdar et al., 2011;

Prakash et al., 2013). This unique structure also aids to quick evaporation of moisture and provides an unfavourable environment for bacteria (Chen et al., 2007; Filiz 2011; Majumdar et al., 2010).

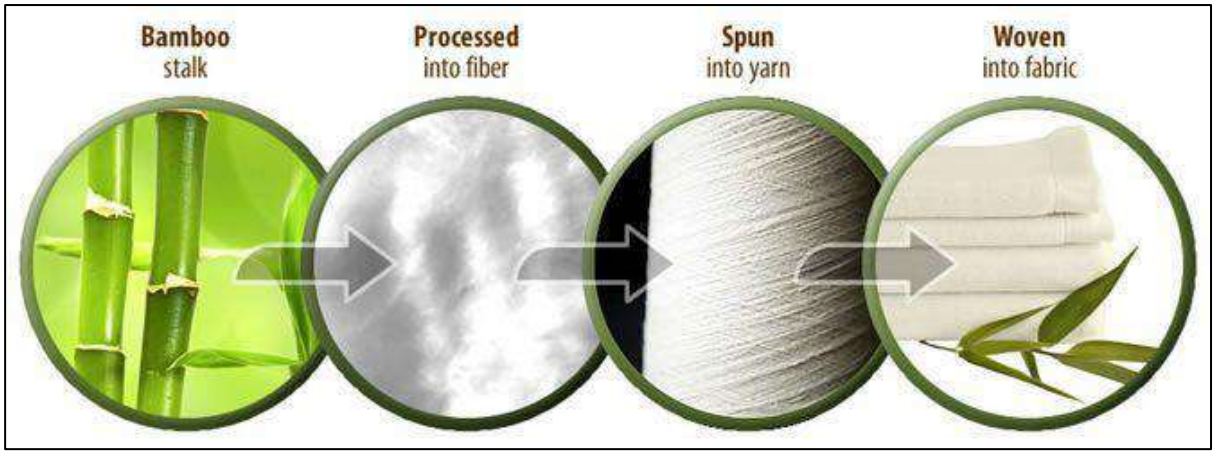

Figure 1: Bamboo viscose fibres are used in the production of apparels, sanitary hygiene products, geotextiles, composites and filtration fabrics.

Bacterial cellulose is a noteworthy alternate natural fibre source. It is also referred to as bacterial nanocellulose and microbial cellulose. Bacterial cellulose (BC) is a natural, non toxic, biocompatible, and stable hydrogel withunique properties produced by several species of bacteria (Gama et al., 2013), the most important of which is *Gluconacetobacter xylinus*. Bacterial cellulose has a three-dimensional network free from plant components like hemicelluloses, pectin and lignin, and thus has unique mechanical properties and also does not require additional processing to get rid of the above mentioned impurities.

Among various BC forms, one particular type can be very easily and effectively synthesized from **Kombucha** (Zhu et al., 2014). Kombucha is a popular health drink, a lightly acidic beverage derived from the fermentation of sweetened tea using a **symbiotic colony of bacteria and yeast (SCOBY).** During the fermentation process, a translucent, gel-like

cellulose membrane is produced at the air/liquid interface of the fermenting tea (Zhu et al., 2014). This flat cellulose membrane when removed and dried resembles a leather like material which can be used as a sustainable raw material for appar

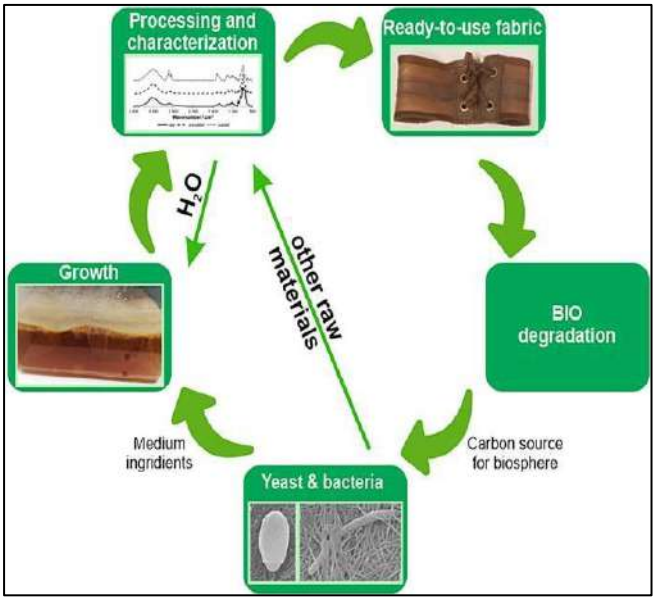

Figure 2: Hydrogel bacterial cellulose: a path to improved materials for new eco-friendly textiles.

Hemp, like bamboo, is considered a sustainable crop. It requires little water to grow, and it is resistant to most pests and diseases. The hemp plant's broad leaves shade out weeds and other plant competitors, and its deep taproot system allows it to draw moisture deep in the soil. Unlike cotton, many parts of the hemp plant have a use. Hemp seeds are processed into oil or food. Hemp fibre comes in two types: **primary and secondary bast fibres**. Hemp fibres are durable and are considered strong enough for construction uses. Compared to cotton fibre, hemp fibre is approximately 8 times the tensile strength and 4 times the durability.

Hemp fibres are traditionally coarse and have been historically used for ropes rather than for clothing. However, modern technology and breeding practices have made hemp fibre more pliable, softer, and finer.

Fig 3 : Hemp fibre

Milk Fibre was firstly introduced in 1930 in Italy & America to compete the wool. It is the new innovative fibre. Cyarn milk protein fibre dewaters and skim milk and manufactures the protein spinning fluid suitable for wet spinning process by means of new bio-engineering techniques. In April 2004, it passed Oeko-Tex Standard 100 green certification for the international ecological textiles. It can be used to create top-grade innerwear , shirts, T shirts, loungewear, etc. It is also healthy for skin, comfortable, with bright colours due to good dyeability. It contains seventeen amino acids & natural anti-bacterial rate is above eighty percent. Hence milk fibre has sanitary functions.

Cactus and agave silk

Textile fibres obtained from various kinds of the Agave species can be used in making vegetable silk, mats, as fillers, and even can be used as bio-composites, in substitute to glass fibres. The cactus silk, also known as **organic or vegan silk**, Agave silk or the more local name **Sabra silk** is a cruelty free fabric unlike mulberry silk. This silk is made in Morocco using vegetable dyes and the cacti used are found in the desert of Sahara. The cactus hails from the **Aloe Vera species of the Agave family**. The fibres have high strength, are durable and aesthetically pleasing. Sabra silk is hand loomed in Morocco and hence is an expensive product. The manufacturing process of this vegetable silk remains traditional and ages old.

Weber tequilana more widely recognized by the name of **Blue Agave** is essentially used to make tequila and a natural sweetener. The waste from manufacturing tequila is known as **bagazo** and it is used to make textile fibres, and is also used as animal feed, biofuel, and paper. This plant is found in the semi-arid regions of **Jalisco state of Mexico**.

Agave fibres are rigid and coarse and hence need to be blended with a softer yarn for application in textile fabrics and garments. Usually a blend is created of cotton yarn and 10-15% agave fibre.

The Agave plant has an age span of seventy years. The plant needs little water and external inputs, and can be grown in other semi-arid regions. Different kinds of Agave plants are used to obtain fibres like **Sisal, Henequen, and Tampico** which are used to make products like ropes, mats, and brushes respectively.

Sisal fibres are an eco-friendly substitute to glass-fibres to be used in furniture, automobiles, boats, water pipes, and tanks. The fibre can be employed in geo- textiles. The major manufacturers of sisal are Brazil, Tanzania, and Kenya. China is a large producer as well as consumer of the fibre. Sisal is hundred percent bio-degradable

Fig 4: Blue Agave silk plant

LivaEco – a sustainable textile fibre by the Aditya Birla group in India

Liva is a leading ingredient consumer brand of the Aditya Birla Group. In 2019, the brand unveiled a new sustainable, eco enhanced variant of the Liva fabric. It is a sustainable, natural based **viscose fibre** which is soft to touch and also provides fluidity to the garment. The natural viscose is made from wood fibres sourced from sustainably managed forests.

BENEFITS

The manufacturing process of these fabrics is a **closed loop process**, which means it is more environmentally friendly.

- **Low water consumption** – the process of manufacturing the LivaEco fabric saves up to 900 litres of water compared to other natural fibres.
- **No land and water pollution** – pesticides and chemical fertilizers are not used in the raw material, hence there won't be any surface run off of harmful chemicals which are a major source of land and water pollution.
- **Low greenhouse gas emissions** – up to 300 gm less emissions as compared to other fibres.
- **Very fast biodegradability** – degrades within 6 weeks in soil, water, marine or compost.

- **Traceability of source** – a molecular tracer helps in tracing the garments to the source at any stage. This can be done by simply scanning the QR code on swing tag of the garment.

CONCLUSION

Textile industry plays a vital role in the Indian economy. It constitutes nearly 30 per cent of India's exports. Even though many fabrics are available we can bring out new innovations in the field of eco-textiles which protect our environment from further depletion. It's right time for Indian Dyestuff manufacturers to adopt environmentally safe processes and products to minimize/eliminate the adverse impact of industrial pollution on the environment and human health. It's time to give Top Priority to Environment,

Health and Safety issues. "Fashion is not only about clothes, it is about all kind of **change." So let us go green and make fashion more eco-friendly which will make the environment to keep green and clean**.

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Article 3

THE EFFECTS OF CLIMATE CHANGE ON THE GENETIC EVOLUTION OF PLANTS

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ABSTRACT –

Rapid climate change is a unique opportunity to study the genetic basis of evolution in all organism, especially plants. Several impacts have been noticed on both macro and micro levels of plants. Genetic mutations that are favourable for survival under stressful conditions are being selected for at a large scale. Studying the effects to climate change on the evolution of plants might hold the key to preserving the genetic diversity of plants.

KEYWORDS – Climate Change, Genetic Adaptation, Evolution, Phenotypic Plasticity, Plant Physiology, Genetic Diversity.

INTRODUCTION –

Human induced climate change is the biggest problem of the 21st century. The whole planet is facing a mass extinction event due to extreme stress and changes in temperature. The effect of such an enormous event is observed in every living organism, both at external and molecular levels.

Examination of plants, one of the most resilient organisms in history, might be insightful as to how evolution has been affected by rapid climate change. Plants have been affected in both positive and negative ways, genetically and in terms of evolution. The coping mechanisms seen in plants might help scientists come up with ways to preserve their genetic diversity.

EVOLUTIONARY IMPACTS OF CLIMATE CHANGE –

Climate change has both direct and indirect influences over abiotic and biotic processes. It represents a very powerful source of selection pressures for adaptive evolution. Climate change can impact several facets of evolution such as patterns of hybridization, population size, gene flow, migration patterns and flowering times.

In order to have evolutionary impact on organisms or populations, climate change must directly or indirectly influence a population's genetic constitution.

Direct Genetic Effects –

Direction or strength of natural selection or an organism's traits can get affected by the changes in the abiotic and biotic environmental conditions. However, most of the adaptive shifts involve quantitative traits.

Indirect Genetic Effects –

By changing the effective population size and patterns of gene flow, climatic conditions can influence a population's genetic constitution. In changing climatic conditions, for populations to stay adapted in a given area the mechanism for it is provided by gen flow through migration. Climate change is leading to new contact zones between related species, with hybridization now being documented in several cases. (Muhlfeld et al., 2014; Taylor et al., 2014)

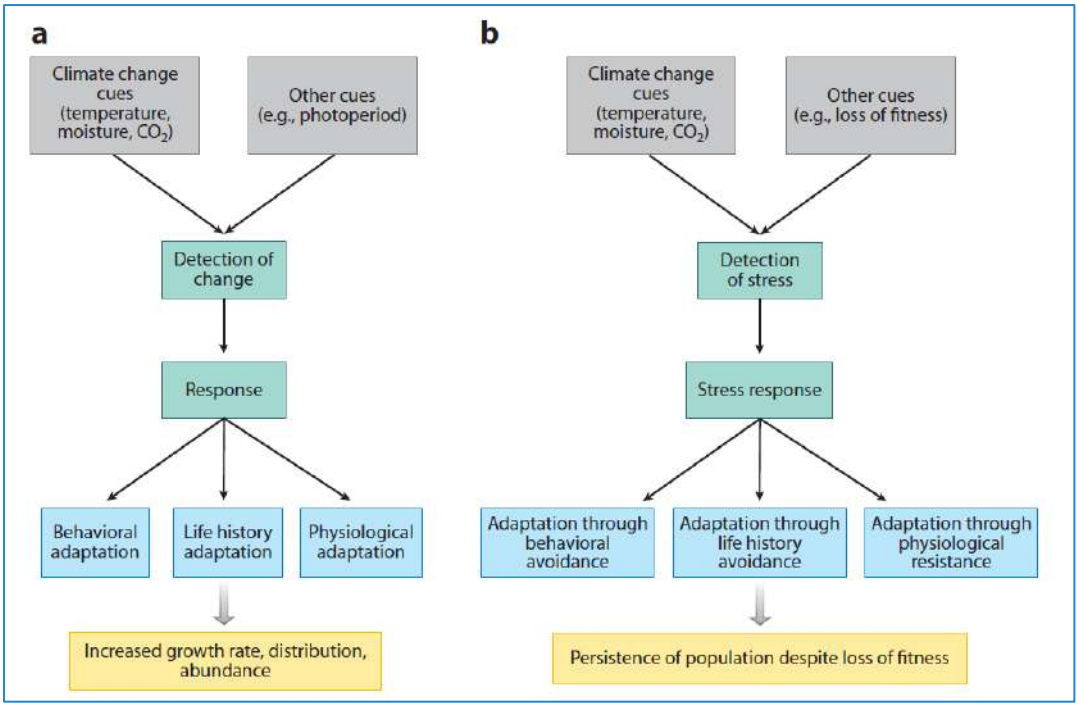

Figure: Direct and Indirect reasons of genetic adaptation (Franks & Hoffmann, 2012)

Phenotypic Plasticity –

Phenotypic plasticity can be defined as an organism's ability to change its phenotype in response to environmental pressures. When faced by changing climates, populations may adjust their phenology, morphology or physiology to adapt and maximize their reproductive capabilities.

Features of plasticity –

Three conceptual features of plasticity are important to properly evaluate the significance of plasticity for evolution (Dubnau and Losick 2006; de Jong et al. 2011; Ackermann 2015).

1. The phenotypic variation of plastic traits can be continuous or discrete, the discrete variation resulting in alternative phenotypes.
2. Phenotypically plastic traits can either be adaptive or nonadaptive

3. The threshold response of plasticity might be regulated in a conditional or a stochastic manner.

Role of plasticity –

Three predictions have to be fulfilled to support the role of plasticity as facilitator or evolutionary novelty and diversity (Merilä & Hoffmann, 2016)

1. Novelty of adaptive evolution depends on plasticity.
2. Development of new alternate forms of genes and the molecular basis for climate change adaptation is provided by plasticity.
3. Assimilation of new forms of genes into populations also occurs due to plasticity.

PHYSIOLOGICAL RESPONSES AND GENETIC CHANGES IN PLANTS –

Climate change has many negative effects on the plants. Climate change changes the pattern of growth of the plants, its physiological activities like photosynthesis, respiration, etc. and genetic structures and functions all in a negative way.

Effects of Climate Change on Plant Physiology –

Plant physiology is the physical, chemical and biological functions of a plant to survive. For example – photosynthesis, respiration, circadian rhythm, plant hormone regulation, dormancy, germination etc. Climate change affects these processes very badly.

1. Effects on Photosynthesis –

Global warming increases the level of CO_2 in the atmosphere which amps up the photosynthesis. Plants would require less water to photosynthesize. Researchers thought that it would result in increased availability of freshwater, but later it was proved completely wrong.

A CO_2 rich atmosphere increases the temperature and plants in warmer temperature get more growth season. Plants will grow more and the number of leaves on them will be more than average. That means when it will rain the bigger plants will absorb more water than normal. But, an increase in CO2 in the atmosphere will result in less opening of plant stomata, checking transpiration. Hence it will result in shortage of water or drying of soil.

2. Effects on Respiration –

Plants respire in the absence of light. Climate change increases the average temperature of the atmosphere. At higher temperature the cellular respiration of plants increases by many folds. In respiration plants giveaway almost 50% of the CO_2 assimilated by photosynthesis during daytime. So increased cellular respiration will increase the release of CO_2 in the atmosphere.

3. Effects on Circadian Rhythm –

Circadian rhythms are observable biological oscillations that occur with a 24-hour periodicity within all organisms. They are based on an endogenous transcriptional clock, which is reinforced by environmental cues such as variations in light, temperature and wind.

Circadian clock is affected by external conditions like high temperature and high level of CO_2 which changes the behaviour of the plant. The interaction of the plants with the environment changes. For example, the heat tolerance, plant pollinator interaction changes.

4. Effects on Plant Dormancy –

Dormancy was evaluated in seeds buried in field soils. Under a global warming scenario, dormancy relief and seedling emergence declined and seed mortality increased as soil temperature increased along a thermal gradient. Seedling emergence advanced with soil temperature, peaking 8 days earlier under 2080 conditions (Footitt *et al*, 2018).

Seeds need much water to germinate. They imbibe water. But, due to global warming and rise in average temperature the seeds are not getting adequate moisture, as a result of which they lose their dormancy.

5. Effect of Climate Change on Plant Pollinator Interaction –

High concentration CO_2 in the atmosphere changes the photosynthesis and respiration pattern which results in abnormal sugar production and degrades the quality of the nectar. Also due to less water the size of the blooming flower reduces. These create disturbances in the smooth plant pollination interaction.

Effects of Climate Change on Plant Genetics –

Climate change has many negative effects on the genotype and phenotype of plants. Climate change affects the genetic variation or genetic diversity of plants, it's plasticity, its gene flow etc.

1. Plants Losing the Wild Variant –

The wild type of a species or organism refers to the phenotype of the typical form of a species as it occurs in nature. Due to the changes in climate like high temperature, high CO2, loss in soil moisture, the plants are migrating to new habitats with new genetic characters. Hence their wild variety is getting lost.

2. Phenotypic Plasticity –

Phenotypic plasticity is the ability of an individual organism to alter its physiology/morphology in response to changes in environmental conditions (Carl D. Schlichting, 1986). Climate change is changing the availability of resources to plants like mineral nutrition, soil fertility etc. which are resulting in the phenotypic plasticity of the plants. It changes the overall phenotypic behaviour of the plants.

3. Adaptive Evolution –

Adaptive evolution is the phenomenon of increase in frequency of beneficial alleles and decrease in deleterious alleles due to selection. For adaptive evolution to happen, plants should have a good genetic diversity so that extinction does not happen. But climate change is making plants lose their genetic diversity which is resulting in the extinction of many less diverse plants.

4. Gene Flow –

In genetics, gene flow (also known as gene migration or allele flow) is the transfer of genetic material from one population to another. Two populations can be considered to be a single effective population if they have the same allelic frequency which can be achieved by a high gene flow.

Due to climate change, the gene flow from the habitant species to the migratory species can benefit the later one as the adapting traits will be in favour of their survival in the migrated area. But this can also hinder the evolution of the migratory species by maladaptation of alleles as they were already lagging behind in their adaptive responses to climate change.

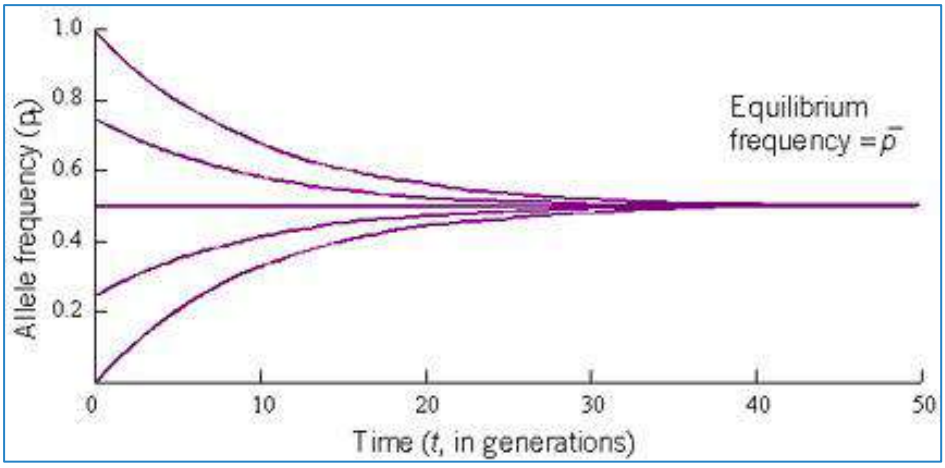

Figure: Gene Flow observed in two populations (Source – American Pathology Society)

GENETIC BASIS OF CLIMATE CHANGE ADAPTATION –

Under normal environmental conditions, evolution occurs by selection of favourable and heritable traits over long periods of time. But in recent times, there have been examples of adaptive evolution occurring due to climate changes in traits like body size, thermal responses, reproductive timing and spore dispersal. These observations bring up the possibility of climate change causing heritable epigenetic changes, which might serve as an alternative and more rapid mode of evolution. This new approach might prevent a mass extinction event and preserve genetic biodiversity.

The following section is a conceptual model of the mechanism of genetic changes seen due to climate change adaptation.

Empirical examples –

Two well studied examples of genetic changes due to climate change adaptation are – sea beet and field mustard. In sea beet (*Beta vulgaris* ssp. *maritima*), flowering times changed heritably due to shortening time of vernalization caused by global warming. In the field mustard *Brassica rapa*, another well-studied case of climate-driven evolution, there was no evidence for evolutionary change in environmental responsiveness, despite available genetic variation (Kelly, 2019). There drought-driven adaptation was noted which resulted in the reduction of mean flowering time.

Approaches and Techniques –

The tools for analysing of genetic adaptation under the stressful conditions of climate change are limited. The following table shows a few experimental approaches to understanding the changes in observed populations –

Table 1: Methods for isolating genes involved in adaptive genetic responses to climate change, their potential advantages, and their limitations (Franks & Hoffmann, 2012)

Source Material	Description	Advantage	Limitations
Methods based mostly on comparisons of natural populations			
Clinal comparison	Comparisons of populations from along clines	Possible to collect data immediately, takes advantage of past evolution and local adaptation	Not always possible to determine if patterns reflect climate change or other factors
Natural evolution	Comparisons based on changes in natural populations across time	The only source material in which populations have actually adapted in contemporary time to changes in climate	Often difficult to find natural examples of rapid evolution
Genome scan of populations	Population comparisons involving a large number of markers to determine which markers are more	Relatively easy to apply, can use a number of different types of markers, allows a direct comparison between effects of selection	Differentiated marker may be distant from marker under selection depending on linkage disequilibrium

	differentiated than expected from neutrality	and population processes	
DNA sequence comparison of natural populations	DNA hybridization on microarrays or other techniques to identify highly diverged areas of the genome, often involving cline ends	Provides an unbiased assessment of parts of the genome involved in adaptive divergence	Can be difficult to link to adaptive phenotypic variation; differences may also be related to historical processes
Methods based mostly on comparisons of manipulated populations			
Artificial selection and experimental evolution	Traits related to climate change adaptation selected directly (artificial selections), or populations are placed in environments simulating climate change effects (experimental evolution)	Traits can be accurately defined, selection pressures set, and crosses and sequence comparisons used to assess the genetic basis of selection reflecting natural environments; can assess potential rates of adaptation	Limited by base population for selection, may not reflect intermittent selection and trade-offs, depends on species with short generation time

Quantitative trait locus mapping (QTL mapping)	Strains are made genetically homozygous and compared for quantitative traits	The same set of strains can be scored for multiple traits, including correlated responses, sequenced strains can be used for a variety of purposes, covers whole genome	Strains are typically inbred to make them homozygous before sequencing, which means that mapping may partly relate to effects of inbreeding depression; adaptive changes may have a different genetic basis, particularly if evolution acts as a filtering mechanism

Identifying relevant genes and Regulation –

In plants and animals across the world, adaptive changes have appeared in the form of point mutations. If the mutations are advantageous for the organism to survive under the stresses of climate change, they are spread across a population. If a point mutation is found in a sufficiently large fraction of a population, it is called SNP (single nucleotide polymorphism).

SNPs produced in response to the stresses of climate change can be isolated and classified as candidate genes, to be studied. Such SNPs have appeared in several plant populations

belonging to several climates. In boreal black spruce (*Picea mariana*), 26 SNPs of 25 genes were found and in *Arabidopsis thaliana*, a large number of SNPs were found across diverse climates. The broad variety of the SNPs and the fitness of the environment the plants grew in showed a strong correlation – the mutations are selected for in environments that are under the stresses of climate change.

Epigenetics –

Epigenetics refers to heritable changes to the phenotype of an organism which are not a direct result of genetic variation. As the environment can influence gene expression, climate change adaptations can cause epigenetic changes. Epigenetic mechanisms may lead to increase resistance to a wide variety of stresses, including thermal extremes. Mobilization of transposable elements due to stressful conditions may lead to widespread genetic alternations. Although epigenetic changes have the potential to cause very impactful variations, they are unpredictable and cannot be depended upon for a solution to loss of genetic diversity.

Case study of *Arabidopsis thaliana* –

One of the most significant changes seen due to climate change adaptation in plants is the changing in flowering time. Reproduction is a critical stage in the life cycle of a plant. Plant species have been naturally selected and evolved to flower at the most favourable environmental conditions. As the length of the seasons are changing, many plants are missing environmental cues and failing to produce viable offspring.

Arabidopsis thaliana has adapted to the changes in climate as shown by QTL mapping of its flowering time genes. In *Arabidopsis thaliana*, the flowering time gene network consists of more than 60 genes, which are regulated by four pathways: photoperiod, autonomous, vernalization, and gibberellin. These four pathways integrate extrinsic and intrinsic signals to promote flowering at appropriate times during the growing season (Anderson *et al*.,2012).

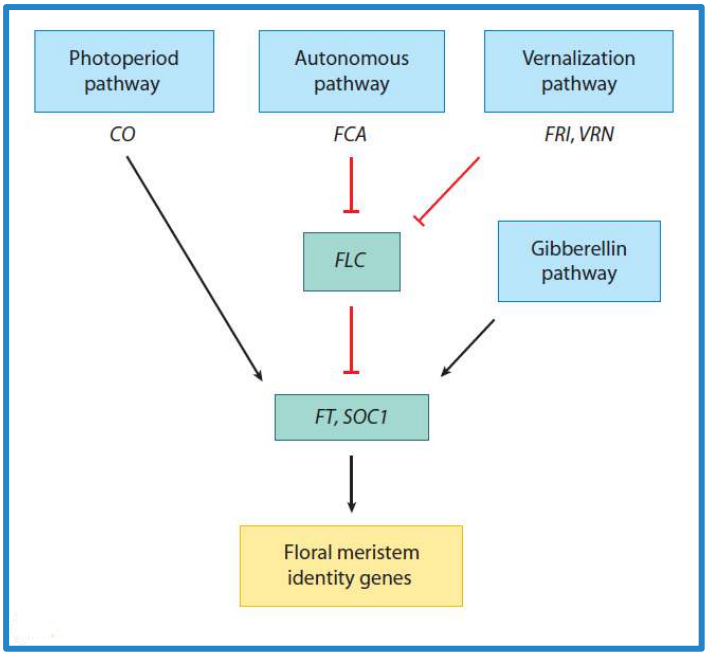

Figure: Genetic regulatory network of flowering time in *Arabidopsis thaliana* (Franks & Hoffmann, 2012)

Many studies done with *Arabidopsis* have shown a correlation between flowering times, variations in the flowering time genes and climate. Loss-of-function mutations and mutations related to water efficiency have been noted. Orthologs of *Arabidopsis* flowering time genes are also present in many monocotyledonous and dicotyledonous species and it signals the transition to reproductive phase.

The changes seen in the flowering time gene locus of *Arabidopsis* are heritable, under selection by environmental factors. Flowering time in several plants containing the orthologs of the genes have the potential to evolve following severe climate change.

GENETIC DIVERSITY AND CLIMATE CHANGE –

Climate change, i.e., global warming not only affects a species' external affairs like their interactions with the environment, events of biological clock but also effect their genetic diversity. Many species show response towards climate change by changing their local

adaptations or by changing their distribution limits along the altitudinal or latitudinal gradients (Range shift).

It is quite important for a species to maintain its genetic diversity to survive both in long term and short term. It is quite important to keep the genetic diversity of a species intact. Genetic variety of wild plant species are used by breeders to imbue inherent disease and pest resistance in cultivated varieties.

When studying genetic diversity, a group of Norwegian scientists studied wild diversity in 9581 samples from 1200 populations of 27 species for assessing the results of range reduction and potential association species traits. We can discuss the variation of loss in genetic diversity by the means of dispersal adaptations and genetic differentiation among populations. The loss of genetic diversity varied among species. A study showed that due to long distance dispersal some herbs were lacking adaptations were estimated to lose genetic diversity than dwarf shrubs who were adapted to long distance dispersal.

A 2016 study published by Andrés J. Cortes showed that globally some of the largest impacts of climate change are expected to occur in alpine environments which are dominated by plants with longer life span. In the alpine region snow cover and summer temperatures are the major drivers of composition of vegetation. The survival species led to patterns of upward migration for the last decades due to increasing climate temperature. Strong elevation gradients in temperature and local topography characterized the heterogeneous alpine region. Species that occur in heterogeneous habitats such as small-scale variations can have implications for their response to climate change.

Another study from scientists of plant research International shows that wild animals and plants are not able to keep up with climate change. Due to their moving from their natural habitat for too much temperature their genetic variation is staying behind in their original habitat, that

means most of the genetic variation are staying in the southern habitats and the species of northern habitats are lacking variations.

Another study showed that two distantly related trees interior spruce and lodgepole pine use the same set of their 47 Gene to deal with temperature precipitation and other climate variables. This way they migrate slowly over generations.

A long-term research on genetic variation in Cottonwood trees stated that the genotype of a tree affects the 700 species of insects that depend upon the plant i.e. effect the microbes, bacteria, lichens and bird's that feed on them. Drastic changes in genetic diversity i.e., genotype disappear could change ecological community in unexpected ways.

CONCLUSION –

There are varied responses to climate change seen in plants of different climatic ranges. All the responses provide vital insight into the future of plant evolution. Isolated candidate genes and certain species of plants can help in further research. It is unlikely that rapid evolution will be seen in plants as a result of global climate change. Nevertheless, the changes observed are enough to conclude that preserving the genetic diversity of plants is not impossible with enough research, conservation and other scientific endeavours.

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Article 4

Orchid World – A Shunned Beauty

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- **Abstract**

The Plant Kingdom has a number of mysteries and puzzles yet to be solved within it, the major part of the exploration of the kingdom is left, where one of the most intriguing mysteries have been related to the vast family, Orchidaceae. Comprising of more than 25,000 species, Orchidaceae is the biggest family in the Kingdom. Due to the striking appearances and spectacular beauty, many orchids have gone extinct, mostly endangered due to illegal poaching and a high level of economic value. Yet there have been numerous studies on the species revealing many different secrets and elucidating their bizarre ways of surviving amidst all the chaos. They are the first indicators of any Ecosystem disturbance, since they only grow in fresh ecosystems, and are mostly confined to limited geographical areas in the wild, hence the rarity. Truly they are the best examples of Mysterious Beauties of the Plant Kingdom.

Keywords: orchids; vast family; ways of surviving; fresh ecosystem; rarity

Introduction:

In a letter to Jospeh Hooker, Charles Darwin, had written,“I never was more interested in any subject in my life, than in this of Orchids.” (David L. Roberts, Kingsley W. Dixon)

Orchidaceae, world's largest plant family, comprising of 25,000 known orchid species represents about 10% of the World's flowering plants (D.L. Roberts, K.W. Dixon) making it one of the "most species-rich plant families".

Their floral diversity and pollination biology has been an island of curiosity for the evolutionary biologists since a very long time. There are numerous secrets being cradled in the Orchid World yet to be discovered by humans. Being rare in occurrence and mostly out of sight in the urban world. orchids usually grow in fresh ecosystems in the wild and majority being limited to specific geographical areas [K.W.Dixon, S.P.Kell, R.L.Barrett and P.J. Cribb (eds). 2003].

Approximate 73% of all Orchid species are epiphytic. An experimental research paper published in "The Royal Society" on 7th October 2004, on 'Epiphytism and Pollinator

~~specialization: drivers for Orchid diver~~sity?' elucidate results which reveal the species richness of the Epiphytic genera, than the Terrestrial genera of both Orchids and Non-Orchids (Barbara Graver ndre Schuiteman). They grow in all terrestrial ecosystems except for the poles and deserts, and being the most highly valued plant in commercial horticultural production, Orchid species are prone to illegal poaching. *Paphiopedilum vietnamese* first described in 1999 was found to be extinct by 2003 due to poaching (D.L. Roberts, K.W. Dixon).

Orchids have a spectacular floral beauty, flowers almost resembling creatures, one of the prime reasons being the Masters of Deception in the Plant Kingdom. This article speaks few interesting facts about these Mysterious beauties.

Masters of Deception:

"FRAUDS ARE IMPOSTERS" (James D. Ackerman).

One of the most important aspects of Flowering is Pollination and survival mechanisms in Orchids are bizarre. Being rare and selective in geographic area, they have mastered the art of Deceptive Pollination, under the influence of Environmental conditions.

i. **Territorial Defence:**

Centris Bees are extremely defensive of their own territory and this behaviour might have been taken advantage of by the *Oncidium* species of Orchids. Long panicles produced by the plants have the flowers blooming all along the panicles, and as the wind blows, they sway along. The gesture alerts the bees of "territorial intruders" and as they attack the flowers, the pollinia get attached to their appendages. Repeated attack on different flowers of the same species, pollinates the orchid.

***Oncidium** sp.*

(Source: https://animals.sandiegozoo.org/plants/orchid)

ii. **Brood sites:**

There are a number of Orchid species which mimic the food substrate of larval insects to feed upon.

One classic example is that of *Satyrium pumilum*. This species of orchids attracts Flesh flies by liberating a weak smell of rotten flesh. Flesh fly larvae feed upon dead and rotten corpse of animals. As the flies are attracted and they settle on the labellum of the

male flower, the orchid dusts a lot of pollen on them. The flies are then further directed by the same smell to the female flower and thus pollination takes place.

So the Orchid mimics a “Road Kill”. The flies are rewarded with nothing in return.

Dusting pollen on the Flesh fly, *Satyrium pumilum* mimicking a “Road Kill” for its own benefit.

(Source: https://www.livescience.com/13231-orchids-stink-roadkill-flies-110314.html

Image: © Dennis Hansen)

In a similar way, *Corybas sp.*, *Dracula sp.* and *Cypripedium sp.* of orchids emit “Mushroom-like-odours”. The fungus gnats are misled to lay eggs on the flower instead of the Mushroom substrate which is a food source for their young. The flowers are pollinated by this mimicry, but in return the gnats’ larvae die since the flower is inedible.

Fungus gnat visiting its brood site, the mushroom-like-labellum of *Dracula sp.*

(Source: https://phys.org/news/2016-02-mystery-dracula-orchids-mimicry-unraveled.html

Credit: Bitty Roy, University of Oregon)

iii. <u>Sexual Deception</u>:

The most well known orchid to exhibit a sexually deceiving behaviour, is the *Ophrys sp.* which acts as a dummy female of a bee or wasp species (depending on the type of *Ophrys sp.* present).

Although, there is one particular species which is not as popular as the *Ophrys sp.*, but exhibits the character of sexual deception in orchids very intricately.

Chiloglottis trapeziformis mimics the female of its male wasp pollinator *Neozeleboria cryptoides*, so accurately that it even releases the exact female sex pheromones in amount ten-folds to the actual females, to ensure the attraction of its male, which exhibits pseudocopulation behaviour with the labellum of the flower.

Marinus de Jagar and Rod Peakall had experimentally found out that the Labellum is of great importance in ensuring the pollination of this Orchid. A longer labellum means a longer time of pseudocopulation, ensuring the picking up of a pollinium, while a shorter labellum pose lesser chances of picking up of a pollinium of the Orchid. While the male wasp tries to mate with the pseudo-female, a pollinia gets attached to its genital

clasps. When defeated, it moves on to another flower to perform the same action, thus unknowingly pollinating it.

[Source:https://www.botany.one/2018/10/orchid-seeks-the-most-passionate-pollinators/]

The male-wasp pollinator (*Neozeleboria cryptoides)* of *Chiloglottis trapeziformis* , exhibiting pseudocopulation behaviour on the labellum of the flower.

(Source: https://www.botany.one/2018/10/orchid-seeks-the-most-passionate-pollinators/

Photo: Rod Peakall)

iv. <u>Foraging behaviour</u>:

Perhaps most of the orchid species take advantage of the food-foraging behaviour of insect pollinators. These orchids mimic flowers which are bright in colour and contain a reservoir of nectar and pollen oil, thereby attracting insect foragers and deceiving them to perform pollination.

Species of Orchids under the sub-tribe Oncidiinae, mimics Malpighia flowers in the Bahamas to deceive oil-collecting female *Centris* bees.

(Source: https://royalsocietypublishing.org/doi/10.1098/rspb.2013.0960)

Striking resemblance between *Stigmaphyllon* sp. (Family: Malpighiaceae) (in the centre) with the two Oncidiinae species, *Rossioglossum ampliatum* and *Trichocentrum ascendens* (right and left).

Deception in Orchids actually contributes to their "Fitness". Since no energy or resource is being used in the making of rewards for the pollinators (such as nectar and pollen-oil), they are in turn being used for their own "Fitness" to survive (D.L. Roberts, K.W. Dixon).

The Seed-Destroyer of Orchids:

70% of the total Orchid population in Japan is endangered due to over-harvesting and development. Now, a team of Japanese researchers have discovered a new threat to the native orchids. A fly named *Japanagromyza tokunagai*, has been discovered to feed upon the seeds of five different varieties of blooming orchids. It potentially destroys almost 95% of the total seeds inside the fruit.

The fly lays its eggs inside the young fruit of the blooming orchids. Since a healthy fruit and an infested fruit both grow to the same size, it is not possible to differentiate in between. The larvae feed upon the seeds and form pupae inside the fruits. It is only at the time when the flies hatch out, making a hole in the fruit to escape, is when differentiation is possible between a healthy and an infested fruit.

"Going forward, we want to shed lighter on the damage caused by *J. tokunagai*," said Kenji Suetsugu from Kobe University Graduate School of Science.

"We plan to do this by quantifying the damage in other areas of Japan, and by testing the theory that *J. tokunagai* is a non-native species through genetic analysis."

[Source: https://www.courthousenews.com/seed-eating-fly-threatening-endangered-japanese-orchids/]

Japanagromyza tokunagai , the seed eating fly on the Golden Orchid bloom.

(Source: https://www.courthousenews.com/seed-eating-fly-threatening-endangered-japanese-orchids/)

The pupa of the fly present inside the fruit (right) and the holes on the fruit through which the adult fly comes out (left).

(Source: https://www.courthousenews.com/seed-eating-fly-threatening-endangered-japanese-orchids/)

6.

Orchids share a similar symmetry with Humans:

"When someone looks at an Orchid, it looks back at you."

- *Smithsonian Gardens' Orchid Collection Specialist, Tom Mirenda.*

Most orchids have a striking resemblance to different things (Eg. Lady's slipper orchid) or even creatures (Eg. Monkey Orchid), perhaps being one of the reasons they appear so attractive and intriguing to humans.

Orchids share a bilateral symmetry with humans, that is, if a line is drawn right through the middle of the flowers, it separates them into two equal halves.

Owl Orchid
(Miltoniopsis Lila Fearneyhough)

(Source: https://fineartamerica.com/featured/owl-orchids-ruben-carrillo.html

Photo: Ruben Carrillo)

Coleman's crested coralroot:

A beautiful, rare and endemic species of Southern Arizona, *Hexalectris colemanii* (commonly known as Coleman's crested coralroot or Coleman's coralroot) is a leafless orchid, devoid of roots as well. It obtains its nourishment from a symbiotic association with mycorrhizal fungi that colonize the roots of trees and shrubs. It is therefore known as a myco-heterotrophic orchid. Since it is completely dependent on the mycorrhizal association for its nourishment, any threat to the fungus is a threat for the Orchid.

The orchid spends most of its time underground, giving rise to floral shoots only when the environmental conditions are favourable.

[Source: goorchids.northamericanorchidcenter.org]

Hexalectris colemanii (Coleman's crested coralroot)

(Source: https://nmrareplants.unm.edu/node/248

Photo: Ron Coleman)

Conclusion:

Orchids are strong indicators of a healthy ecosystem. The family comprises of numerous mysteries yet to be uncovered, starting from their symbiotic association with Mycorrhizas for germination to numerous other ways of deceptive pollinating and food-foraging mechanisms. Not much of a research is conducted on them, since most species of Orchids are on their way to extinction, some still falling under the endangered category of IUCN. True, they are the "Selfish Family" but the most diversified and beautiful family in the Plant Kingdom.

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Article 5

COVID-19 outbreak: the use of Medicinal Plants as herbal ingredients in the formulations for Medicine

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INTRODUCTION

Currently, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, formerly known as 2019-nCoV) has rapidly spread across China and around the world. Our special issue is focused on the use of Medicinal Plants as herbal ingredients in the formulations for Medicine.

Medicinal plants are considered as rich resources of ingredients which can be used in drug development pharmacopoeial, non- pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world.

There are many benefits of Herbal Medicine like being easier to obtain than prescribed medicine, stabilizes hormones and metabolism, cause natural healing, strengthen immune system, Fewer side effects. Considering the importance of immunity boosting measures during the COVID-19, it is very important to consume supplements in the form of immune nutrients such as vitamin A, C, E, D, B-complex, Zinc and copper that will support your body to fight against the pathogens. Application of modern technologies and methodologies in herbal medicine research and development using the accepted Western scientific and ethical standards

can have a significant impact on the scientific validity, quality improvement, and standardization of herbal medicines.

DISCUSSION

- **Antiviral Activity of Herbal Medicines and Phytochemicals against Coronaviruses**

Four extracts exhibited moderate to potent inhibition effects against SARS-CoV: *Lycoris radiata* (red spider lily), *Pyrrosia lingua* (a fern), *Artemisia annua* (sweet wormwood), and *Lindera aggregata*, which is an aromatic evergreen shrub, member of the laurel family. The antiviral effects of these extracts were dose-dependent and ranged from low to high concentrations of the extracts, depending in the herbal extract considered. In particular, L. radiata exhibited the most potent antiviral activity against the virus strain.Other medicinal herbs and plants and culinary spices that have been described to have antiviral properties against SARS-CoV are Japanese honeysuckle (*Lonicera japonica* Thunb.) , the commonly known Eucalyptus tree, and Korean ginseng (Panax ginseng), the last one through its active secondary metabolite ginsenoside.

Japanese honeysuckle

Lycoris radiate

Pyrrosia lingua

Artemisia annua

- **Mode of Antiviral Action**

Many investigations and studies of plant extracts and pure molecules have been carried out with different strains of coronavirus. Proteins involved in coronaviral replication and the conductance of ion channels and proteases were the main targets . Several researchers have discovered plant formulations that inhibit in vivo and in vitro viral replication .Many natural anti-CoV phytomedicines include an aqueous extract of fish mint (Houttuynia ordata) , which has been demonstrated to mediate several antiviral mechanisms against SARS-CoV, e.g., inhibition of viral RNA-dependent RNA polymerase and suppression of the function of the viral 3CL protease .

Houttuynia ordarta

- **Phytomedicine and Clinical Trials for Coronavirus Infections**

Three investigations followed focusing on Chinese medicine for the prevention of SARS. None one of the participants in these studies who received herbal remedies became infected with SARS. Based on those data, 23 territories in China released COVID-19 prevention strategies using appropriate herbal medicines used in Chinese medicine:

Radix astragali (dried root of Astragalus membranaceus (Fisch.) Bunge and Astragalus mongholicus Bunge (Fabaceae)) is a popular traditional Chinese medicine, and its active compounds may help fortify the immune system and decrease inflammation. Astragalus is occasionally also administrated as an injection in hospitals.

Radix glycyrrhizae (dried roots and rhizomes of Glycyrrhiza glabra) or liquorice root is one of the 50 important plants used in phytomedicine. Radix saposhnikoviae, *Saposhnikovia divaricate*, recognized as fángfēng meaning "defend against the wind" in Chinese, is the single species in the genus *Saposhnikovia*.

Atractylodis macrocephalae rhizome is hailed as "the most essential Qi herb (vital energy in Chinese medicine) that tonifies and enhances the spleen". It is the dried rhizome of *Atractylodes lancea* (Thunb.), *Atractylodes chinensis* Koidz, or any other nearby plant like *Japonica atractylodes*.

Lonicera japonica Flos, member of the family Caprifoliaceae, is among the most widely used traditional medicines. It includes bioactive components such as caffeic acid derivatives, essential oils (EOs), flavonoids, iridoid glycosides, and terpenoids and it has anti-inflammatory, antimicrobial, anticancer, antioxidant, and immune-modulating properties.

(dried root of vegetative stage of perennial herb Saposhnikovia divaricate (Turxz.)

Radix Astragalus

Astragalus is an herb. The root is used to make medicine.

Radix glycyrrhizae

It is the dried roots and rhizomes of **Glycyrrhiza** uralensis or G. glabra or G. inflata from the Leguminosae/Fabaceae family. It has been used for centuries in traditional medicine as a life enhancer, for the treatment of coughs and influenza, and for detoxification

CONCLUSION

Many viral infections are still lethal and/or are not yet treatable, even though some can be kept under control with life-prolonging agents, which, however, are expensive and outside the reach of most people. Thus, the discovery and development of safe, effective, and low-cost antiviral molecules is among the top universal urgencies of drug research. Therefore, scientists and researchers from divergent medical fields are studying aromatic herbs and ethnomedicinal plants, with an eye to their applicability as antiviral drug.

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Article 6

Plants In Chernobyl

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ABSTRACT

On 26th April, 1986, the Chernobyl nuclear power plant exploded causing devastation on a massive scale and resulting in numerous deaths and all sorts of damages to the organisms in that area. The effects of the explosion can be felt to this day in various forms. This article gives us a glimpse of how some local plants (*Arabidopsis* sp., soybean and flax) developed mechanisms to evade the damaging effects of the high energy radioactive particles in the surroundings. A case (carrot seeds) where the plants failed to adapt satisfactorily to these conditions has also been considered.

Keywords: Chernobyl, radiation, *Arabidopsis* sp., soybean

On **26th April 1986, reactor no.4 of the Chernobyl power plant** exploded causing radioactive materials to be released into the surroundings and spread all across the countryside. Consequently, life forms in the immediate surroundings were either wiped out or severely affected. Surprisingly, when it comes to **vegetation cover, all but the most vulnerable and exposed plants survived.**

Chernobyl's radioactive materials are unstable as they **emit high energy particles** that **destroy the cell structure or release toxic chemicals capable of damaging the cellular machinery**. In animals including humans, this is fatal as their body systems are greatly specialized and inflexible. Plants are comparatively flexible and can adapt to such changes. They are immobile and must adapt to their surroundings or die. Plants are usually capable of renewing dead cells quite easily if needed. Even if the radiation can cause tumours in plant cells, they are usually incapable of spreading from one part to another due to the rigid and interconnecting nature of cell walls. Such tumours are not lethal in most of the cases as the plant finds ways to work around the affected tissue. The plants may also **develop extra mechanisms** to become more damage resistant and also have repair systems in place (if the mechanism of resistance does not work).

SOME IMPORTANT SCIENTIFIC STUDIES:

The study by Kovalchuk et al. assessed the adaptability of native *Arabidopsis* plants obtained from areas with various levels of contamination around the Chernobyl power plant from 1986-92. Their resistance to MMS (methyl methane sulfonate), a radiomimetic agent, and a free radical producing agent RB (Rose Bengal) was evaluated. The plants used were collected from 3 experimental plots (Tolstoy Les, Yanov 12-6, Chernobyl) and a control plot. It was found that the progeny of the plants grown in high contamination areas were more resistant to mutagens, surviving concentrations that killed the progeny of plants from low contamination zone. One possible mechanism of adaptation is genome stabilization. The homologous recombination (HR) frequency was measured to determine the genome stabilization. It was found that the HR frequency is lesser in plants from higher contamination zone than the control plants. Increased resistance to such mutagenic substances could also be due to modification in the expression of important housekeeping and DNA repair genes acquired by these plants over

many generations of exposure to radiation. This may have given rise to progenies capable of adapting to high radioactivity.

In a study by Danchenko et al. on local soybean variety, the seeds were sown in a contaminated and a control field in Chernobyl. The mature seeds were collected and their proteins were subjected to two-dimensional gel electrophoresis (2-DE). After a few more necessary procedures, identified differentially expressed proteins were grouped into 6 metabolic classes.

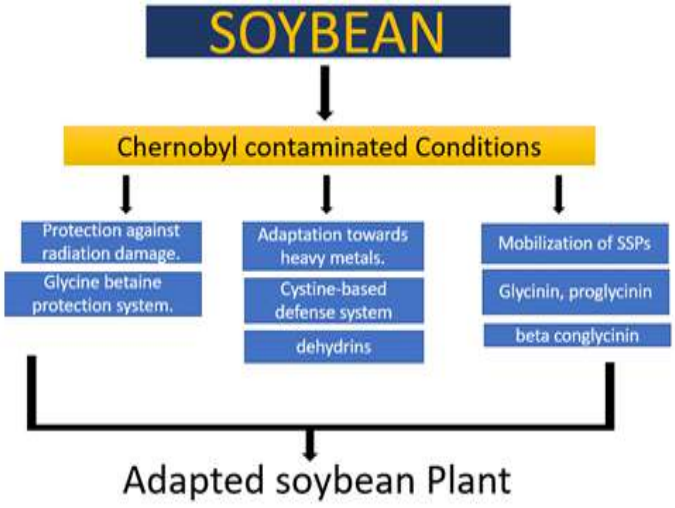

Fig: The working model of plant adaptation proposed towards the highly contaminated soils of Chernobyl based on comparative proteomic studies.

The classes associated with proteins' destination and storage were present in the largest amounts in the seeds from the contaminated field. Based on the study, a working model was suggested which indicated adaptation towards heavy metal stress, protection from radiation damage, and mobilization of seed storage protein are primarily involved in plant adaptation mechanisms against radioactivity in the Chernobyl region.

Another similar study conducted on local flax plants yielded almost the same results as the above-mentioned experiment except for the fact that while seed storage proteins are mostly affected in soybean, flax showed signalling proteins as mostly affected. This difference could be due to the fact that soybean seeds accumulate more radioactivity than the flax seeds.

Fig: Soybean plant being grown near contaminated zone showing how it has adapted to the high radioactivity in Chernobyl

However, not all plants can adapt to the highly radioactive environment in Chernobyl. For example, a study was conducted where 660 seeds originating from 33 wild carrots were collected near the Chernobyl nuclear power plant. The maternal plants had been exposed to varying levels of radiation depending on their site of collection. The seeds from such plants were then grown in an uncontaminated greenhouse. The results of the experiment revealed strong negative effects of high radiation on the timing and rates of germination of seeds. The later stages of development and timing of the emergence of consecutive leaves were delayed

too. Also, in many cases, plants either succumbed immediately upon exposure to the radiation or suffered debilitating damages.

CONCLUSION:

In stressful situations, most organisms will have to choose either between flight or fight. However, as plants cannot move from one place to another, they must fight (adapt) and overcome the stressful conditions, which is quite apparent from the above discussions. These studies show how even in one of the Earth's most radioactive places, some plants have managed to survive and give rise to progeny. Such plants can be said to exhibit what survival of the fittest really means.

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Article 7

EXPERIMENT WITH BITTER GOURD- A SIMPLE CASE STUDY

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INTRODUCTION

Momordica charantia L. is most common vegetable crop, belongs to family Cucurbitaceae, and commonly known as Bitter Melon, Bitter Gourd, and Karela in India. It is widely cultivated and utilized as nutritious as well as medicinal plant in households of Indian – subcontinents.

Cucurbits are monoecious; there are separate male and female blossoms on the same plant. The male flowers tend to open first, followed by the female flowers (This phenomenon is known as Protandry). It is only when both the male and female flowers are open pollination can occur.

Pollination is the act of transferring pollen grains from the anther of a male flower to the stigma of a female flower to produce offspring. Pollination in Cucurbits occurs through Entomophily i.e. insects like bug, beetle, bees and moth are the pollinating agents.

FIGURE: BOTANICAL ILLUSTRATION OF PLANT PARTS

OBJECTIVE

To artificially pollinate a Bitter Gourd plant in absence of insects using household tools at Balcony garden of a high-rise apartment.

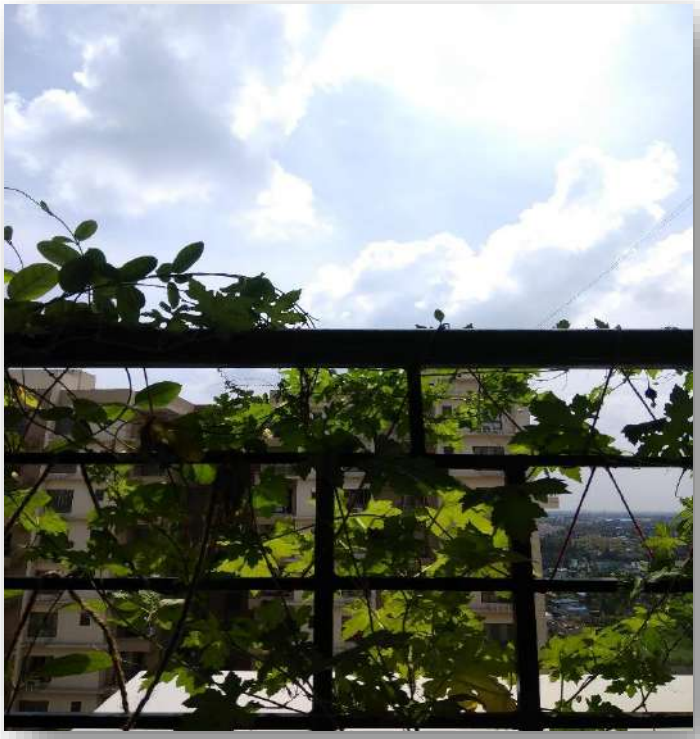

FIGURE: HERBACEOUS, TENDRIL-BEARING VINE OF BITTER GOURD PLANT GROWS UP TO 5 M (16 FT) IN LENGTH. IT BEARS SIMPLE, ALTERNATE LEAVES 4–12 CM

MATERIALS REQUIRED

1. A clean, dry and dust-free Round paint brush (Size no – 2)
2. A clean, dry and dust-free Tissue paper

EXPERIMENT

1. Male and female flowers of the same plant are identified. The white male blossoms will be four times more in number and contain only anthers full of bright yellow pollens, whereas, the white female blossoms will contain a single stigma, greenish yellow in color with a swollen base.
2. Brush is used to gently rub the anthers of male blossom which results in adhering of pollens on the brush. Tissue paper is placed right below the male flower to collect pollens which are dusted off while executing the process.
3. Very delicately the same brush is rubbed on the stigma of a female blossom. Make sure the pollens stick on the surface of stigma. After pollinating, do not disturb the flower anymore.

FIGURE: ILLUSTRATION OF MALE AND FEMALE FLOWERS OF BITTER GOURD

OBSERVATION

1. In time span of 2-3 weeks, the petals of the female flower will wither, the swollen basal part will increase in size and gradually formation of fruit will take place.
2. The fruit has a distinct warty exterior and an oblong shape. It is hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large, flat seeds and pith. The fruit is most often eaten green, or as it is beginning to turn yellow. The skin is tender and edible. Seeds and pith appear white in unripe fruits.

FIGURE: GREEN EDIBLE FRUIT AND ORANGE RIPPEN DEHISCED FRUIT WITH RED SEEDS OF BITTER GOURD PLANT

RESULT

Formation of a healthy Bitter Gourd fruit.

CONCLUSION

Successful artificial pollination of a Bitter Gourd plant in absence of insects using household tools at Balcony garden of a high-rise apartment.

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CHAPTER 2

This section include science articles from alumni of the department. These are glimpses of research and dissertation work carried by them in different Post Graduate and Research Institutes in India and abroad.

Article 8

Nanoparticles as Emerging Environmental Threats

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Abstract

The field of nanotoxicology has evolved over the past three decades owing to the escalating demands for various nanoparticles (NPs). The advantageous characteristics of small size , and large surface area to volume ratio of NPs are utilized for their applications in industry, agriculture, medicine, cosmetics, and theranostics. This review aims to highlight the types of NPs, their market demands and methods in nanotoxicology testing. Literature survey was carried out by employing the NCBI PUBMED database with various keywords and time points. With the rising market demand, increasing number of studies on their toxicology are being published every year. Ratio analyses of the number of publications revealed a scarcity of information on the toxicology of NPs in plant systems. Moreover, a major portion of the studies were performed using *in vivo* animal models and *in vitro* human cell lines. Plants are exposed to various natural and anthropological NPs due their sessile habitats. Since they are the primary producers of every ecosystem, it is the need of the hour to assess the toxicology of NPs in plant systems as an integral part of their environmental safety appraisal.

Keywords: nanoparticles, nanotoxicology, cytotoxicity, genotoxicity, *in planta*

Introduction

In the past few decades, the rapid development in the field of nanotechnology has propelled an upsurge in the use of various natural and engineered NPs for industrial, clinical, and agricultural applications."Nanoparticle" is an umbrella term comprising particles within the range of 10^{-7} to 10^{-9} m (1-100 nm) (Bhatia, 2016). In solution, they often undergo agglomeration (formation of loose clumps) and /or aggregation (formation of tight clumps) to form larger bulk particles. Hence, the prefix "nano" can be acceptably used for particles less than 500 nm in diameter, and ≤ 100 nm in at least one dimension (European Commission, 2011). Particles in the nanoscale possess the unique feature of small size and large surface area to volume ratio. This enables novel anthropogenic applications. Today, nanotechnology is omnipresent and forms an integral part of human life. The high demand for nanoparticles (NPs) in industry, medicine, diagnostics, material science, lifestyle products, agriculture, and food has led to their extensive production to cater to the ever-increasing consumer needs. Such widespread applications gave rise to the field of nanotoxicology, which encompasses the systematic toxicity evaluation of various NPs for their safe usage. In this review, the following aspects of nanotoxicology are elucidated:

1. Classification of nanoparticles
2. The growing market demand for nanoparticles
3. NPs as emerging environmental threats
4. Methods in nanotoxicology testing

1. Classification of nanoparticles

Size is the first parameter for the classification of NPs. Fine particles cover a broad size range of 100-2500 nm. NPs in the size range of 1-100 nm are termed as ultrafine particles. The nanoscale size range of 1-100 nm provides particles with the fundamental characteristic feature

of high surface area to volume ratio. As the particle size decreases to the nano scale, higher percentage of constituent atoms are exposed to the surface. This unique feature is enhanced at an even smaller size range of 1-10 nm, where particles exhibit unique surface specific quantum properties. These particles are also known as quantum dots or nanodots. The classification of NPs based on size is illustrated in Figure 1.

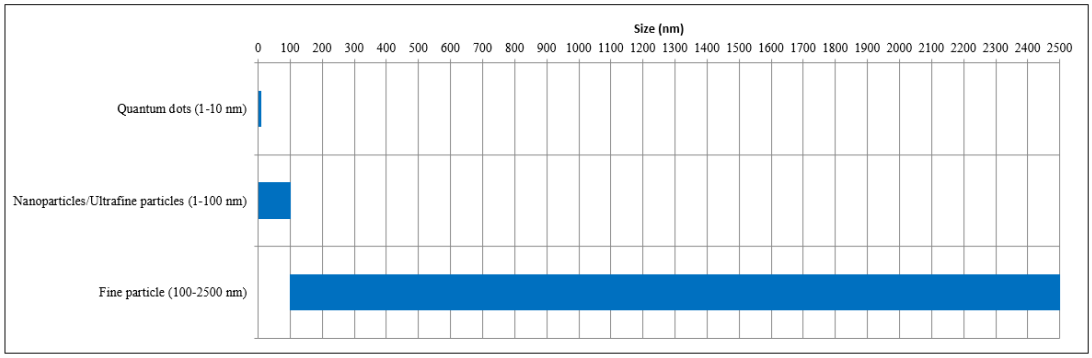

Figure 1. Classification of Nanoparticles based on size (Ghosh, 2018)

NPs have been broadly categorized as natural and engineered depending on their sources (US EPA, 2014). Figure 2 demonstrates the classification of NPs. It is well established that nature itself is a nanotechnologist. Combustion products of volcanic eruptions and wild fires, weathering, soil erosion, hot springs, sea sprays, minerals in soil and rocks are classified as non-biological natural NPs (US EPA, 2017; Griffin et al., 2017; Smita et al., 2012). For example, different forms of iron such as magnetite (Fe_3O_4) and maghemite (Fe_2O_3), manganese oxides, fullerenes/buckyballs (C_{60}, C_{20}, C_{70}) naturally occur in nano-forms in the earth's crust (Singh et al., 2010; Trpkovic et al., 2012). Biological NPs include natural products such as those synthesized by various bacteria, algae and plants. Numerous bacterial species synthesize iron and manganese NPs (also known as magnetosomes), within their cells by specialized mechanisms (Schüler, 2008).

Unwanted emissions of most metallic, non-metallic, and carbon-based NPs designed to meet the growing industrial and consumer needs are classified in the unintentional engineered

nanoparticle category. They are released from diesel exhausts, industrial effluents, disposal of cosmetic products, welding fumes, biomedical and laboratory wastes. Gebel et al., (2014), in an elegant review, further divided them into the following categories based on their toxicity:

Category 1: NPs whose toxicity is caused by their specific chemical components which also include released ions or functional groups from the surface. E.g.Cd based quantum dots, ZnO, Ag NPs, Fe and most metal oxide NPs.

Category 2: NPs that are rigid, biopersistent, respirable and fibrous with high aspect ratio and a specific geometry fall under this category. E.g. multi-walled carbon nanotubes (MWCNT) and single-walled carbon nanotubes (SWCNT).

Category 3: Granular biodurable particles (GBP) which are respirable and can cause inflammation, secondary mutagenicity leading to lung cancer. E.g. TiO_2 NPs and fullerene soot.

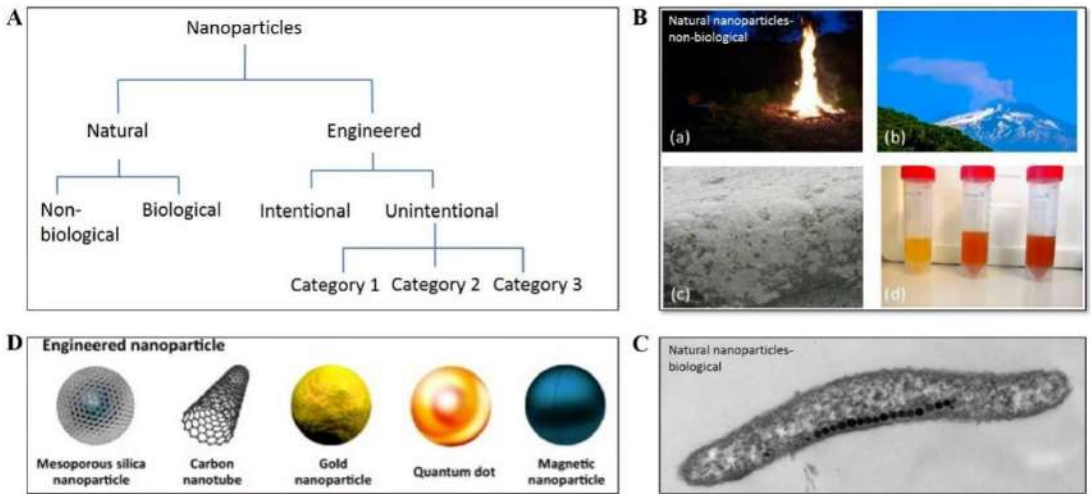

Figure 2. Classification of NPs. (A) Types of NPs based on sources. (B) Natural sources of NPs (a) near open fire; (b) as result of volcanic activity; (c) in the form of precipitates; and (d) as reductively formed deposits of elements (Griffin et al., 2017) (C) Natural organic NPs-magnetosomes formed by magnetotactic bacteria (Schüler, 2008). (D) Types of engineered NPs. (Ghosh, 2018)

2.The growing market demand for nanoparticles

The differing physico-chemical characteristics provide unique features that facilitate novel applications leading to their rising market demand. The global annual production of NPs reached 1000 tons in 2004 and is projected to rise to over 1 million tons by the end of 2020, to attain a staggering value of $ 125 billion by 2024 as per conservative market estimates (US EPA, 2007; **Maurer-Jones et al., 2013; Global market for metal oxide NPs 2020, 2013;** Maynard et al., 2006; Sizochenko et al., 2014). There are already more than 1800 nano-based consumer products in the market, and hundreds are expected to appear in the years to come (US EPA, 2014; US EPA, 2017; Rejeski and Lekas, 2008). At least 44 elements of the periodic table are being synthesized in the nano-form by more than 169 producers worldwide to cater to the escalated demand (Chen et al., 2004; Gaffet, 2012). Figure 3 shows the diverse applications and rising production of NPs.

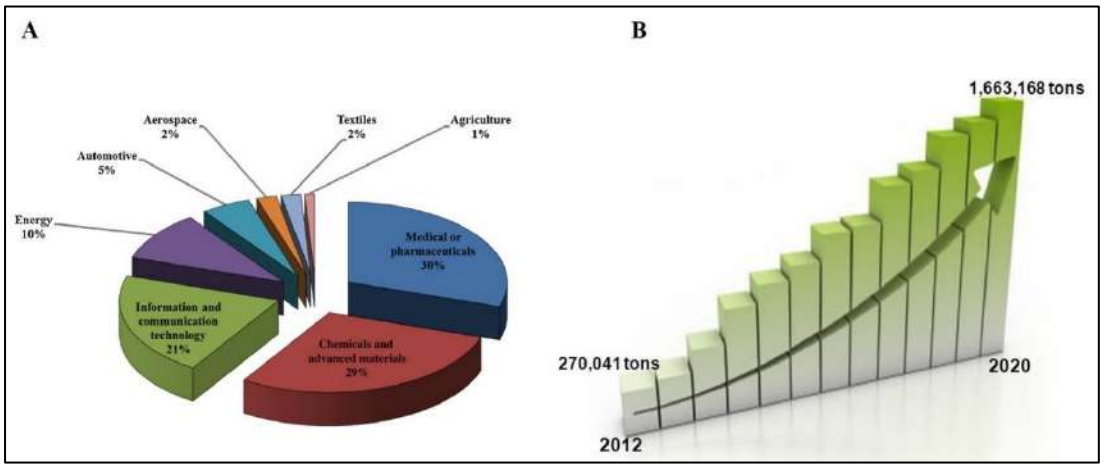

Figure 3. (A)Applications of NPs. (B) Rising production of NPs (Global market for metal oxide NPs 2020, 2013).

3. Nanoparticles as emerging environmental threats

NPs differ from their bulk counterparts in terms of their physicochemical properties (Figure 4) of size, shape, unique surface chemistry, charge, ion dissolution, pH, conductivity, and uptake/internalization within biological systems. Hence, they pose higher toxicological implications compared to their bulk forms (Buzea et al., 2007).

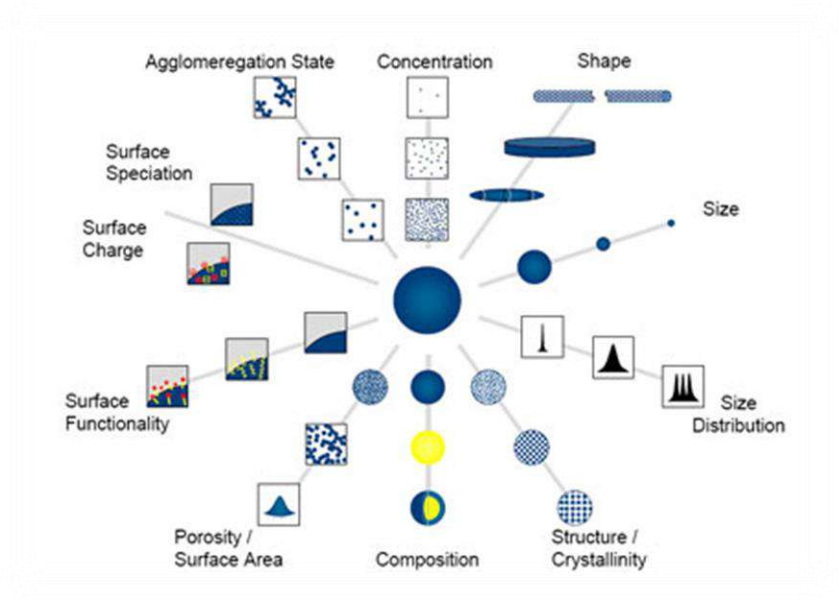

Figure 4. Physico-chemical characteristics of NPs (Adapted from Hassellöv and Kaegi, 2009)

Literature survey revealed an increase in publications on nanotoxicity profiling over the past three decades (Figure 5). Based on the number of hits obtained, studies in plant systems are relatively scarce (Figure 6A). Maximum nanotoxicity studies are based on *in vivo* animal systems (Figure 6A). In case of animal cells, the percentage of studies on human cells ranks higher than other animal cells. Among the various human cells, least nanotoxicity assessments are performed on blood cells (lymphocytes and erythrocytes). In the category of plant systems, most nanotoxicity assessments employ *Allium cepa* as the model system of choice (Figure 6B). While most nanotoxicity analyses involve cytotoxicity assessments, the number of studies on

genotoxicity is highly limited (Figure 7A). Moreover, genotoxicity studies in plant systems hold a minor fraction (Figure 7B).

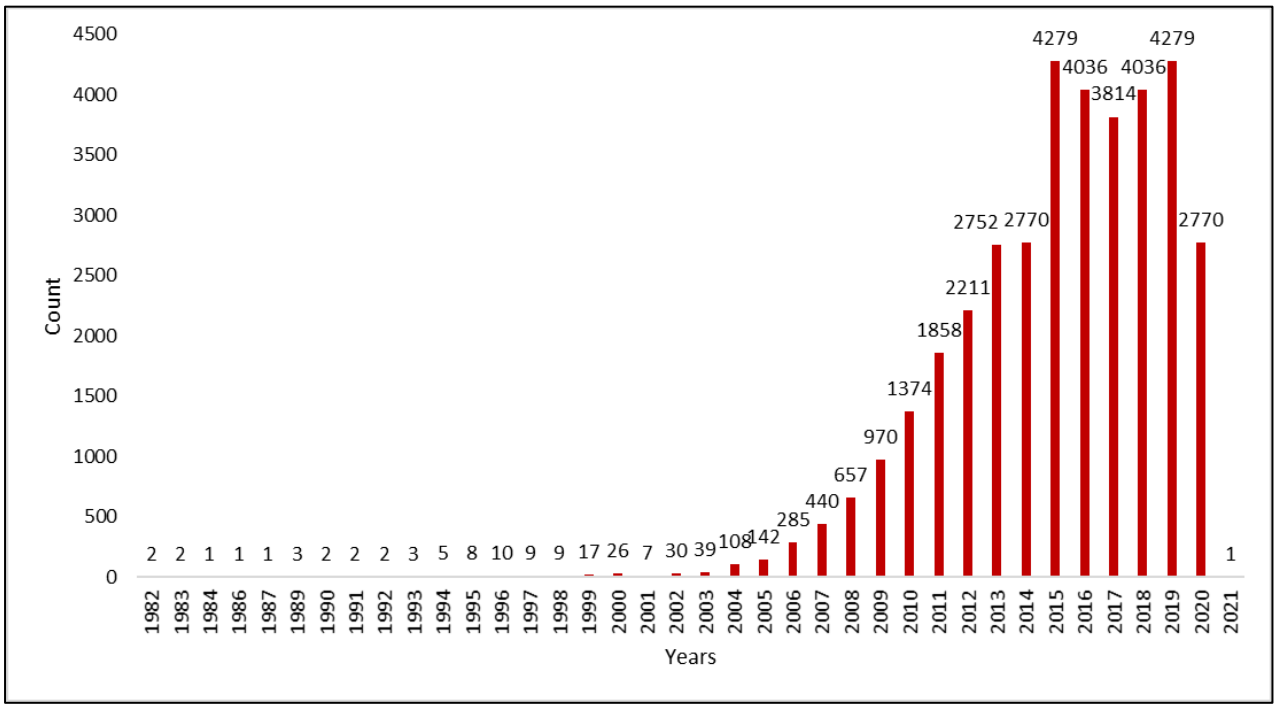

Figure 5. Toxicity of nanoparticles- Year wise number of hits with the keywords "Nanoparticles AND Toxicity". Source- NCBI PUBMED (1982-2021)

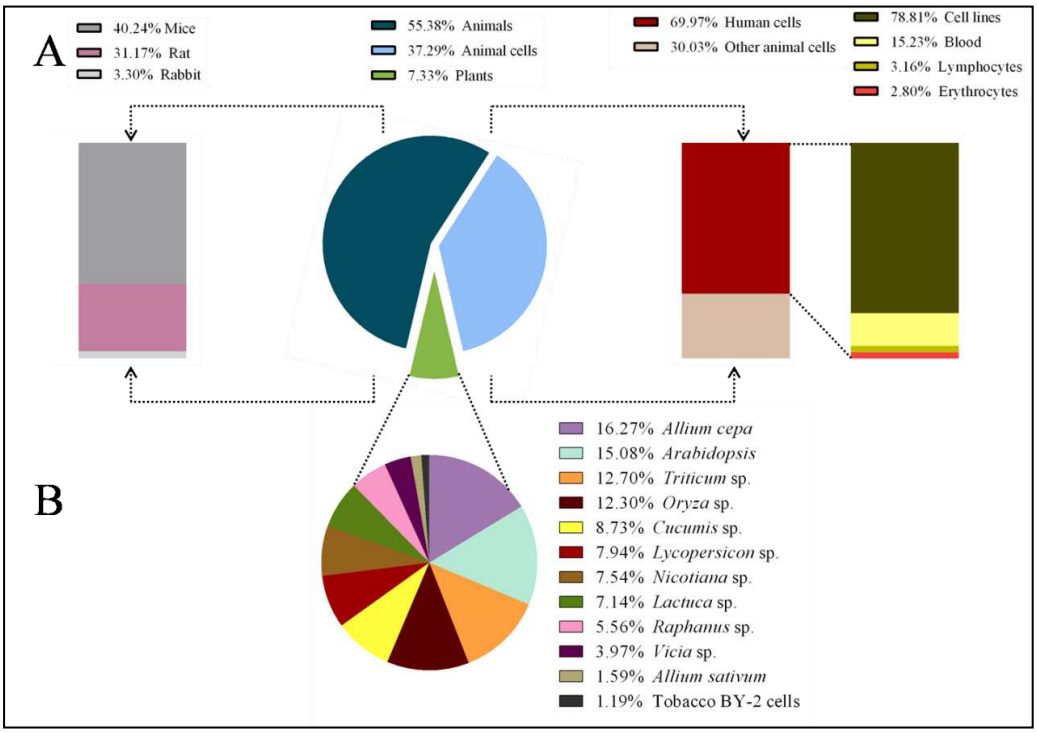

Figure 6. Literature survey on the biological systems used in nanotoxicity studies. (A) Ratio charts showing the percentage of hits on animals ("Nanoparticles toxicity AND Animals"), animal cells ("Nanoparticles toxicity AND Animal cells") and plants ("Nanoparticles toxicity AND Plants"). Highest percentage of hits is from animal systems, within which maximum works are on mice. In the category of animal cells, most studies are performed using human cells. Among the human cells, various cell lines are widely used in nanotoxicity assessments. A small fraction of studies is on lymphocytes and erythrocytes (B) Pie chart showing the percentage of hits on different plant systems used in nanotoxicity studies. Maximum hits are on *Allium cepa* and the least number of studies are on tobacco BY-2 cells. Source- NCBI PUBMED (1988-2018) (Ghosh, 2018)

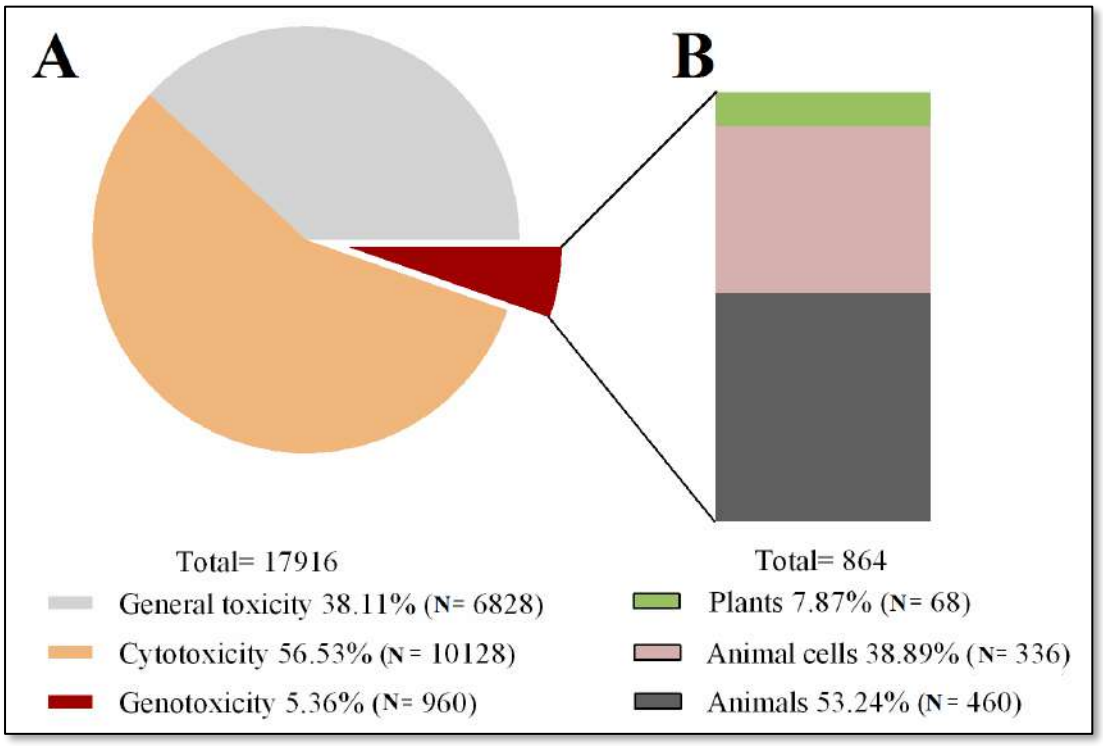

Figure 7. Different types of nanotoxicity studies. (A) Percentage of hits with the keywords "Nanoparticles AND Toxicity"; "Nanoparticles AND Cytotoxicity" and "Nanoparticles AND Genotoxicity". (B) Percentage of hits with the keywords "Nanoparticles AND Genotoxicity

AND Animals"; "Nanoparticles AND Genotoxicity AND Animal cells" and "Nanoparticles AND Genotoxicity AND Plants". Source- NCBI PUBMED (1988-2018) (Ghosh, 2018)

With the growing dissipation of NPs into the environment, further studies are needed to fully understand their toxicity in both plant and animal systems. In view of the dearth of genotoxicity studies and the scarcity of such works in plant systems, it is the need of the hour to assess the toxicity of environmentally relevant NPs in plants.

4. Methods in nanotoxicology testing

A prerequisite to all nanotoxicology evaluation is physico-chemical characterization. Primary characterization can be done using electron microscopy (TEM, SEM) or atomic force microscopy (AFM). Hydrodynamic characterization in solution at experimental time points are performed by dynamic light scattering (DLS) (Ghosh et al., 2018). Nanoparticles are taken up by cells through different forms of endocytosis mechanisms. They are then encapsulated within vesicles for biodistribution and internalization within organs and /or cellular organelles (Ghosh and Mukherjee, 2020). The smaller size and higher dispersity of nanoparticles in suspension enable increased diffusion across membranes compared to their bulk counterparts (Shang et al., 2014). Methods such as Inductively coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS) can be used to quantify nanoparticle adsorption, uptake, and internalization.

Numerous methods have been developed over the years to assess nanotoxicity at the physiological, biochemical, and molecular levels. Two of the most important categories of nanotoxicity analyses are genotoxicity and cytotoxicity. The use of genotoxicity assays of mutagens gained momentum since the 1970s and 1980s, due to high demands of the pharmaceutical industries for inexpensive alternatives to existing carcinogenesis bioassays (Borràs and Nadal, 2004). With the advent of nanotechnology, genotoxicity bioassays were incorporated as a vital part of nanotoxicology assessments with a thrust in scientific literature,

since 2012. These endpoints involve the estimation of DNA single and double strand breaks and /or oxidative DNA damage. Figure 8 shows some of these widely used assays. Cytotoxicity endpoints reply on the study of membrane integrity, cellular metabolic activity and type of DNA fragmentation to assess the mode of cell death. Figure 9 demonstrates some of the important cytotoxicity endpoints.

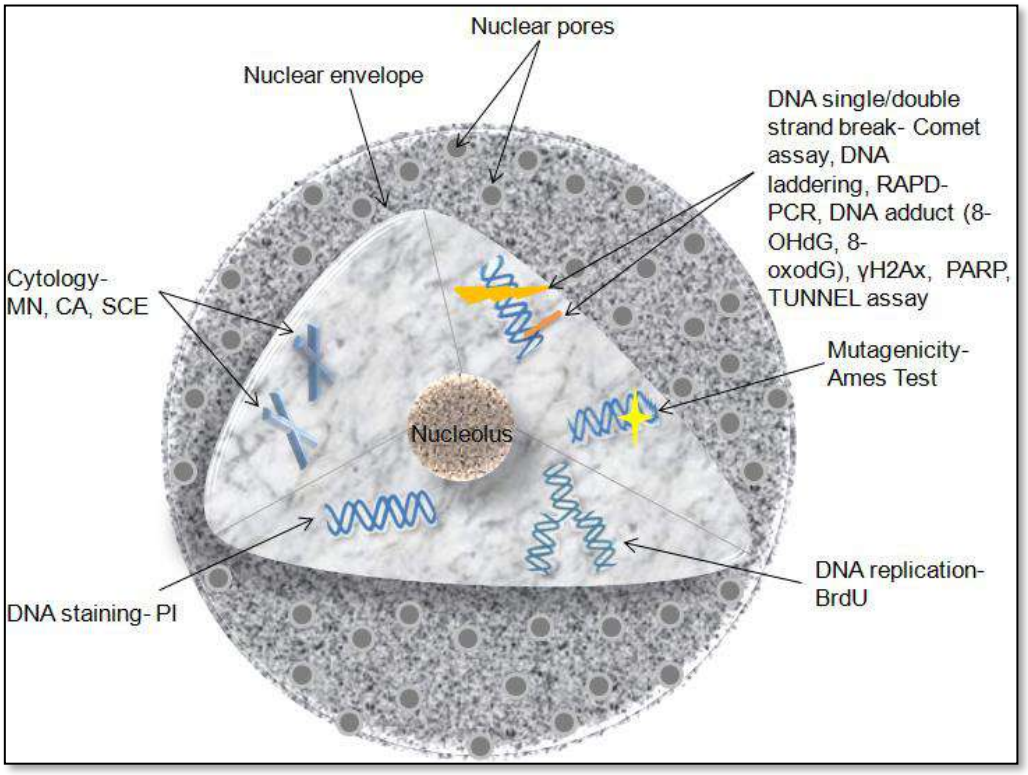

Figure 8. Diagrammatic representation of the widely used multi-endpoint genotoxicity analysis methods used in nanotoxicology. The cytogenetic parameters of micronuclei (MN), chromosome aberration (CA) and sister chromatid exchange (SCE) determine changes in cell division, nuclear organization/structure, chromosome structure, movement and number. PI staining of DNA and BrdU mediated detection of DNA replication is used to study genotoxicity. Mutagenicity is detected by Ames Test. DNA single and double strand breaks are detected by comet assay, TUNNEL assay and DNA laddering. RAPD-PCR is carried out for qualitatively studying genome integrity as a result of DNA damage, DNA adduct analysis

(8-OHdG and 8-oxoG) for oxidative DNA damage and γH2Ax and PARP assays for the study of DNA repair (Ghosh and Mukherjee, 2020).

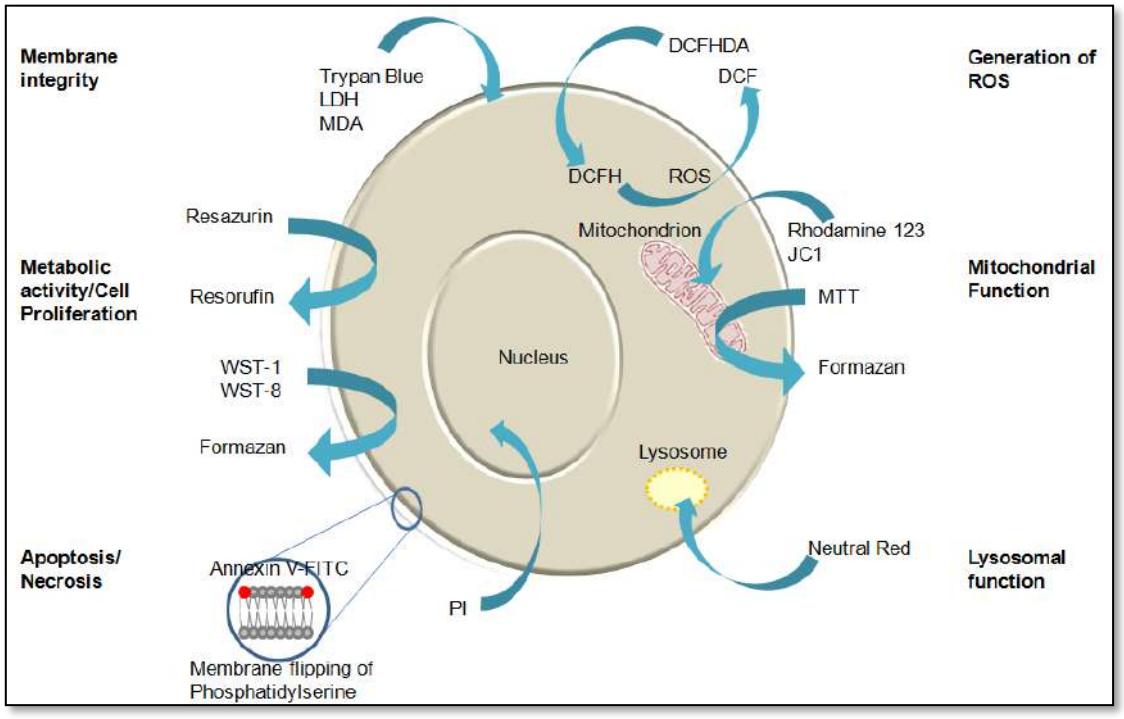

Figure 9. Multi-endpoint cytotoxicity analysis- NP induced membrane disruption can be studied by trypan blue dye exclusion test, lactate dehydrogenase (LDH) assay and assessment of malondialdehyde (MDA) content. Metabolic activity and cell proliferation can be detected by the dyes Resazurin, WST-1 and WST-8. Detection of apoptosis/necrosis is carried out by Annexin V-FITC/PI double staining method that detects membrane flipping of Phosphatidylserine as a mark of apoptosis. Neutral red staining is carried out to detect lysosomal function while Rhodamine 123, JC 1 and MTT are used to study mitochondrial function. Generation of ROS is studied by 2', 7'-dichlorofluorescein diacetate (DCFHDA) staining. All the above methods rely on spectrophotometry, fluorimetry or flow cytometry (Ghosh and Mukherjee, 2020).

In view of the unique properties of each NP, and their alternating physico-chemical characters in various media, it is important to develop modified nanotoxicity endpoints coupled with

proper characterization in order to fully understand the effect of NPs in different biological systems.

Conclusion

The growing usage of NPs in industry, agriculture, cosmetics, medicine and theranostics has elicited their environmental exposure. This leads to the higher propensity of toxicological manifestations in plant and animal systems. With the rising demand for NP-based products/systems, their fate within biological systems is obscure and information on the influence of varying physico-chemical properties on toxicity is scarce. Overall, results of this literature survey indicate that comprehensive toxicological analyses *in planta* are required to fully understand their ecotoxicology and effects on environmental health.

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Article 9

Potential of Plant Biotechnology in the fight against SARS-CoV2

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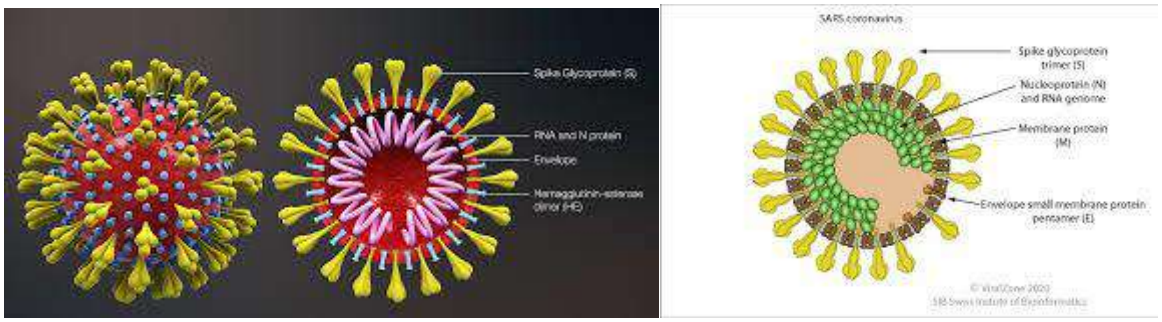
Abstract

The coronavirus is an RNA virus which was first detected in China and soon after an alteration was identified in its RNA sequence. This alteration made it different from SARS-CoV and it was named as SARS-CoV2 which could spread more efficiently in humans. The coronavirus probably arose from mutation and modification of pre-existing virus. Before SARS-CoV2, six other coronaviruses have been known to infect humans. Two of them SARS-CoV and MERS-CoV cause severe disease and death and the other four HKU1, NL63, OC43 and 229E cause mild symptoms.

Introduction

Viruses cannot live independently and need a host cell to multiply where it uses the host cell's machinery for its survival and multiplication. The coronavirus has spike proteins on its surface which helps it to gain entry into the host cell by grabbing and penetrating the cell. The spike protein has two major components: receptor binding protein (RBD) and cleavage site. RBD acts as a hook which helps in grabbing the host cell and cleavage site helps in penetrating. The RBD portion of coronavirus shows five alterations as compared to the genome of SARS-CoV which enables it to bind human cells more efficiently to a particular protein. This protein is

known as ACE-2 or angiotensin converting enzyme 2 which regulates blood pressure. People suffering from heart disease and diabetes are treated with ACE inhibitors which increases the amount of ACE2 proteins in the body and this in turn provides more particles for coronavirus to adhere and enter the lungs. The cleavage site also has certain modifications, however, no such detailed study has been carried out as of yet.



Further in order to trace back the origin of coronavirus, the SARS-Cov2 is more than 90% similar to the bat coronavirus rather than to other coronaviruses. However, the RBD domain is considerably different and it is reported that the bat coronavirus cannot bind to ACE2 as effectively. However, the alterations found in the RBD portion is very similar to the ones present in pangolin coronavirus.

Observation

Since the outbreak of coronavirus, there is a keen interest in the development of vaccines and antiviral drugs for the control of its spread and bring lives back to normal. A part of this effort is to bring all researchers working with plants together to achieve the rapid supply of antigens and antibodies and speed up the process of production of antiviral drugs. For more than 30 years, plants have been used as a platform for the development of diagnostic reagents and proteins of pharmaceutical importance in an approach known as molecular farming. The main advantages of the use of plants for the development of antiviral drugs is its ease in scalability, safety and economy, yet, even today research is focused on the use of mammalian cell lines

and microbial lines. However, plants have made their mark as effective contributors in a number of cases with the production of compounds with favorable glycan configurations (taliglucerase alfa) (Mor et al., 2015), production of HIV microbicides (Sabalza et al., 2013) and are particularly useful when transient expression systems are used so they can be scaled up rapidly upon demand.

Discussion

Soon after the genome sequence of SARS-CoV2 was released in the database, the first detection system that arose was RNA based detection with the help of a set of primers. This led to extensive research for a positive control in RT-PCR. A group at the John Innes Centre (JIC, Norwich, UK), led by George Lomonossoff and Hadrien Peyret initiated their research to develop a control reagent for COVID-19 based on the virus like particles (VLPs) from Cowpea mosaic virus (CPMV). VLPs have the same structure as SARs-Cov2 except they lack the genome and are unable to replicate. Another method of detection of virus particles requires the production of ligands in the form of antibodies that can be detected by ELISA. The transient expression of SARS-CoV2 material packaged inside VLPs may help in scaling up the production of such ligands and detection system.

In case there is any apprehension, plant derived antibodies are stable and functional, produced in large quantities as can be deciphered from the report in Nature paper of the production of a plethora of antibodies in tobacco (*Nicotiana tabacum*) about 30 years ago.

Apart from the use of plants in the development of detection systems, plants can contribute effectively for the development of vaccines. Scientists propose that a quicker way for the development of vaccines would be against individual subunits of SARS-CoV2 introduced as antigens with a suitable adjuvants in different boosts. Among the subunits of SARS-CoV2, the S1 subunit varies with other CoV viruses and allows entry into the body. Blocking its entry

would prove to be very effective in the control of the virus. Many such subunit vaccine candidates have already been produced in plants, such as those for pandemic strains of influenza virus produced by transient expression in tobacco (Vaquero et al., 1999). Kentucky Bioprocessing, based in Owensboro, KT, USA is taking the lead in developing a COVID-19 vaccine with the expression of SARS-CoV2 protein subunit expression in tobacco plants.

VLP based system is very effective for the production of vaccines as they efficiently trigger the adaptive immune system, can be produced in large quantities and cannot replicate in humans even in the native form (Rybicki et al., 2020). A platform for the use of VLP in the production of vaccines was developed by Medicago Inc. (Quebec, Canada) and they were successful in developing 10 million doses of vaccine against H1N1 influenza virus (D Aousta et al., 2010). They are now involved with the development of vaccines against COVID19 (Phillip Morris International, 2020).

The production of antibodies in plants can also be used in passive immunotherapy just in the same way the plasma serum from cured patients can be used for those who are infected. The path for this was laid when a mixture of three neutralizing antibodies known as ZMapp was made against the outbreak of ebolavirus in West Africa in 2014 (Hiatt et al., 2015) and the only way for the rapid large scale production of antibodies was via the production of transgenic plants (Capell et al., 2020). Plants could also be used for the large scale production of antibodies against the cytokine storm that follows SARS-CoV2 infection.

Lastly, antivirals can slow down the virus replication process and help the body fight the infection. Some proteins can be directly used as antivirals such as plant based lectins that block the glycan structures on the surface of the virus particle (Mazalovska et al., 2018). Griffithsin is a 121-amino-acid lectin produced by red algae of the genus *Griffithsia* that is effective as an entry inhibitor against multiple viruses such as HIV, Zaire ebolavirus, and the coronaviruses

responsible for the original SARS outbreak (SARS-CoV) (Capell et al., 2020). Scytovirin is a 95-amino-acid lectin from the cyanobacterium *Scytonema varium* that is also active against multiple viruses, including HIV, Zaire ebolavirus, Marburg virus, and SARS-CoV (Capella et al., 2020). However, their effectivity against SARS-CoV2 is yet to be recognized.

Plants can be very effective in the production of diagnostic reagents, vaccines and antiviral proteins as well as for the scaling up of components in times of such crisis when we need more and more kits and vaccines by the hour. Two major H2020 projects of EU consortia Pharma Factory and Newcotiana are developing plant based platforms for their industrial applications. Plants have the potential for the development of a model that may not only help fight the present pandemic situation but also prepare us for future pandemics.

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Article 10

Can phytochemicals rescue the world from COVID-19 pandemic?

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Abstract

Coronaviruses are the causative agents of diseases such as severe acute respiratory syndrome (SARS caused by SARS-CoV), Middle East respiratory syndrome (MERS, caused by MERS-CoV), Coronavirus disease of 2019 (COVID-19 caused by SARS-CoV-2). However, a dearth of effective antiviral representatives for many coronavirus strains makes the diseases difficult to handle. With the ongoing global pandemic COVID-19, there is an urgent need of a compelling therapeutic strategyto outcast the global flare of the highly infectious and life-threateningaffliction. Some remedies include synthetic anti-viral chemical compounds (Lopinavir, Ribavirin, Chloroquin, Rapamycin), siRNAs, monoclonal antibodies (anti-RBD mAbs), cyclophilins (Alisporivir), peptides and small molecules, vaccine based therapy (DNA plasmid, recombinant protein subunits, live attenuated virus). Current worldwide statistics of COVID-19 has marked over 23 million SARS-CoV-2 infections with greater than 0.8 million deaths and about 6.6 million active cases of COVID-19 (as and on 25th August 2020). India occupies the third position with the most number of confirmed and active cases of COVID-19. The country reports maximum number of infections in a day signifying the uncontrolled spread of the virus.

Introduction

A massive field of alternative and complementary medicine has often come to escape in times of trouble to the medical fraternity. The naturally existing compounds provide riches of chemical diversity that may have utility as therapeutic agents against coronaviral infections as there are plenty reported anti-viral compounds in literature. The PubMed database was searched with keywords - coronavirus, SARS, MERS, COVID-19, traditional medicine, herbal, natural compounds, anti-viral phytochemicals. Some significant scientific reports have been compiled here in an introductory mini-review highlighting plants and the compounds or their extracts which are known anti-virals or possess the potential of treating coronavirus infections.

Traditional herbal medicines/plants and purified phyto-compounds could be developed or might help in paving ways for novel anti-viral drugs. A study suggested that a good number of patients in Chinese hospitals received herbal medications tagged with modern medicines (Wan et al. 2020). The traditional drugs could be very well used for prevention (Luo et al. 2020) or treatment (Yang et al. 2020). Natural compounds with anti-oxidant activities act as direct viral inhibitors and block the interaction of the viral particles with the human protein receptors. The main druggable targets of SARS-CoV-2 (also in case of SARS-CoV/ MERS-CoV) are $3CL^{pro}$ (Wu 2020) and PL^{pro}.

Natural compounds with anti-viral activity such as Quercetin, Luteolin, Betulinic acid, Epigallocatechin gallate,Aloe-emodine are enzyme inhibitors in functioning of 3CLpro in SARS-CoV functional at remarkably low IC_{50} (4 to 500 μM) (Nyugen et al. 2012, Ryu et al. 2010). Quercetin 3-β-d-glucoside, Herbacetin, Isobavachalcone and Helichristine could block $3CL^{pro}$ activity in MERS-CoV (Jo et al. 2020).

Methodology &Observation

Zhang and team (2020) used in silico tools to study 26 Chinese herbal plants and their reported phyto-compounds with anti-viral roles which were docked with coronavirus protein targets previously studied on SARS-CoV and MERS-CoV. Some included Kaempferol, Cryptotanshinone, Quercetin, N-cis-feruloyltyramine which docked with low binding energies with 3CLpro (3C-like protease) and PLpro (papain-like protease) viral proteins.

The aqueous root extracts of *Isatisindigotica*contains several phenolic compounds such as Sinigrin and Hesperitin that potentially inhibit $3CL^{pro}$ SARS-CoV (Lin et al. 2005). Ghosh et al. (2020) made an interesting finding about active compounds of green tea namely Epigallocatechin gallate and Epicatechingallate that interact with catalytic residues of M^{pro} (main protease) of SARS-CoV-2.

1,3,5-Trihydroxybenzene from *sargassum spinuligerum* (brown algae), Bieckol and Diekol compounds from *Ecklonia cava* (brown algae) are also said to inhibit M^{pro}. Phloroglucin oligomers from the plant are promising viral inhibitors (Gentile et al. 2020).

Extracts of Chinese traditional plants, *Gentianascabra, Cassia tora, Dioscorea batatas, Taxilluschinensis*could inhibit replication in SARS-CoV-2 (Wen et al. 2011). Wen's team investigated 200 Chinese herbs, some including *Rhizomacibotii, Dioscoreae rhizome, Cassiae semen, Loranthi ramus, Gentianae radix* showing very low viral inhibition concentrations of SARS-CoV infected Vero E6 cells. Lung's group (2020) screened 83 Chinese medicinal compounds virtually in search of activity against RNA polymerase of SARS-CoV-2 and identified Theaflavin as a potent inhibitor. Extracts of the plant *Artemisia annua, Lycoris radiata, Lindera aggregate* exhibited remarkably low effective concentration of antiviral activity over SARS-CoV.

In a review work by Keyaerts and co-workers in 2007, lectins from the diaminopropane extracts of *Allium porrum, Morusnigra, Listera ovata, Nicotiana tabacum, Urticadioica, Polygonatum sp*. exhibited very low virus inhibitory doses as determined by CPE assay. The same assay showed essential oils like β-pinene, α-pinene, 1,8-cineole from *Laurus nobilis* in SARS-CoV FFM1 strain could inhibit viral replication. A-cedrol and α-pinene from *Thujaorientalis* showed the same function (Loizzo et al. 2008).

Procyanidin A2 from *Cinnamomisp* could interfere with the entry of SARS-CoV-PUMC01 F5 as determined by plaque reduction assay (Zhuang et al. 2009). Emodin from the root extracts water extracts of *Rheum officinale*could inhibit binding of viral S-protein to human ACE2 receptors (Ho et al. 2007). Liu and Zhou (2005) identified 18 compounds from marine natural products that could inhibit $3CL^{pro}$ in SARS-CoV $3CL^{pro}$ strain through computer modelling. The technique could also identify Sabedinine as an inhibitor of CoV protease from *Veratrum sabadilla* (Toney et al. 2004) and Aurantiamide acetate from Artimisiaannua (Wang et al. 2007).

A group of Tanshinones and Rosmariquinones (Park et al. 2012) acted as SARS-CoV viral enzyme inhibitors from the ethanol extracts of *Salvia miltorrhiza* found using $3CL^{pro}$ inhibition assay. Amentoflavone, Bilobetin, Ginkgetin, Sciadopitysin were isolated from the ethanol extracts of *Torreya nucifera* using the same assay and found to non-competitively inhibit CoV $3CL^{pro}$ (Ryu et al. 2010a). A similar work by the author's team (Ryu et al. 2010b) reported about the competitive inhibition of CoV protease by Celastrol, Tingenone, Iguesterin and Pristimerein isolated from the methanol extracts of *Tripterygium regelii*.

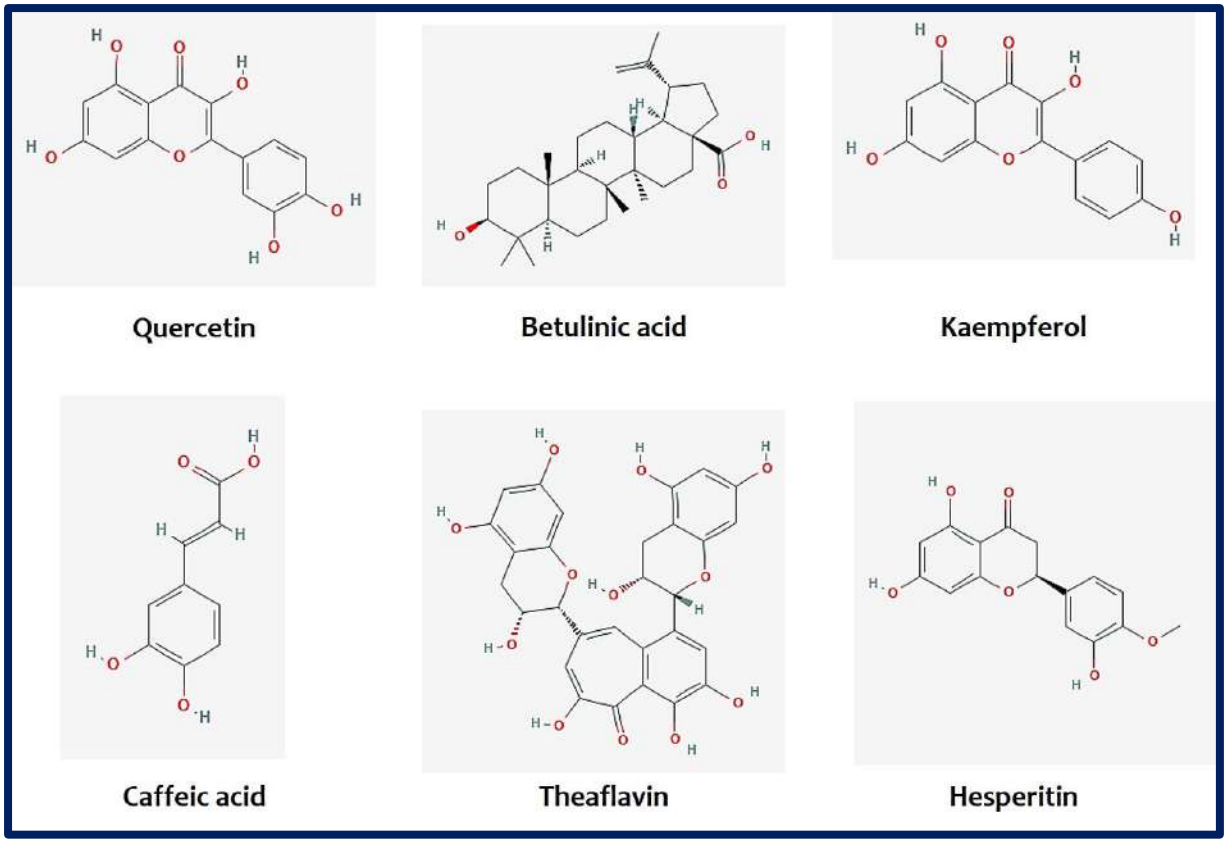

Figure: Some commonly known phytochemicals with anti-viral activities. (Structures obtained from PubChem)

Conclusion

Naturally occurring phytochemicals are anessential and powerful resource displaying anti-viral properties. Chemical modification of the reportedcompounds, virtually aided by computerised docking simulations, may perhaps increase their potency and/or viral selectivity. Some compounds seem promising for the treatment of coronavirus in humans includingpolyphenolics like Quercetin, yricetin, Caffeic acid, Psoralidin and Isobavachalcone, lectins such as Griffithsin, Lycorine and others like Scutellarein, Silvestrol, Tryptanthrin, Saikosaponin B2. Though these compounds need to go through tests of cytotoxicity and hence *in vitro* and *in vivo*assessmentsare required to determine the safe and therapeutic rangesofthese compound before human clinical trials could be performed. Establishment of an truly effective drug or any other therapeutic tool against COVID-19 and allies would be the most awaited scientific

breakthrough. There is a bank of such finely functioning phytochemicals against viruses, all they need is a pinch of limelight.

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Article11

Regulation of MicroRNAs by Phytochemicals in Cancer Therapy

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Abstract

Cancer therapeutics all over the world is in dire need of targeted approaches to prevent any cancer cells from escaping to reduce the chances of cancer relapse. MicroRNAs, the small non-coding RNA molecules, represent such precise cellular sites. Every cancer forms have been identified with such marker microRNAs, either behaving as oncogenes or tumour suppressor genes. They majorly target genes in cell cycle, proliferation, death or other signalling pathways; hence acting as efficient therapeutic targets. Although microRNA modulation using synthesized miRNA mimics/inhibitors is possible, use of natural phytochemicals characterise a safer approach. Naturally accessible bioactive compounds have always topped the priority chart in drug development, and present studies have been focusing on their ability to target microRNAs to inhibit the cancer cell proliferation. This article is an attempt to review the use of natural phytochemicals to modulate the microRNAs expression in cancer therapeutics.

Introduction

Among the multiple types of non-coding RNA molecules discovered so far, microRNAs (miRNAs) represent the ones regulating the post-transcriptional gene expression. They are usually single stranded, conserved in nature and 18-25 nucleotides long. Till date, MicroRNAs have been described to control broad spectrum of cellular activities like development,

proliferation, differentiation, metabolism, survival and cell death (de Planell-Saguer and Rodicio, 2011).

Ambros along with his team reported the first miRNA from *C. elegans*, named as lin-4 (Lee et al., 1993). In present times, several other cellular miRNAs have been garnered into knowledge; 28645 annotated human miRNA precursor genes forming ~35828 mature miRNA products in 223 species are known. Later, Croce's group identified the first implication of miRNA in human cancer (B-cell chronic lymphocytic leukaemia), depicting downregulated or removed miR-15a and miR-16-1 (Calin et al., 2002). It was then later discovered that these two miRNas act as tumour suppressors in malignant B cells and many other malignancies (Cimmino et al., 2005 and Calin et al., 2008). In the next few years, miRNA profiling and sequencing has led to the understanding of miRNA dysregulation in cancer and that these miRNA signatures can act as novel diagnostic biomarkers. Hence, these signature microRNAs represent precise targeted therapeutics to recede the soaring cancer mortality.

With respect to the available chemotherapeutic approaches, natural sources have always served as efficacious and inexpensive source either alone or as adjuvants in combination therapy. Examples include natural products from plants, like vincristine, vinblastine, paclitaxel, camptothecin, or from marine organisms like cytarabine or from microorganisms like dactinomycin, bleomycin, doxorubicin, etc. (Bhanot et al., 2011) Some of the plant derived dietary polyphenols like curcumin, epigallocatechin-3-gallate, resveratrol, catechin, quercetin, genistein, indole-3-carbinol, and its derivative 3, 3'-diindolylmethane have been reported to function through microRNA modulations; hence deemed to be probed further for developing targeted approach and are currently under clinical trials as well.

MicroRNA misbehaviour in cancer

Several deregulated microRNAs have been held responsible for the carcinogenesis of cells (Volinia et al., 2006). It is not merely a random occurrence; loss of function (e.g. deletions, insertions, mutations) of tumour suppressor microRNAs and gain of function (amplifications, translocations) of oncogenic microRNAs enhance the cancer progression (Peng and Croce 2016; Garzon et al., 2010). These microRNAs might not be function specific; like miR-221 acts as oncogene in liver cancer to inhibit the function of tumour suppressor PTEN while its activity of silencing the KIT oncogene in erythroblastic leukemia, makes it function like a tumour suppressor (Pineau et al., 2010; Felli et al., 2005); thus promoting the cancer formation in both the cases. The table below (**Table 1**) lists the reported functions of microRNAs in specific cancer types with their respective targets.

Table 1: MicroRNAs as oncogenes and tumour suppressors and their respective targets

miRNA	Tumour Type	Cellular Targets	References
ONCOMIRS			
miR-21	Colon, breast, pancreatic, lung, prostate, liver, gastric cancers	PTEN, TPM1, PDCD4, Maspin	Bautista-Sanchez et al., 2020
miR-155	Glioma, colorectal, liver, lung, breast cancers, CLL, AML	TP53INPI, PTEN, PDCD4, MAPK13/14, HDAC4, E2F2, SMAD2, etc.	Bayraktar and Roosbroeck, 2018.
miR-17-92	Lung, colorectal, breast, colon, gastric, prostate, nasopharyngeal, pancreatic cancers, lymphomas	PTEN, SMAD2/4, TGFβ, TSP1, CTGF, E2F1, AIB1, p21	Mogilyansky and Rigoutsos,

			2013; Fang et al., 2017
miR-106a	Colon and gastric cancers	RB1	Xiao et al., 2009; Diaz et al., 2008
miR-373	Testicular, breast, gastric, colon, liver, lung, prostate, pancreatic, cancer	LATS2, CD44, RAD52/23B, mTOR, SIRT1, LAMP1, JAK	Wei et al., 2015
miR-197	Lung, liver, pancreatic cancer	P53, NOXA, BMF, FOXJ2, FUS1	Wang et al., 2016
miR-221/222	Glioma, gastric, colorectal, cervical, breast, ovarian, thyroid, prostate, liver cancers, CLL	PUMA, KIT, p27(Kip1), p57(Kip2), PTEN	Song et al., 2019
TUMOUR SUPPRESSOR MIRS			
let-7	Lung, ovarian, breast, prostate, gastric, liver, thyroid and colorectal cancers	RAS, PRDM1, HMGA2, c-Myc. E2F, Cyclin A/D, Twist, Snail, Vimentin, Cadherin	Chirsev et al., 2019
miR-15/16	CLL, prostate cancers	Bcl-2, Wnt3a, WT1, Mcl-1, Cyclin D1	Aqeilan et al., 2010
miR-34	Breast, pancreatic, lung, liver, cervical and colon cancers	MDM4, c-myc, c-met, BCl-1, survivin, E2F3, Notch1, CDK4/5	Zhang et al., 2019; Misso et al., 2014; Geng et al., 2015

miR-17-5p	Prostate, breast, liver, lung, colorectal cancer	AIB1, E2F1, p21, BIM, PDCD4, PTEN, vimentin, TP53INP1	Bobbili et al., 2017
miR-26	Bladder, breast, oral squamous cell, thyroid, liver, prostrate, gastric, colorectal cancer	FUT4, PTEN, EZH2, PDHX, CDK8	Li et al., 2017; Gao and Liu, 2011
miR-29	Lung, breast, bladder, prostrate, hepatocellular, stomach, ovarian, glioma, cervical, AML, CLL cancer	MCL-1, TCL-1, DNMT3s, AKT2/3, CDK6, FOXM1	Kwon et al., 2019
miR-30	Glioma, lung, breast, liver, colorectal, ovarian, gastric, hepatocellular	MTDH, Notch1, Beclin1, vimentin, p53, E-cadherin, cyclin D1/D2	Jiang et al., 2018; Yang et al., 2017
miR-122	Hepatocellular, colorectal, breast, gastric cancer	Bcl-w, CCNG1, IGFR1, CDK4, CREB1	Ma et al., 2010; Wang et al. 2012; Rao et al. 2017
miR-124a	Hepatocellular, breast, pancreatic, gastric, colorectal, prostate, cervical cancer	AQP3, CDK6, FLOT1, Slug, ZEB2, Rac1, ROCK1	Jia et al., 2019
miR-127	Breast, gastric, ovarian, osteosarcoma	Bcl-6	Chen et al., 2013; Guo et al., 2013; Bi et al., 2016

miR-143/145	Colon, prostate, breast, gastric, ovarian, oesophageal, bladder, and osteosarcoma	Ras, ERK5, Akt, MACC1, TGFβ, KRAS, RREB1	Poli et al., 2020
miR-148a	Gastric, colorectal, pancreatic, liver, oesophageal, breast, non-small cell lung cancers, glioma, osteosarcoma	CCKBR, ROCK1, DNMT1, SMAD2, MMP7, BCL2, HPIP, MET, USP4, WNT1	Li et al. 2016
miR-181	Non-small cell lung, pancreatic, hepatocellular, colorectal, ovarian, prostate cancers, leukemia, glioma	Bcl2, PTEN, VCAM-1, Wnt, MKP5, ATM, SMAD7, TGFβ, BIM, Cyclin B1, TCL1	Huang et al., 2015; Indrieri et al., 2020
miR-363-3p/5p	Gastric, hepatocellular, lung, colorectal, ovarian cancers	NOB1, PCNA, NOTCH1, SP1, Sox 4	Song et al., 2015; Lin et al., 2017; Ying et al., 2017; Wang et al., 2017; Hu et al., 2016

MicroRNA based Therapeutic Approaches

MicroRNA based therapies present several benefits over the conventional chemotherapeutics in use. Since they are known to simultaneously regulate more than one gene, they help to achieve a cumulative effect at multiple levels of the same pathway; hence amplifying the therapeutic effect. This is particularly useful in case of a heterogeneous disease like cancer,

where deregulation of more than one pathway is often the underlying reason. Both use of synthesized miRNA mimickers/silencers or natural phytochemicals are within the present research premises which hold its own advantages and disadvantages. Although use of synthesized microRNAs is an upcoming highlight, use of natural products definitely overshadows the synthesized compounds in terms of non-toxicity and easy availability.

MicroRNA regulation by natural agents

Several literature reports till date have demonstrated aberration of miRNA expression in cancer cells after being treated with natural agents singly or in combination with approved chemotherapeutic drugs. These aberrant miRNAs when analysed, were found to be related to either oncogenes or tumour suppressor genes or transcription factors which resulted in anomaly of cell migration, invasion, proliferation and cell death. Many natural agents like curcumin, resveratrol, I3C (indole-3-carbinol), DIM (Diindolylmethane), EGCG (epigallocatechin gallate), quercetin, etc. have been reported to inhibit tumour growth with miRNA alteration ability. Few important phytochemicals and their mode of miRNA regulation have been enlisted.

Curcumin

The polyphenolic compound dietary curcumin is predominantly found accumulated in the rhizome region of turmeric plant (*Curcuma longa*). Along with its significant antioxidant and anti-inflammatory properties (Esatbeyoglu et al., 2012), curcumin has been shown to exert its anticancer activity in breast, cervical, oral, oral, gastric, colon, pancreatic, melanoma, and prostate cancer (Aggarwal and Shishodia, 2006; Saja et al., 2007). With respect to the miRNA regulation, several reports have been made till date in all these forms of cancer as listed in **Table 2**. Curcumin induced miRNA modulation have been often linked to the disruption in cell proliferation, apoptosis induction, reduction of migration/invasion, cell cycle arrest. Few

epigenetic modulations were also noted, such as tumour suppressive miR-203 promoter was found hypomethylated in response to curcumin (Saini et al., 2011).

Resveratrol

Resveratrol belongs to a class of phytoalexin compounds, or more specifically stilbenoid polyphenol, often observed in grape skins or berries, plums, and peanuts. Similar to curcumin, resveratrol treatment to control cancer cell proliferation is also linked with many reported miRNA modulations (**Table 2**). These miRNA modulations have been linked to cellular factors like inhibition of eEF1A2 (Vislovukh et al., 2013), Bcl2 (Liu et al., 2013), TGFβ1 transcript (Tili et al., 2010), PTEN (Dhar et al., 2011), Akt suppression *in vitro* and *in vivo* (Sheth et al., 2012), E2F3 transcription factor and its downstream target sirtuin 1 (Kumazaki et al., 2013). A notable study found upregulation of miR-141 and miR-200c in response to resveratrol helped to inhibit stem cell like characteristics in MDA-MB-231 cells (Hagiwara et al., 2012).

Genistein

Genistein belongs to isoflavone class of compounds, naturally found in fava beans, lupins, and soybeans (Kaufmann et al. 1997). Genistein also exerts its anticancer activity by means of several miRNA changes; associated with NF-$\kappa\beta$, Akt, estrogen/androgen mediated pathways (Banerjee et al., 2008), Notch 1 signalling pathway (Xia et al., 2012), reduced Bcl-XL, caspase-3 and -9 activation (Chiyomaru et al., 2013), MCM suppression for DNA replication blockage (Majid et al., 2010), induction of PTEN (Liu et al., 2013), F-box and WD-40 domain protein 7 (Ma et al., 2013) (**Table 2**). One interesting observation with genistein lies in the connection found between upregulated miR-34a and HOTAIR lncRNA, which when knocked down by genistein induced apoptosis and cell cycle arrest in PC3 and DU145 prostate cancer cells (Chiyomaru et al. 2013).

Epigallocatechin-3-Gallate (EGCG)

The epigallocatechin-3-gallate (EGCG) is also polyphenolic by nature, abundantly observed in green tea (*Camellia sinensis*). It has been reported to have significant anticancer effects along with the ability to alter several miRNA expression (**Table 2**). The miRNA modulations evidently was linked to apoptosis induction (Tsang and Kwok, 2010; Chakrabarti et al., 2012) and cisplatin sensitisation (Zhou et al., 2014).

Indole-3-Carbinol (I3C) and 3, 3'-Diindolylmethane (DIM)

The indole-3-carbinol (I3C) falls under glucosinolate class of compounds, naturally found in *Brassica* clade of vegetables, like broccoli, cauliflower, cabbage, radish, kale and brussels sprouts, while 3, 3'-diindolylmethane (DIM) is formed during I3C condensation reactions in the stomach. The potent anticancer effect of I3C and DIM is linked with several miRNA changes (**Table 2)**, associated with Cdc25A downregulation (Jin, 2011), reduction of EZH2 (Kong et al., 2012), PTEN, PDCD4 (Melkamu et al., 2010), ZEB1, slug, vimentin and E-cadherin changes (Li et al., 2009).

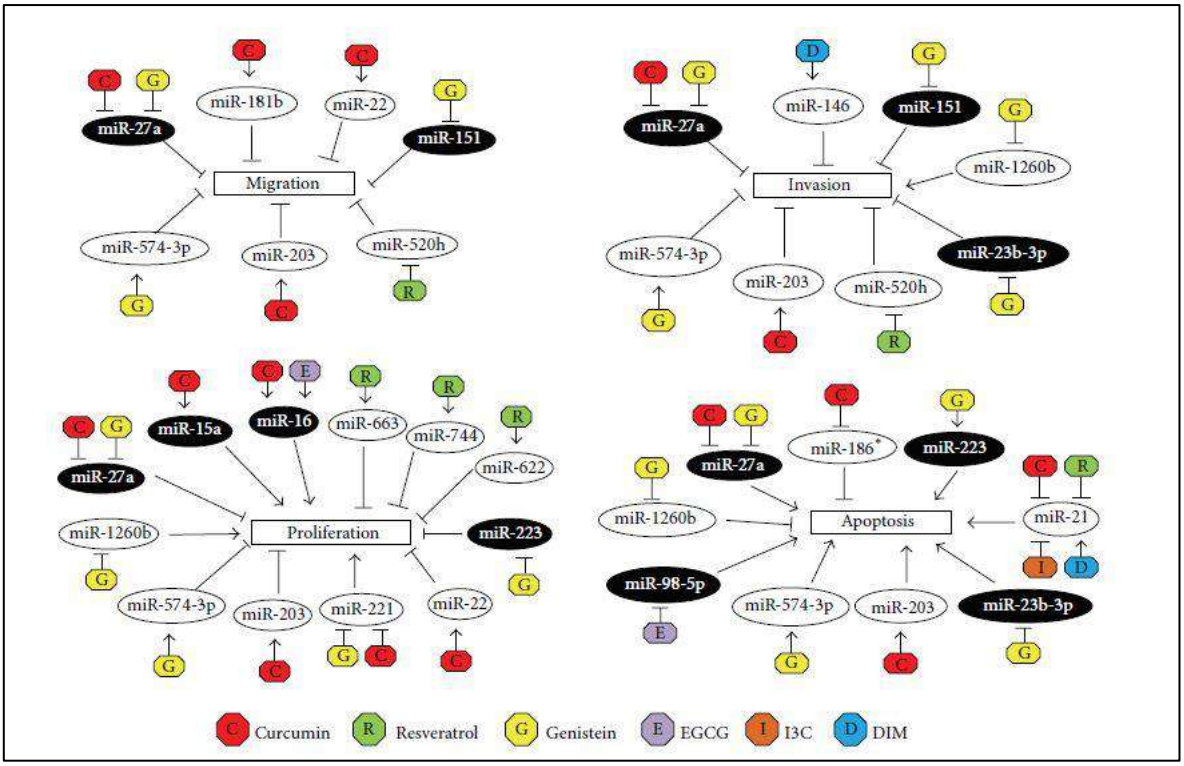
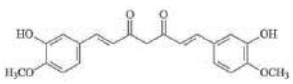
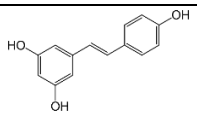
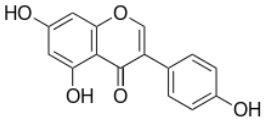
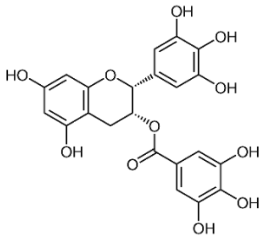


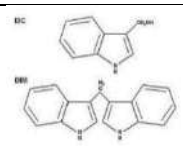
Figure 1. Regulation of miRNAs by natural agents and effects of miRNA inhibition (black ellipse) or overexpression (white ellipse) on cell migration, invasion, proliferation, and apoptosis. Inhibitory relationships are denoted as flat arrow heads, whereas positive interactions are denoted as open arrow heads. (Phuah and Nagoor, 2014)

Table 2: Micro-RNA regulation by Phytochemicals

Natural Agents	Chemical Structure	miRNAs Regulated by Natural Agents	References
Curcumin		miR-7, miR-9n, miR15, miR-16, miR-17-5p, miR-19, miR-20a, miR-21, miR-22, miR -23a/b, miR-24, miR-26a, miR-27a, miR-34a, miR-39, miR-98, miR-101, miR-125b, miR 130a, miR-141, miR-146a,	Gandhy et al., 2012; Mudduluru et al., 2011; Saini et al., 2011; Zhang et al., 2010; Gao et al., 2012; Sun et al., 2008; Milenkovic et al., 2012; Kronski et al., 2014;

		miR-181a/b, miR-186n, miR-195, miR-196a, miR-199a, miR-200b/c, miR-205-5p, miR-320, miR-374, miR-429, miR-510, miR-576, miR-625 and let-7/7a,b,c,d,e,	Yang et al., 2010; Sreenivasan et al., 2012; Subramaniam et al., 2012; Liang et al., 2013; Dahmke et al., 2013; Mirzaei et al., 2017; Momtazi et al., 2016
Resveratrol		miR 662, miR 663, miR 774 , miR 21, miR-17, miR-25, miR-26a, miR-92a-2, miR-103-1 and -103-2, and miR-181a2, miR-17-92 and miR-106ab, miR 520h	Vislovukh et al., 2013; Liu et al., 2013; Han et al., 2012; Tili et al., 2010; Dhar et al., 2011; Sheth et al., 2012; Yen et al., 2018; Kumazaki et al., 2013; Follo-Martinez et al. 2013; Yu et al., 2013; Bae et al., 2011; Sachdeva et al., 2012
Genistein		miR 34a, miR 574-3p, miR 1296, miR 548-3p, miR-15a, miR 221, 222, miR 151, miR 23b-3p, miR 1260b, miR 223, miR 27a, miR-15b, miR-125a, miR-125b, and miR- 320, miR-155, miR-208b, miR-211, miR-376a, and miR-411, miR-494, miR-520g, and miR-542, miR 21, miR-151a-3p,miR-151-5p, miR-100, miR-122a,miR-125b,miR-126,miR-135,miR-135b, miR-136,miR-137,miR-141,miR-152,miR-190,miR-196a,miR-196b,miR-204,miR-205,miR-206,miR-217,miR-22,miR-296, miR-30a-3p,miR-30a-5p,miR-	Hirata et al., 2014; Ma et al., 2013; Sun et al., 2009; Xu et al., 2013; Xia et al., 2014; Rabiau et al. 2011; Zaman et al. 2012; Xia et al., 2012; Chiyomaru et al., 2013; Majid et al., 2010; Liu et al., 2013; Ma et al., 2013

		331,miR-335,miR-342,miR-362,miR-449b,miR-454,miR-497,miR-500,miR-501,miR-503, miR-515,miR-517c,miR-532,miR-565,miR-578,miR-584,miR-585,miR-590,miR-595,miR-625,miR-647,miR-7,miR-765, miR-766	
EGCG		miR 16, miR 210, miR-7-1, miR-34a, and miR-99a, miR-93 and miR- 7-1, miR 330, let-7, miR-18b, miR-20a, miR-25, miR-92, miR-93, miR-221, miR-320, miR 98-5p, miR-30b∗, miR-453,miR-520-e,miR-629, and miR-608, miR-92, miR-93, and miR-106b, miR 21, miR-10a, miR-18a, miR-19a, miR-26b, miR-29b, miR-34b, miR-98, miR-129, miR-181d, miR-467bn, miR-487b, miR-197,miR-805,miR-374n, miR-350,miR-24-1n,miR-137,miR-335-3p,let-7a,miR-222, miR-26b,miR-30c-1n, miR-98,miR-30c,miR-30bn,miR- 32,miR-674n,miR-532-5p,let-7g,miR-18a,miR-192,miR- 302d,miR-30b,miR-802,let-7e,miR-322,miR-720,miR-146b, miR-340-3p,miR-185,miR-425,miR-10a,miR-126-5p,miR- 101a,miR-30en,let-	Tsang and Kwok, 2010; Zhou et al., 2014; Chakrabarti et al., 2012; Siddique et al. 2011

		7c,miR-141,miR-33,miR-29an,miR-199b, miR-450a-5p,miR-21,miR-23a,miR-101b,miR-148a,miR-193, miR-23b,miR-107,miR-140,miR-551b,miR-466c-5p,miR- 106a,miR-590-3p,miR-875-3p,miR-224,miR-292-5p,miR-678,miR-469,let-7bn,miR-463nmiR-574-3p,miR-201,miR-290-3p,miR-181a,miR-302a,miR-429,miR-133a,miR-190b, miR-710,miR-135b,miR-296-5p,miR-191n,miR-188-5p,miR- 298,miR-181a-1n,miR-466g,miR-26bn,miR-466f-3p,miR- 29bn,miR-1224,miR-291b-5p,miR-324-5p,miR-486,miR-128, miR-450b-3p,miR-135an,miR-294,miR-671-5p,miR-878-3p, miR-801,miR-370,miR-1,miR-494,miR-133b	
I3C and DIM		let-7 family, miR 146a, miR-21, miR-31, miR-130a, miR-146b, and miR-377 miR-200b, miR-200c, let-7b, let-7c, let-7d, let-7e, miR-663, miR-638, miR-122, miR-149, miR 221 miR-21, miR-31, miR-130a, miR-146b, miR-377, miR-20b, miR-654, miR 34c	Jin, 2011; Kong et al., 2012; Li et al., 2010; Melkamu et al., 2010; Paik et al., 2013; Li et al., 2010; Ahmad et al., 2013

Conclusion

Since the discovery of miRNAs, they have been associated to be important players in the process of carcinogenesis. Cancer cells harbouring irregular miRNA expression develops the potential to sustain proliferative signalling, evade growth suppressors, resist cell death, activate invasion ad metastasis and induce angiogenesis (Peng and Croce, 2016). MiRNA signature profiles represent unique markers for specific cancer cells, because of which miRNA targeted therapeutic approaches in cancer hold significant potential to achieve targeted therapy for elimination of specific cancer cells, relieving the patients from harmful side-effects. Several recent studies have found that natural agents from dietary components harbour immense potential to carry out miRNA based cancer therapeutics. More studies will be required in future to validate the reality of miRNA based therapeutics, but use of natural phytochemicals provides a safer and specific targeted way of therapeutic approach towards cancer. In addition to the phytochemicals discussed here, several other phytochemicals like quercetin, camptothecin, sulforaphane, silymarin, garcinol, luteolin, lycopene, astaxanthin, ellagitanin, etc. have also been reported to modulate microRNAs to exert their anticancer effects (Kim 2019; Kang, 2019; Debnath et al., 2017; Rafiei et al., 2020; Noratto et al., 2011; Slaby et al., 2013; Li et al., 2014; Moeng et al., 2020; Sahin et al., 2017; Wen et al., 2009; Beretta et al., 2013; Singh et al., 2016). MicroRNA profiles of cancer cells bear diagnostic feature and hence targeting them help to achieve specific elimination of cancer cells. Moreover, the use of natural phytochemicals to target microRNAs provides a safer efficient approach, some of which has already reached clinical trials.

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Article 12

Taxonomic Study of Nodal and Petiolar anatomy of 10 species of Malvaceae.

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Abstract:

A comparative account on the nodal and petiolar anatomy of 16 species of Malvaceae was undertaken to assess the anatomical variation. These variations can be used as a taxonomic tool for species identification. The generalised nodal configuration is trilacunar three traced without any variations. Stipular trace originates as a branch from the lateral traces.The arrangement and number of vascular bundle differ in different elevation point of the petiole. Some variations are observed in the proximal zone of teh petiole in different species.

Introduction:

Malvaceae or the mallow family is a family of flowering plant containing over 200 genera with close to 2300 species (Judd and Manchester, 1997); ca. 22 genera and 93 species in India. Well known of this family include okra, jute and cacao. The largest genera in terms of number of species include *Pavonia* (200 species), *Sida* (200 species), *Dombeya* (225 species), *Sterculia* (250 species) and *Hibiscus* (300 species) (Judd and Manchester, 1997). Popular plants of the family Malvaceae are generally valued for commercial cotton, gorgeous spring blossoms and some for their colourful foliage. It is a globally distributed family with primary concentrations

of genera in the tropical and subtropical regions (Hutchinson 1967; Fryxell, 1975, 1988, 1998; Heywood, 1993; La Duke and Doeby, 1995; Mabberley, 1997).

The structure of angiospermous node and petiole, indeed, has been the subject of numerous and careful studies by different anatomist, and it has been found that in many families and even in certain genera the nodal and petiolar structure are sufficiently peculiar and conservative for use as diagnostic character for the group. Nodal and petiolar anatomy play important role in plant systematic. The Nodal anatomy and its taxonomic significance have been much discussed (Grew, 1675; Sinnott, 1914; Bailey, 1956; Howard, 1979; Esau, 1979). Howard (1962, 1979) advocated the petiolar anatomy along with the combination of nodal vasculature and other associated characters can be of immense importance of systematic studies.

Malvaceae Juss. Magnoliopsida- Malvales. Incl. Bombacaceae, Sterculiaceae, Tiliaceae 113/500 cosmopoliton mainly in tropical region.

The family Malvaceae s.s. is most closely to Bombacaceae, and the two are separated primarily on the basis of pollen characters. In the broader APG circumscription, Malvaceae s.l. corresponds to the four traditional plant families Malvaceae s.s., Bombacaceae, Sterculiaceae and Tiliaceae (Table 2). Thus, the family has expanded to include 250 genera and has been divided into nine subfamilies, one of which is Malvaceae s.s. These families are closely related to Malvaceae s.s. but they are not monophyletic groups as shown by numerous researchers on the Malvales.

In Malvaceae, mainly trilacunar three traced nodes are observed.

Objective:

The present study has been undertaken to represent the valuable anatomical as well as to extent morphological information and characteristic features which can been helpful to enrich the present concept of the classification and generic and specific delimitation' identification as well as to provide some additional information towards the betterment and understanding their systematic position. However, the main objectives are:-

1. Attempts were made to find out how far the nodal and petiolar anatomy is in agreement with the present taxonomic classification and what changes it suggests.

2. To identify the key character that help in identification of different taxa in non floriferous condition as well as fragmented and distorted materials.

MATERIALS AND METHODS:-

Materials :

Fresh material of mature and immature twigs of sixteen (16) wild species as well as cultivated species were collected from different parts of West Bengal, viz. Ballygunge, Baruipur, Kalyani, Kasba, AJC Bose Indian Botanic Garden, Howrah, Baghajatin, *Agri-Horticultural Society* of India, Alipur, Sonarpur, etc. Herberium specimens were prepared for all available specimens collected during this study.

Petioles from the matured leaf of all the samples were examined for studying the petiolar anatomy, the younger nodes were examined for studying the nodal anatomy.

Method:

The anatomical studies of the nodes were done followed by the petiolar anatomy of the same material to get the continuation of of vascularisation pattern. Free hand transverse sections were made. Free hand sections were made. Petioles of shorter size were cut throughout, from proximal to distal regions, while in case of longer petiole 3 regions were

selected. Sections were stained with 2% safranin , some of the very fine sections were double stained (2% safranin in 50% ethanol) and light green (1% solution in absolute ethanol) and mounted on Canada Balsam.

The diagrammatic drawings of all the nodes and petioles as in transverse sections were drawn under microscope with the help of Camera Lucida. Few of the sections were photographed using Leica DM750 compound microscope at Taxonomy and Biosystematics Laboratory, Department of Botany, University of Calcutta. Few photographs were also taken through Nikon Coolpix camera.

OBSERVATIONS:

1. *Abutilon indicum* (L.) Sweet.:

NODE: (Fig:1.C1-C4): T.S. of the nodal region reveals the node to be trilacunar, each lacuna with a single trace. All the traces more or less of equal size. Of these three traces, the median trace cuts earlier than the two lateral traces which cuts at the same levels of the stem. Mucilage canals observed in the pith region.

Median trace undergoes division producing two more traces forming three traces which enter into the petiole as petiolar trace.

The lateral trace contributes to form the stipular trace. Part of the lateral traces travels to the stipules and the other part moves towards the median trace to form petiolar trace.

PETIOLE:

Proximal End (Fig:1.D 1) of the petiole shows a more or less oval configuration. Vascular bundle 9, arranged in a circular manner observed. Mucilage canals observed in the cortical and

pith region**. Middle Part: (Fig:1.D 2)** of the petiole persists similar configuration as in the proximal portion. **Distal End: (Fig:1.D 3)**T.S. of the distal portion of the petiole shows a more or less circular outline. Here the vascular traces unite together to form 2 traces. The vascular bundle towards the adaxial surface more or less semi-lunar whereas that on the abaxial surface more or less triangular shaped. The main vascular bundle divides into 2 xylem segments that remain embedded in sclarenchymtous pith forming accessory vascular bundle.. Mucilage canal absent in this region.

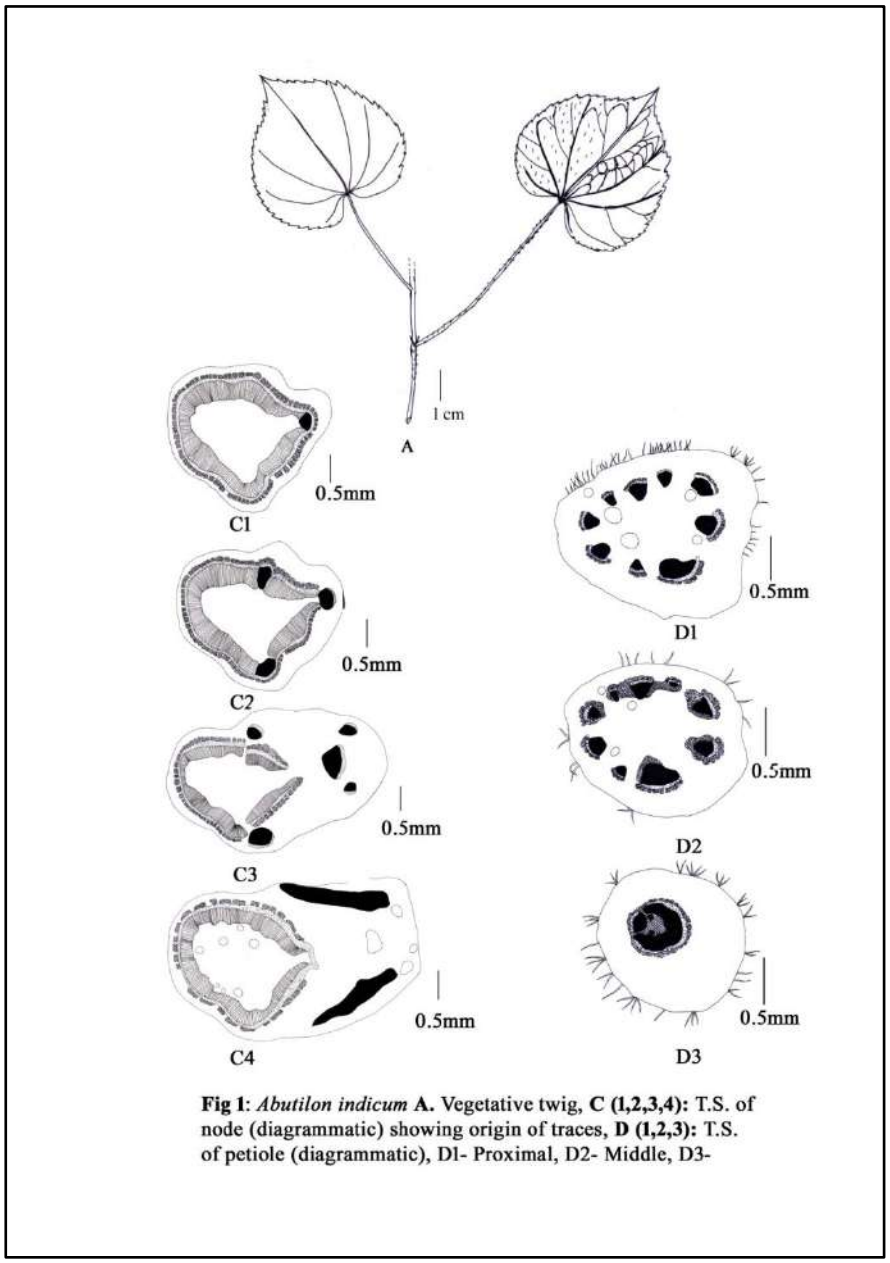

Fig 1: *Abutilon indicum* **A.** Vegetative twig, **C (1,2,3,4):** T.S. of node (diagrammatic) showing origin of traces, **D (1,2,3):** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3-

2. *Abutilon ranadei* Woodrow & Stapf:

NODE:(Fig:2.C1-C3): T.S. of the nodal region reveals the node to be trilacunar, each lacuna with a single trace. The median trace larger than the 2 laterals. All the trace cuts at the same level of the stem. The median contributes to form petiolar traces.

Part of the laterals gives rise to 2 stipular trace that moves to the stipules. Rest of the lateral trace ultimately moves towards the median trace together forming petiolar traces.

PETIOLE:

Proximal End: (Fig:2.D1) of the petiole shows a more or less circular outline. Presence of stellate hairs observed. Vascular bundles 8, arranged in circular manner. All the traces with sclerenchymatous bundle sheath present in patches. Mucilage canals observed in pith as well as cortical region. **Middle Part: (Fig:2.D2)** of the petiole persists similar configuration as that of the proximal part. **Distal End: (Fig:2.D3)** T.S. of the distal portion of the petiole shows almost a circular outline. The vascular bundles 6, remains free but surrounded by sclerenchymatous cells. Mucilage canals observed in the cortical region .

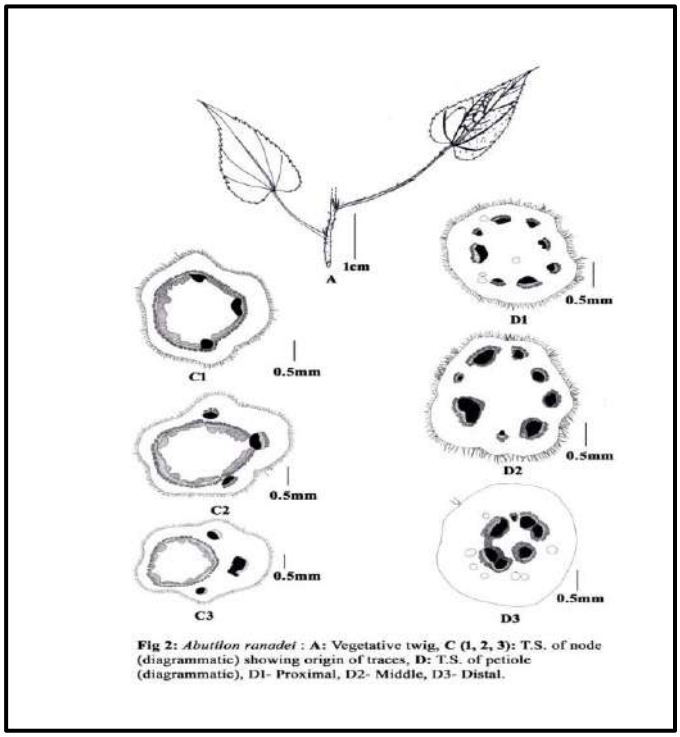

Fig 2: *Abutilon ranadei* : **A:** Vegetative twig, **C (1, 2, 3):** T.S. of node (diagrammatic) showing origin of traces, **D:** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal.

3. *Aloyogyne hakeifolia* (Giord.) Alef. [syn. *Fugosia hakeifolia* (Giord.) Hook.]

NODE: (Fig:2.C1-C3) T.S. reveals the node to be trilacunar, each lacuna with a single trace. The median trace slightly larger than the 2 laterals. The two lateral trace cuts earlier than the median trace . The median ultimately contribute to form petiolar traces.Part of the laterals gives rise to stipular trace that moves to the stipules. Rest of the lateral trace ultimately moves towards the median trace together forming petiolar traces. Presence of stellate hairs observed in the surface.

PETIOLE:

Proximal End: (Fig:2.D1) shows a more or less oval in shape. Presence of stellate hairs observed. The Vascular bundle single, almost circular with a small cut at the right lateral adaxial face. All the traces with sclerenchymatous bundle sheath present in patches. Mucilage canals are observed scattered in the pith region. **Middle Part: (Fig:2.D2)** persists almost similar configuration. **Distal End: (Fig:2.D3)** petiole shows a more or less oval in shape. Here the vascular traces merge to form a central circular trace. The vascular bundles with sclerenchymatous bundle sheath present in patches. Mucilage canals are present in the pith and cortical region.

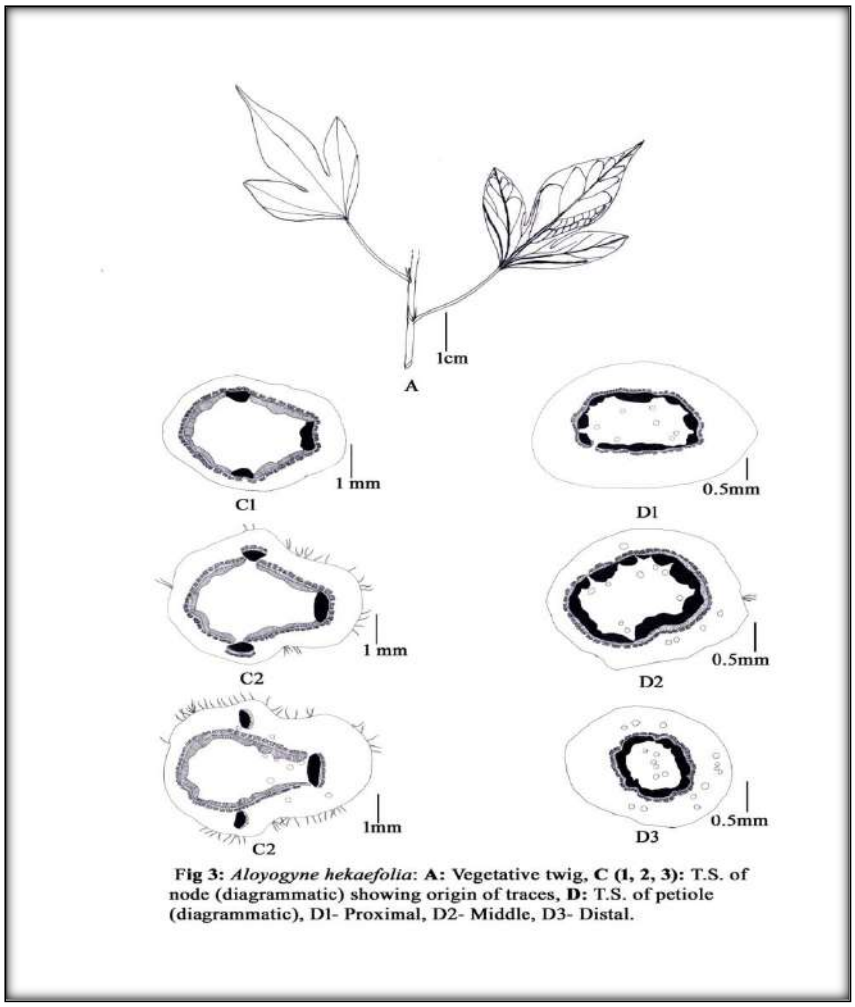

Fig 3: *Aloyogyne hekaefolia*: **A:** Vegetative twig, **C (1, 2, 3):** T.S. of node (diagrammatic) showing origin of traces, **D:** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal.

3. *Hibiscus schizopetalus* (Dyer) Hook.*f*.

Node: (Fig:4.C1-C4) T.S. shows the node to be trilacunar, each lacuna with a single trace. The median trace slightly larger than the two lateral traces. The lateral trace cuts earlier and at different levels of the stem. Of the two lateral trace one cuts earlier than the other and the median trace cuts at later level. The median trace later divides into 2 subsequent traces ultimately forming 3 traces which later forms petiolar trace.The petiolar traces later moves to the median trace after contributing to the stipular trace and ultimately together forms the

petiolar trace. Mucilage canals are observed throughout the cortex and few present in the pith region.

PETIOLE: Proximal End: (Fig:4.D1)T.S. shows a more or less oval outline. Vascular bundles 7, arranged in a circular manner. All the traces with sclerenchymatous bundle sheath in patches. Mucilage canal present in the pith and cortical region in a more or less circular manner. **Middle Part: (Fig:4.D2)** T.S. shows a more or less oval outline. Presence of hairs observed on the surface. Vascular traces 3, unite to form more or less semi lunar in shape. **Distal End: (Fig:4.D3)** T.S. the vascular traces merges together to form a more or less circular vascular bundle.

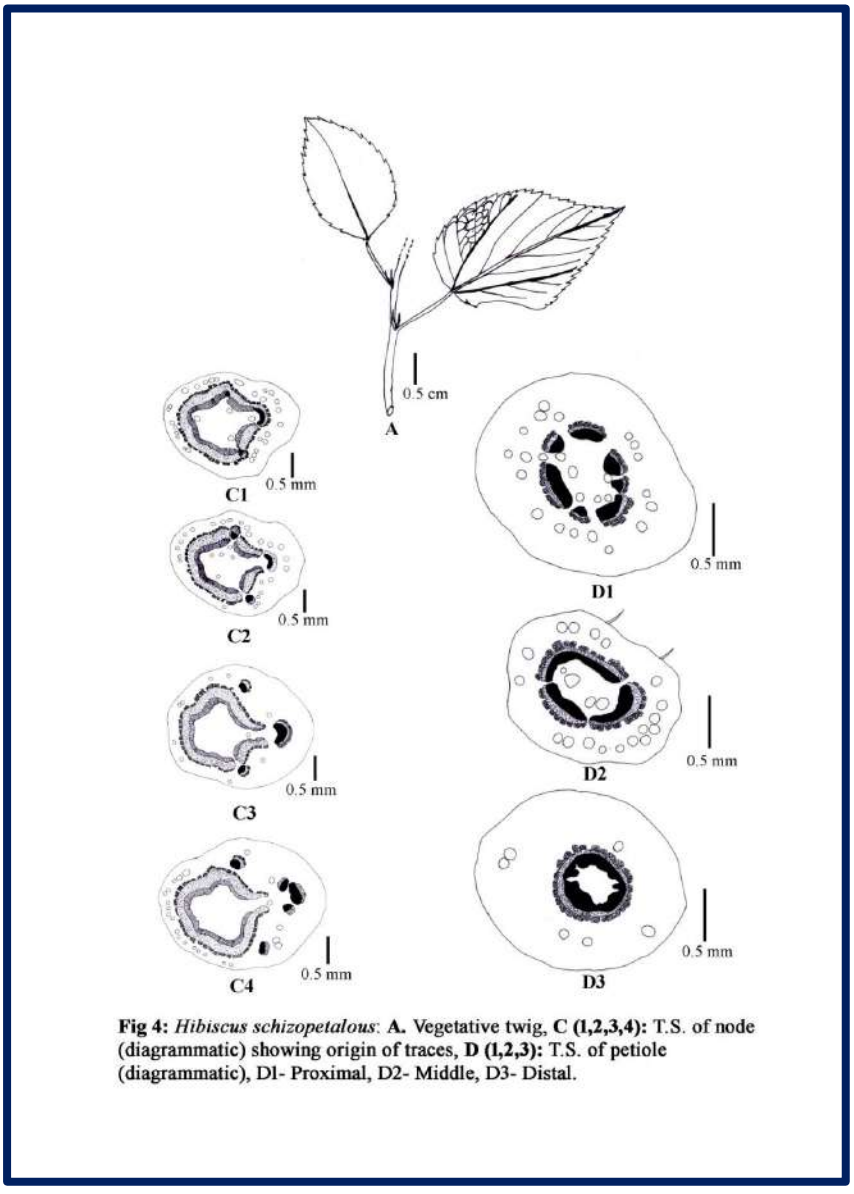

Fig 4: *Hibiscus schizopetalous*: **A.** Vegetative twig, **C (1,2,3,4):** T.S. of node (diagrammatic) showing origin of traces, **D (1,2,3):** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal.

5. Hibiscus tiliaceus L.

NODE: (Fig:5.C1&C2) T.S. the node to be trilacunar, each lacuna traces. The median trace are slightly larger than the two lateral traces. The traces cut almost at the same level. Mucilage canals observed in the pith and cortical region.The median trace moves towards the petiole forming petiolar trace. The two laterals divides and a part of the trace forms stipular trace and then move to middle trace forming petiolar trace.

PETIOLE:

Proximal End:(Fig:5.D1) T.S. circular outline with flattened adaxial surface. Vascular bundles 10, arranged in circular manner.. Mucilage canals present in the pith and the cortical region in a more or less circular pattern. **Middle Part:(Fig:5.D2)** T.S. shows almost circular outline with presence of few hairs on the surface. Vascular bundle 1, traces here merges to form a single vascular bundle. **Distal End: (Fig:5.D3)** T.S shows almost a circular outline. The main vascular bundle invaginates from the adaxial face towards the pith and divides into many segments of xylem that remains embedded within the central phloem. These segments of xylem are the accessory vascular bundle. The thickness of the xylem in the main bundle more compared to the phloem. Mucilage canal and bundle sheath absent.

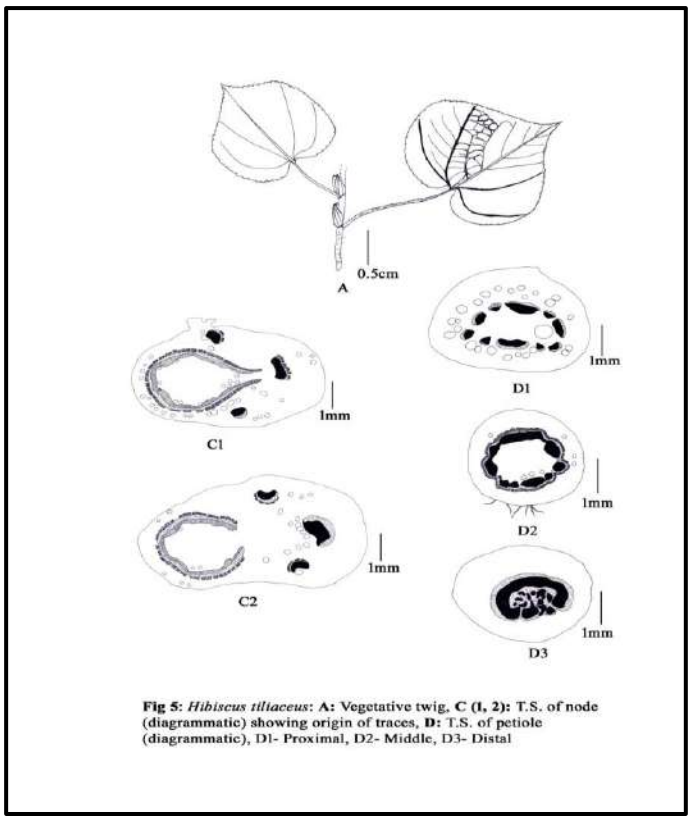

Fig 5: *Hibiscus tiliaceus*: **A:** Vegetative twig, **C (1, 2):** T.S. of node (diagrammatic) showing origin of traces, **D:** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal

6. *Hibiscus hirtus* L.

1. **NODE: (Fig:6.C1-C4)**T.S. shows node to be trilacunar, each lacuna traces with single trace. The median trace are slightly larger than the two lateral traces. The traces cuts almost at the same level. Mucilage canals observed in the cortex and pith. The median trace later moves to the petiole forming petiolar bundle.Of the two lateral traces, one divides earlier than the other and a part of the trace forms stipular trace and then move to middle trace forming petiolar trace.

PETIOLE:

Proximal End: (Fig:6.D1) T.S. shows a more or less oval outline with flattened adaxial surface. Vascular bundles 4, arranged in a circular manner. Presence of hairs observed. **Middle Part: (Fig:6.D2)** T.S. shows a more or less circular configuration with flattened

adaxial surface. Vascular traces 4, sparsely arranged however connected by a cambium layer. Presence of long stellate hairs are observed. **Distal End: (Fig:6.D3)** T.S. shows almost circular outline with a slight flattened adaxial surface. Vascular traces merges together to form 3 bundles. The vascular bundles surrounded by sclerenchymatous tissue. Presence of long stellate haits are observed

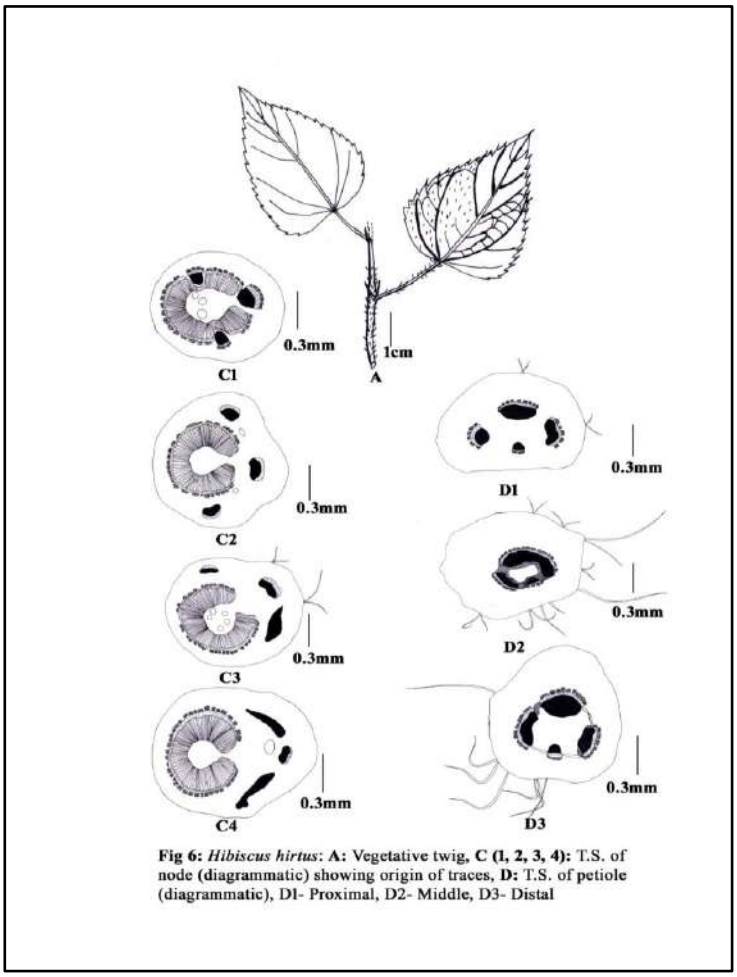

Fig 6: *Hibiscus hirtus*: **A:** Vegetative twig, **C (1, 2, 3, 4):** T.S. of node (diagrammatic) showing origin of traces, **D:** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal

7. *Hibiscus mutabilis* L.

NODE: (Fig:7.C1-C3) T.S. reveals the node to be trilacunar, each lacuna with a single trace. The median trace slightly larger than the 2 laterals. All the traces cuts at the same levels of the stem. The median trace undergoes further division and ultimately divides further into 2 more trace which ultimately contribute to form petiolar traces.Part of the laterals gives rise to stipular trace that moves to the stipules. Rest of the lateral trace ultimately moves towards the median trace together forming petiolar traces.

PETIOLE:

Proximal End: (Fig:7.D1) T.S. shows a more or less oval in shape. Presence of stellate hairs observed. Vascular bundles 8, arranged in a circular manner. **Middle Part: (Fig:7.D2)** T.S. shows a more or less oval in shape. Presence of stellate hairs observed. Vascular bundles 4, present in circular pattern. Few traces remains free in this region. **Distal End: (Fig:7.D3)** T.S. shows a more or less oval in shape. Presence of stellate hairs observed. Here the traces merge to form a single trace at the centre. Mucilage canals present in the pith and cortical region.

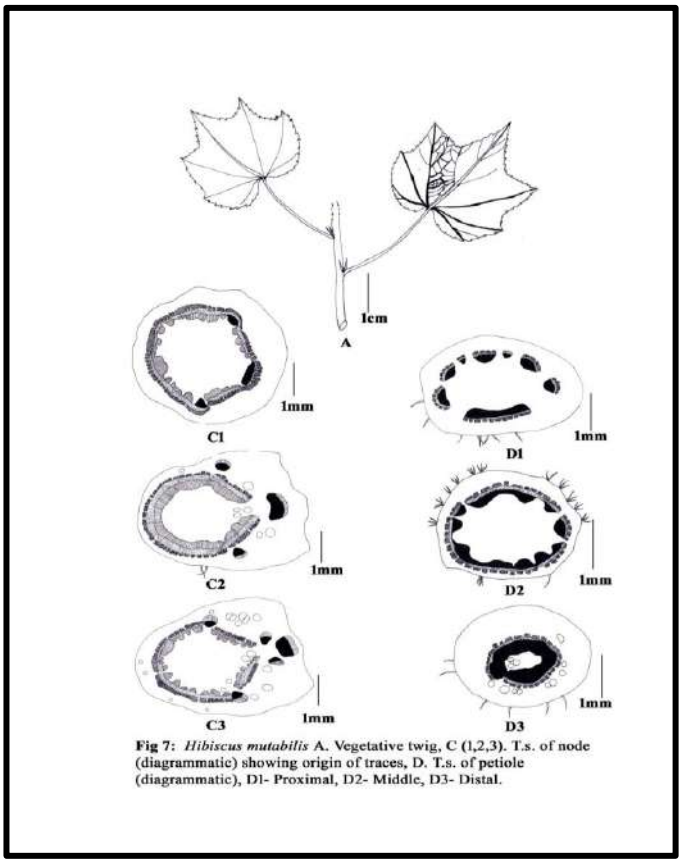

Fig 7: *Hibiscus mutabilis* A. Vegetative twig, C (1,2,3). T.s. of node (diagrammatic) showing origin of traces, D. T.s. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal.

8. *Hibiscus rosa-sinensis* L.

NODE: (Fig:8.C1-C4) T.S shows the node to be trilacunar, each lacuna with single trace. The median trace is slightly larger than the two laterals. The laterals of equal size. The two lateral traces cut earlier than the median trace and at the same level. Median trace divides into 2 subsequent traces thus forming 3 traces that ultimately forms petiolar trace. The lateral traces divide and a small part of it contribute to form stipular trace and the rest moves towards the middle trace ultimately forming petiolar bundle.

PETIOLE:

Proximal End: (Fig:8.D1) T.S. shows circular outline. Vascular bundles 7-10, arranged in a more or less circular manner. Mucilage canals observed in the cortical region in a more or less circular pattern. **Middle Part: (Fig:8.D2)** T.S. shows circular outline with adaxial surface slightly flattened. Vascular bundles 2, abaxial one larger, semi-lunar; adaxial smaller. elongated. **Distal End: (Fig:8.D3)** T.S. shows the vascular bundles merges together to form a more or less circular vascular bundle. The thickness of the xylem layers uneven and more than that of the phloem the even. Mucilage canals absent.

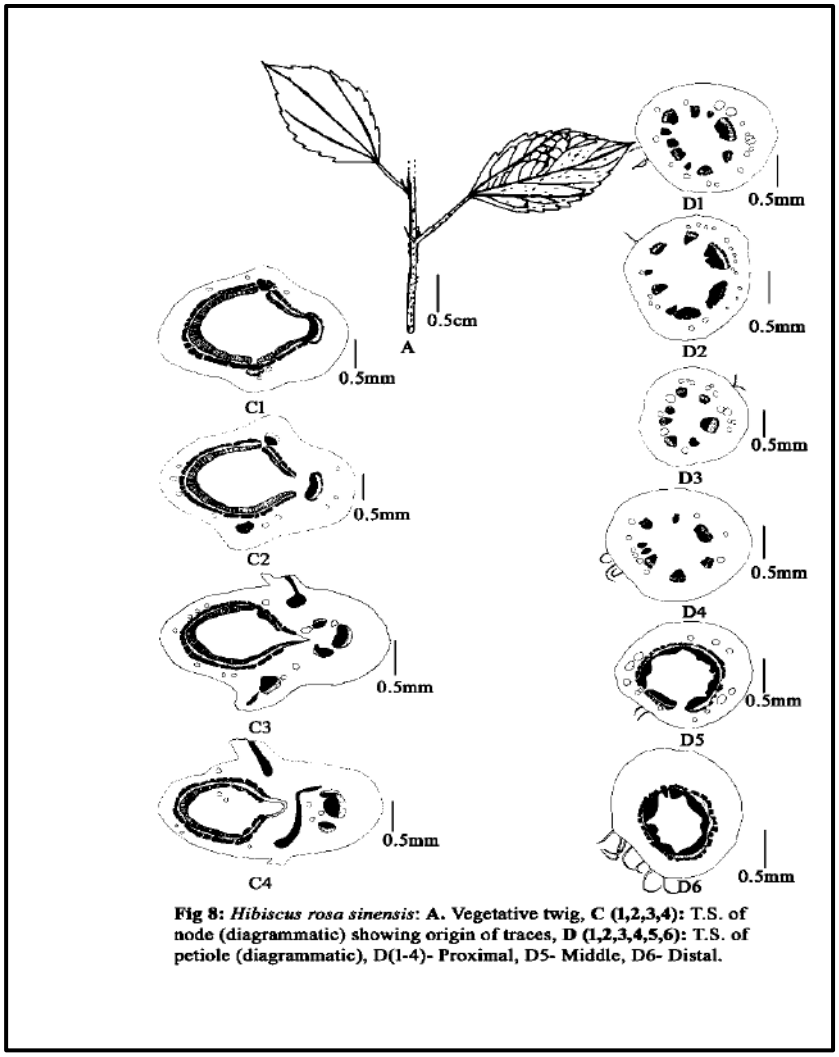

Fig 8: ***Hibiscus rosa sinensis*****: A. Vegetative twig, C (1,2,3,4): T.S. of node (diagrammatic) showing origin of traces, D (1,2,3,4,5,6): T.S. of petiole (diagrammatic), D(1-4)- Proximal, D5- Middle, D6- Distal.**

9. *Malachra capitata* (L.) L.

NODE: (Fig:9.C1-C4) T.S. reveals the node to be trilacunar, each lacuna with a single trace. The median trace larger than the 2 laterals. All the trace cuts at the same level of the stem. The median ultimately divides into 2 more traces that ultimately contribute to form petiolar traces.

Part of the laterals gives rise to 2 stipular trace that moves to the stipules. Rest of the lateral trace ultimately moves towards the median trace together forming petiolar traces.

PETIOLE:

Proximal End: (Fig:9.D1) T.S. shows a more or less oval outline. Presence of stellate hairs observed. Vascular bundle 6, arranged in circular manner. **Middle Part: (Fig:9.D2)** T.S. persists similar configuration as that of the proximal part. Mucilage canals observed in the cortical region. **Distal End: (Fig:9.D3)** T.S. shows almost a circular outline. Presence of

stellate hairs observed. The vascular bundle single, somewhat distorted in appearance as xylem remains in two segments, followed by discontinuous phloem, though the bundle sheath remain continuous. Sclerenchymatous cells occupy the central pith completely.

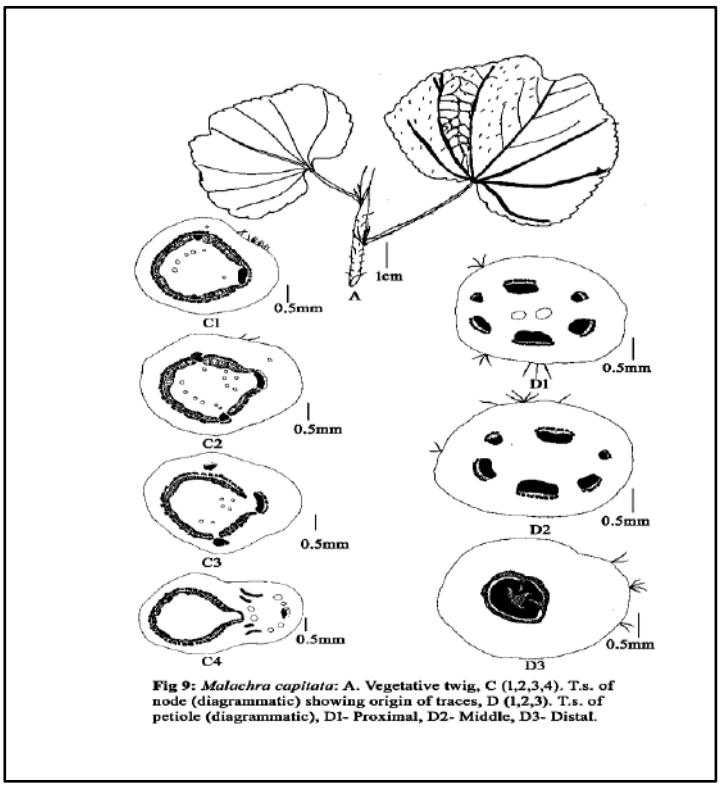

Fig 9: ***Malachra capitata*****: A. Vegetative twig, C (1,2,3,4). T.s. of node (diagrammatic) showing origin of traces, D (1,2,3). T.s. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal.**

10. *Malvaviscus arboreus* Cav.

NODE: (Fig:10.C1-C3) T.S. reveals the node to be trilacunar, each lacuna with a single trace. The median trace slightly larger than the 2 laterals. The median trace cuts earlier than the laterals. The median and the lateral trace ultimately contributes to form petiolar trace.

PETIOLE: Proximal End: (Fig:10.D1)T.S shows a more or less circular outline. Presence of hairs observed at the abaxial surface in a row. Vascular bundles 12, arranged in a circular manner. **Middle Part: (Fig:10.D2)** T.S. persists the similar character as that in the proximal region of the petiole except the stellate haris observed in the abaxial side. **Distal End: (Fig:10.D3)** T.S. shows a more or less circular outline. Presence of hairs observed at the abaxial surface. Vascular bundles 7, arranged near the centre in a circular manner.

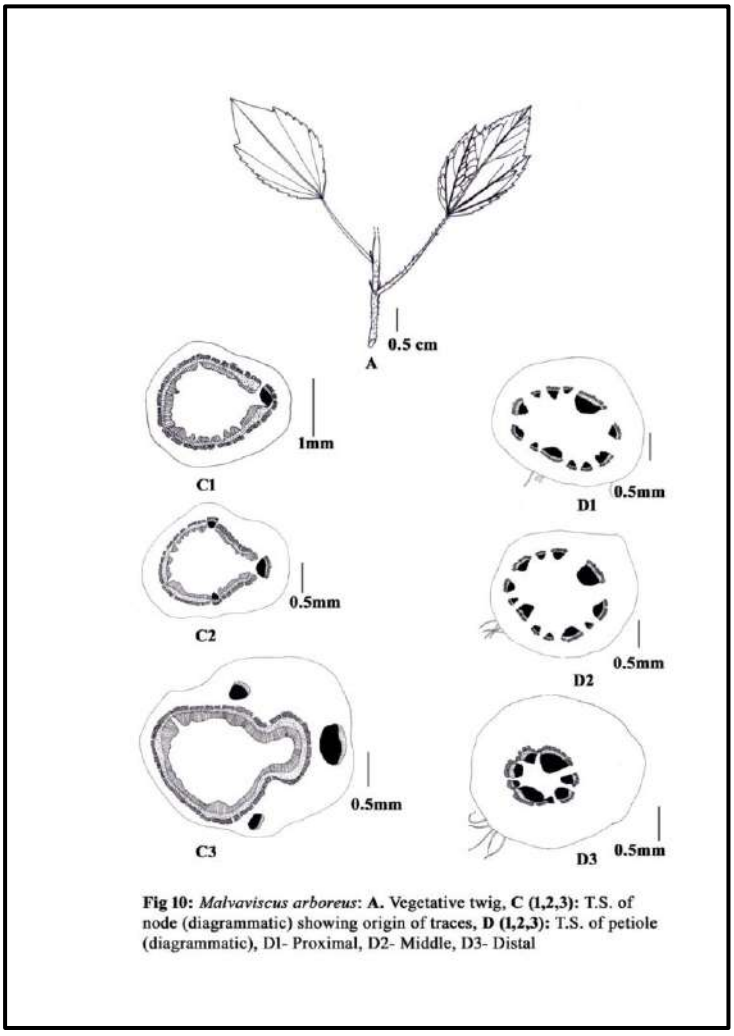

Fig 10: *Malvaviscus arboreus*: **A.** Vegetative twig, **C (1,2,3):** T.S. of node (diagrammatic) showing origin of traces, **D (1,2,3):** T.S. of petiole (diagrammatic), D1- Proximal, D2- Middle, D3- Distal

Discussion and Conclusion:

Anatomical features are widely used in systematic for identification, for placing anomalous group in a satisfactory position in classification and for indicating the pattern of relationship that may have been obscure by superficial convergence in morphological features.

The Nodes of the members of Malvaceae are uniformly trilacunar, having one trace to each lacuna as also recorded earlier by Sinott (1914), Metcalfe and Chalk (1950). Sinott (1914) has concluded that the trilacunar three traced condition of node to be primitive. Presently a total of 16 species of the family Malvaceae have been studied, of which all the members showed the trilacunar three traced condition.

The petiole is a specialized structure of leaf because its vasculature maintains the continuity between the stem and leaves. A complete survey of petiolar anatomy particularly the configuration of the vascular traces in T.S. can be used for a proper and easy identification up to generic and species level and also clarification of taxonomic relationship. In petiolar anatomy three zones- Proximal End, Middle Part, Distal End holds equal significance. The presences of mucilaginous canals are observed in all the specimens throughout in the cortical and the pith region seen in the T.S. of nodal and petiolar region. In all of the studied species, the vascular bundles of nodal and petiolar region encircled by discontinuous patches of sclerenchymatous bundle sheath observed.

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Article 13

BRIEF OVERVIEW ON THE DYNAMICS OF SIGNAL TRANSDUCTION CASCADE OF PLANT HORMONE JASMONIC ACID AND ITS MULTIFACETED ROLE IN PLANT DEVELOPMENT AND DEFENCE.

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1. Introduction

The noteworthy research carried out using large scale analyses of gene sequences (genomics), expression (transcriptomics), protein patterns (proteomics), metabolite profiles (metabolomics) and lipid compounds (lipidomics) resulting in the generation of enormous amount of data has given us an understandingas to how plants carry out various developmental programs simultaneously along with response to biotic or abiotic stress. A vital interplay carried out by plant hormones provide an indispensable link to such dynamic processes. Jasmonic Acid (JA), its methyl ester (MeJA) and isoleucine conjugate (JA-Ile) are derivatives of a class of alpha linolenic acid collectively referred to as jasmonates (JAs) is one such key molecule, that fine tunes a plethora of processes required all throughout the life cycle of a plant. In this article, the pathway from initiation of the signal cascade to its perception and response has been discussed. The article intends to highlight the involvement of JA in various developmental and stress related response thereby maintaining harmony within the plant cells.

2. Jasmonic Acid Biosynthesis And Response In Plants

2.1 Initiation of Jasmonic Acid Signaling

Systemin, a polypeptide signal molecule consisting of 18 amino acids, responded insect inflicted damage (biotic stress) in *Lycopersicon esculentum* (tomato). Systemin produced upon hydrolysis from prosystemin, a precursor protein consisting of 200 amino acids, is transported to other cells via the apoplast and unites with the cell surface receptor SR160 (a protein rich in leucine repeat units) to activate the JA signaling pathway alongside oligosaccharide signals induced by pathogens and fungal elicitors. AtPEP1, consisting of 23 amino acids, was identified in Arabidopsis thaliana. Analogous to the production of systemin, mechanical damage or pathogen infection prompts the hydrolysis of the precursor protein PROPEP1 (consisting of 92 aminoacids) to AtPEP1, that binds to an enzyme rich in leucine repeat units receptor PEPR1 present on the plasma membrane, activating the JA signaling pathway. Several phospholipases , including PLA2 in tomato and DAD1, DGL, and PLD in Arabidopsis are induced by systemin and AtPEP1 respectively, that acts on the galactolipids present in the chloroplast membrane to release linolenic acid, a precursor of JA synthesis.

2.2 Biosynthesis of Jasmonic Acid

In Arabidopsis, there are two parallel pathways of JAs, including the octadecane pathway and the hexadecane pathwaystarting from alpha linolenic acid (18:3) and hexadecatrienoic acid (16:3) respectively. Both the pathways require the three compartments as reaction sites (figure:1). Firstly, the chloroplast, where the synthesis of 12-oxo-phytodienoic acid (12-OPDA) or deoxy methylated vegetable dienic acid (dn-OPDA) from unsaturated fatty acid takes place. Secondly, at the peroxisome where the OPDA is converted to JA. And finally JA is metabolized andinto active compounds, namely MeJA, JA-Ile, cis-jasmone (CJ), and 12-hydroxyjasmonic acid (12-OH-JA) in the cytoplasm.

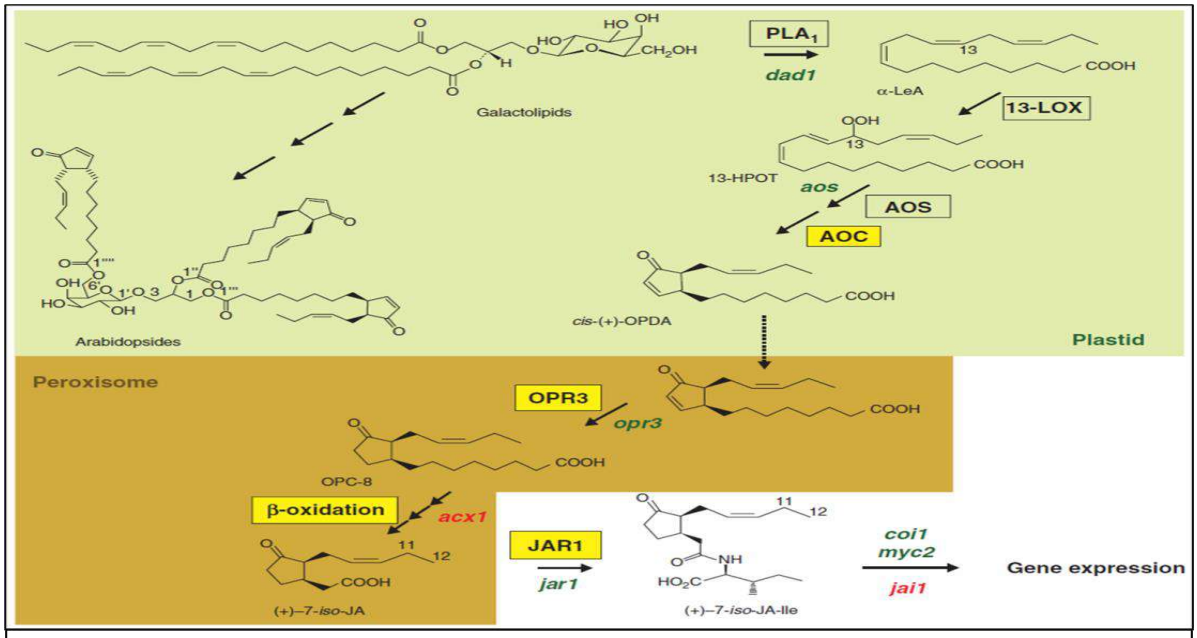

FIGURE1: Biosynthesis of jasmonic acid (JA)/JA-Ile from a-linolenic acid from galactolipids.
Steps impaired in mutants of Arabidopsis (green) or tomato (red) are indicated. Acx1: acyl-CoA-oxidase1; AOC:allene oxide cyclase; AOS: allene oxide synthase; coi1: coronatine insensitive1; dad1: delayed anther dehiscence1; 13-HPOT, (13S)-hydroperoxyoctadecatrienoic acid; jai1: jasmonic acid insensitive1; JAR1: JA-amino acid synthetase; a-LeA, a-linolenic acid; 13-LOX: 13-lipoxygenase; myc2: bHLHzip transcription factor MYC2; OPR3: OPDA reductase3; OPC-8: 3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid; cis-(+)-OPDA; cis-(+)-12-oxophytodienoic acid; PLA1: phospholipase A1.
SOURCE: Wasternack & Hause — Biosynthesis and action of jasmonates; Annals of Botany 111: 1021–1058, 2013

2.3 Signal Transduction

The traumatic signal triggers a defence response including both short-distance transmission and long-distance transmission of JA signals depending on whether it is a local defense response in the vicinity of the wound and/or a systemic acquired resistance (SAR) at a far off uninjured site, as well as induced defense responses from adjacent plants. transient accumulation of JA at the site of injury as response to insect feeding or mechanical injury activates the genes involved in local defense. Short-distance transmission may ply in one of the two ways where the systemin produced by the wounding acts as a signalling molecule to the adjacent site through the apoplast and phloem to activate the JA cascade reaction pathway or JA and JA-Ile induced by systemin act as signalling molecule that are transported to adjacent sites for defensive responses.

Similarly, in the long-distance transmission of JA signal is transduced via two pathways: vascular bundletransmission and/or airborne transmission. Studies conducted on JA mutants revealed that it is JA and MeJA are systemically transmitted in plants. Radioisotope labeling experiments tracked movement of MeJA through the phloem and xylem in vascular bundles alongside resynthesis of JAs during transport which was confirmed by the localization of various JA synthetases such as LOX and AOS in the companion cell–sieve element complex (CC-SE) of tomato vascular bundles and the sieve molecules in the phloem have the ability to form the JA precursor OPDA. An interesting experimental analysis demonstrated that the flow rate of the tomato phloem signal is 1–5 cm per hour whereas the accumulation of JA and JA-Ile can be detected in the whole plant within 15 min after mechanical damage. Ring-cutting experiments revealed even when the vascular bundle transmission was blocked, yet there was a rapid and strong defense gene expression in the distal leaves. In a wide range of plants, such as *Arabidopsis thaliana, Nicotiana tabacum, Phaseolus lunatus and Artemisia kawakamii*, MeJA being a volatile, easily penetrates the cell membrane spread by airborne diffusion to distant leaves and adjacent plants.

2.4 Perception of JA Signal

AtJAT1/AtABCG16, an ABC transporter identified in *Arabidopsis thaliana*, localises on the plasma and nuclear membrane of plant cells and aids in JA transport across the membrane. JA crosses the barrier of plasma membrane via the transporter into the cytoplasm where it is converted to the bioactive JA-Ile. Bioactive JA-Ile is then ferried across the inner membrane of the nucleus to activate JA responses.AtJAT1/AtABCG16 not only perceives the signal but also acts to regulate and maintain homeostasis of JA levels within a plant cell (figure2).

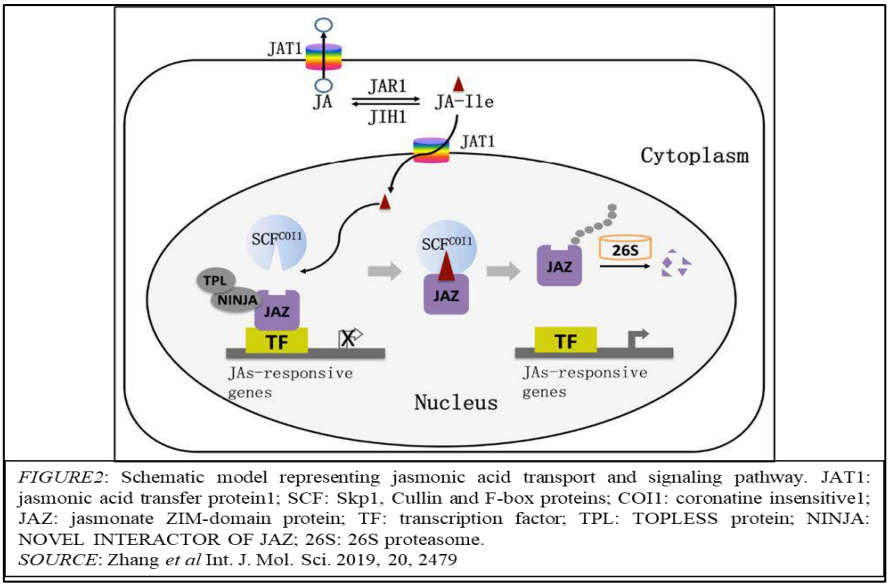

FIGURE2: Schematic model representing jasmonic acid transport and signaling pathway. JAT1: jasmonic acid transfer protein1; SCF: Skp1, Cullin and F-box proteins; COI1: coronatine insensitive1; JAZ: jasmonate ZIM-domain protein; TF: transcription factor; TPL: TOPLESS protein; NINJA: NOVEL INTERACTOR OF JAZ; 26S: 26S proteasome.
SOURCE: Zhang *et al* Int. J. Mol. Sci. 2019, 20, 2479

Mutant study in the *Arabidopsis* coronatine insensitive1 (coi1) lacking all JA responses indicated that the COI1 gene encodes an F-box protein which associates with the SKP1 protein and Cullin protein to form the SCF-type E3 ubiquitin ligase that is referred to as SCFCOI1 targeting the repressor proteins for degradation by ubiquitination. The repressor, Jasmonate Zinc finger Inflorescence Meristem (ZIM)-domain (JAZ) protein family interacts with COI1 via the Jas domain and interacts with MYC2 via the ZIM domain. COI1 and JAZ are coreceptors of JA signaling pathway having high affinity for the bioactive JA-Ile. The repressor proteins are thereby degraded after being transferred to the 26S proteasome, allowing the activation of transcription factors (TFs) bringing about activation of downstream response related genes.

2.5 Transcription Factors Regulating JA Signalling

JAZ-COI1 represses the MYC2 until, JA-Ile binds to the former and allows the activation of the latter. Transcription factors MYB, NAC, WRKY and ERF are also involved in JA signalling regulating the activation of genes directly involved in growth and development along with genes in combatting stress. JA pathway interacts with other hormones such as ethylene,

Abscisic acid (ABA), salicylic acid (SA) by activating the MAPK and calcium ion signalling pathways to regulate developmental processes in plants.

3. JA In Plant Growth, Development and Defense

3.1 JA in vegetative growth phase

Seed: In seed development, JA participates by inhibiting the seed germination via the OPDA which has been identified as an inhibitory compound.

Root: Root growth inhibition induced by JA occurs in orchestration with the effects of auxin in root growth and development. JA- MYC2 repression of PLETHORA, the key player in root stem cell niche activity, induced by JA affects the auxin. Auxin induced lateral root formation is inhibited by the conjugate of JA with tryptophanstrongly indicating a role of JA in lateral root formation. This is further backed up with the evidence where JA-insensitive mutant coi1-16 produces fewerlateral roots.

Leaf: Leaf senescence is a process that depends on various factors like the light/dark conditions, nutrients, biotic and abioticstresses, and numerous hormones. In *Arabidopsis thaliana*, the gene encoding the CHLOROPHYLLASE1 is strongly induced by JA and Rubisco-activase is down regulated by JA in a COI1-dependent manner. Transcription factors WRKY, WRKY54 and WRKY70 active in leaf senescence are linked to JA. Hyponastic growth or the upward leaf movement is yet another phenomenon stimulated by JA.

Trichome: In Arabidopsis, JA controls trichome initiation in a dose-dependent manner via targets of JAZ proteins are TFs such as MYB75, GL3 andEGL3.In *Gossypium barbadense*, the cotton fibre is a special type of single-cell seed trichome whose initiation and elongation are under hormonal control including JA.

3.2 JA in development of reproductive organs

In *Arabidopsis thaliana*, JA biosynthetic mutants, dad1, dde1, dde2, aos and opr3 and JA response related mutants coi1, myc2, have one defect in common, i.e., they are male sterile. They are characterised by defective anther, and/or non-viable pollen grains. Fertility in the plants could be restored by exogenous JA treatment. MYB21 andMYB24 were further identifiedas targets of JAZ repressors in opr3 mutants. In coi1 mutant background, the overexpression of MYB21 could partially restore the delayed anther dehiscence suggesting a vital role of MYB21 in stamen and pollen development. Cross-talk between JA–auxin governs flower development where ARF6 and ARF8 regulate JA biosynthesis in anther filaments (figure3). In tomato, homologue ofCOI1, jai1 impaired is female sterile, suggesting that JA signalling plays distinct roles in flower development in *Arabidopsis* and tomato. JA is involve in the stages of petal growth which are greatly dependent on cell proliferation along with cell expansion. Cytoplasmic male sterility (CMS), a maternally inherited phenomenon causing pollen abortion, is linked with JA biosynthesis.

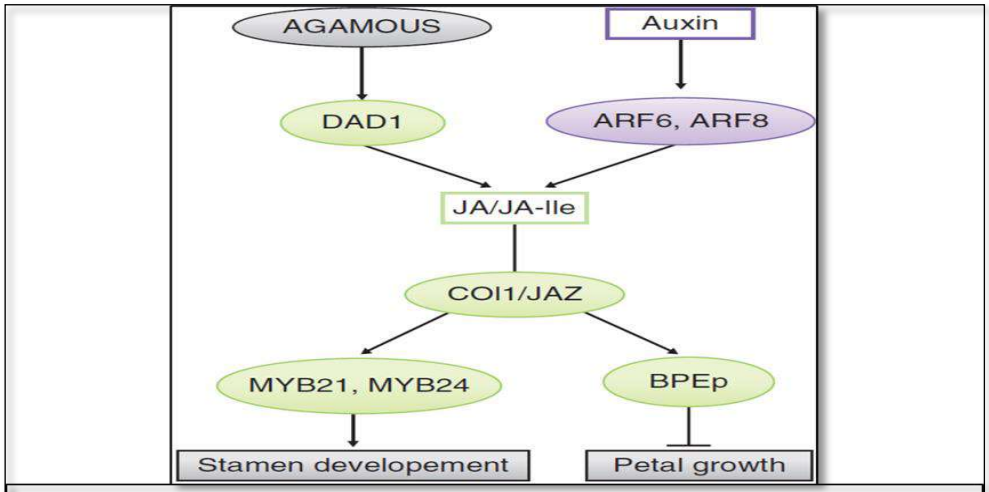

FIGURE3: The role of jasmonic acid (JA)/JA-Ile in flower development. ARF: Auxin Response Factor.
SOURCE: Wasternack & Hause — Biosynthesis and action of jasmonates; Annals of Botany 111: 1021–1058, 2013

3.3 Role of JA In Defense

Pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs) and develop PAMP-triggered immunity (PTI), suppressed by pathogen effectors. Conversely, the resistance gene products that recognize the effectors lead to Effector Triggered Immunity (ETI). The JA induced signalling cascade via SCFCOI1 –JAZ is the pillar for JA induced immunity against the necrotrophic pathogen, herbivores and phloem-feeding insects. In *Arabidopsis*, defence against herbivores is coordinated by circadian JA accumulation with circadian insect behaviour. JA acts in synergy with ABA during herbivory via MYCs to incur defence response through the expression of VSP2 and with Ethylene upon attack by necrotrophic pathogens via the ERF1 and PDF1.2. it was also suggested that 9-LOX oxylipins, confers resistance against biotrophic pathogens via JA signalling. Presumably the role of JA in Systemic Acquired Resistance (SAR) remains less explored.

4. Conclusion

JA signalling cascade is triggered by both external as well as internal stimulus. The absence of JA represses the responsive genes that may be triggered upon the need of the hour. JA was previously known to be induced as a result of defense response against pathogenic attack but later a breakthrough marked its involvement in the developmental processes. JA Biosynthetic mutants exhibit sterility at various levels highlighting the importance of JA in progression to the next generation. Apart from the functional aspects of JA mentioned in this article, there are many roles of the same that remain undiscussed. JA is also known to play part in governing light signalling, gravitropism, in establishing and maintaining arbuscular mycorrhiza (AM). Extensive study on JA biosynthetic and response pathway has been carried out in the past three

decades yet there are innumerable questions regarding the signalling cascade and regulatory process which remain unanswered.

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Article 14

A Study of Phytoremediation Potential of Some Selected Phototrophs Using Energy Dispersive X Ray Fluorescence Technique: A Reflection on Evolutionary Significance

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Abstract

An increasing trend of contamination of land, surface waters and groundwater from different sources (viz. industrial, military and agricultural) is emerging as an alarming environmental problem either due to ignorance, lack of vision, or carelessness. The build-up of toxic pollutants (metals, radionucleides and organic contaminants in soil, surface water and ground water) not only affects the quality of natural resources but also results into strained ecosystems. Phytoremediation can be defined as "the efficient use of plants to remove, detoxify or immobilise environmental contaminants in a growth matrix (soil, water or sediments) through

the natural biological, chemical or physical activities and processes of the plants" (Newsletter and Technical Publication Fresh Water Management Series No.2 ; Phytoremediation : An Environmentally Sound Technology For Pollution Prevention,Control and Remediation). Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix; that can be soil or water. Algal samples (*Spirogyra hyalina*), pteridophyte samples (*Pteris vittata*) and Angiosperm samples *(Lemna minor* and *Eicchornia crassipes*) were collected to estimate elemental accumulaton. Energy Dispersive X Ray Fluorescence (EDXRF) technique, a multielemental, fast and non-destructive technique was used to determine the elements present. The results showed that the algal mass was the highest accumulator of heavy metals whereas the angiosperms reported the lowest concentration. This preliminary study shows bioremediation of environmental pollutants through plants as a means to clean up sites having moderate to high contamination due to metals from different sources.

Keywords: Phytoremediation, Hyperaccumulation, trace elements, heavy metals.

2. Introduction

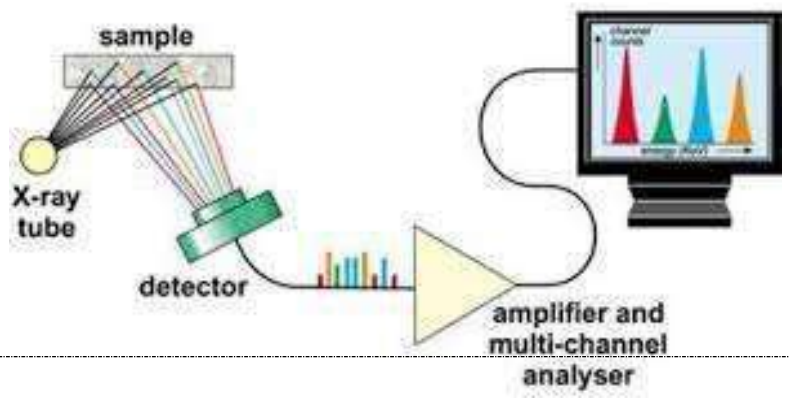
Environmental pollution caused by heavy metals is a major global issue that has attracted increasing concern and research attention worldwide. Heavy metals, though being natural constituents of the environment, has been indiscriminately added to our environment through anthropogenic activities which altered bio-geochemical cycles and balance. Excess release of heavy metals into natural resources from the industries, agriculture and domestic wastes pose serious threat to the environment and its biotic components through food web accumulation and bio-magnification. Remediation using conventional physical and chemical methods is uneconomical and generates large volumes of chemical wastes. Bioremediation is a cost

effective and eco-friendly technique for converting the toxic pollutants into environmentally benign products through the action of various biological treatments. Bioremediation of hazardous metals has received considerable and growing interest over the years as it is a promising technology for mitigation of environmental pollution. Plant-based bioremediation (phyto-remediation) though still at a nascent stage, has shown immense promise as an alternative approach for cleaning up of the contaminated biosphere .

3. **Aim**: This study aims at probing into the potential if any, of phototrophs as an effective heavy metal bioremediatory**.**

4. The Principle of XRF

Direct excitation is a process by which atoms in a specimen are excited by primary photons from external sources, such as an X-ray tube, radioactive source, and synchrotron beam, to produce primary fluorescence. An alternative process is indirect excitation, in which the observed fluorescence is produced as a secondary process by photons or particles (electrons) originating from direct excitation or other secondary processes within the specimen. X-ray is an electromagnetic radiation generated by high-energy particles bombarding atoms. This radiation has wave-particle duality. X-ray fluorescence (XRF) spectrometry uses primary X-ray photons or other microscopic particles to excite the atoms in the test material to produce secondary XRF for material composition analysis and chemical state research. Qualitative analysis of X-ray spectroscopy is based on Moseley's law. The following figure(1) explains the mechanis



5. Materials and Methods

- *Sampling Sites:*

The sampling sites were chosen in and around Kolkata (22◦30_N 88◦30_E). Algal samples (Spirogyra hyalina) from 2 spots in Subhasgram and Baruipur, pteridophyte samples (Pteris vittata) from 3 spots in Rajpur and angiosperm samples (Lemna minor and Eicchornia crassipes) from 2 sampling spots near Dhakuria lake were collected to estimate elemental accumulation.

- *Collected Samples:*

Algal sample Spirogyra hyalina were collected from moist and damp soil surface. Pteris vittata were collected from the open fields using stainless steel knives . Lemna minor and Eicchornia crassipes were collected from the surface water of lake with the help of scalpel

NAME OF SPECIES	FAMILY	ORDER
Spirogyra hyalina	Zygnemataceae	Zygnematales
Pteris vittata	Pteridaceae	Polypodiales
Lemna minor	Alismataceae	Alismatales
Eicchornia crassipes	Alismataceae	Alismatales

Fig(2): Description of collected sample

- *Sample Preparation:*

After removing the remains of dirt with a small brush from the samples, they were freeze dried and were powdered and homogenized using a mortar and pestle.

Pellets (1 mm thick and 13 mm in diameter) were prepared using a tabletop pelletizer (Pressure: 100– 110 kg/cm2) for 1 min. Three pellets per sample were prepared.

The elemental analysis of samples was carried out using a Xenemetrix Ex3600 Energy dispersive X-ray fluorescence (EDXRF) spectrometer, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV, current 1 mA).

6. Results and Discussion

It has been found that ***Spirogyra hyalina*** shows highest accumulation of **Mn** and **Fe.** However **,** ***Pteris vittata*** shows highest accumulation of **As** and **Pb**. ***Lemna minor*** is more prone to accumulation of high metals than ***Eicchornia crassipes*** although both belong to family Alismataceae of class Monocotyledons. The phylogenetic analysis reveals that ***Spirogyra hyalina*** and ***Pteris vittata*** (i.e. algae and pteridophyta) show **less than 15% of similarity** in accumulation of heavy metals andv **Pteridophyte** and **angiosperms** show **more than 50% of similarity** in accumulation of heavy metals (Fig 3)

Both sori and leaf of ***Pteris vittata*** show similar pattern in accumulation of heavy metals, though there are few variations in case of **Ba** and **Pb** accumulation (Fig4).

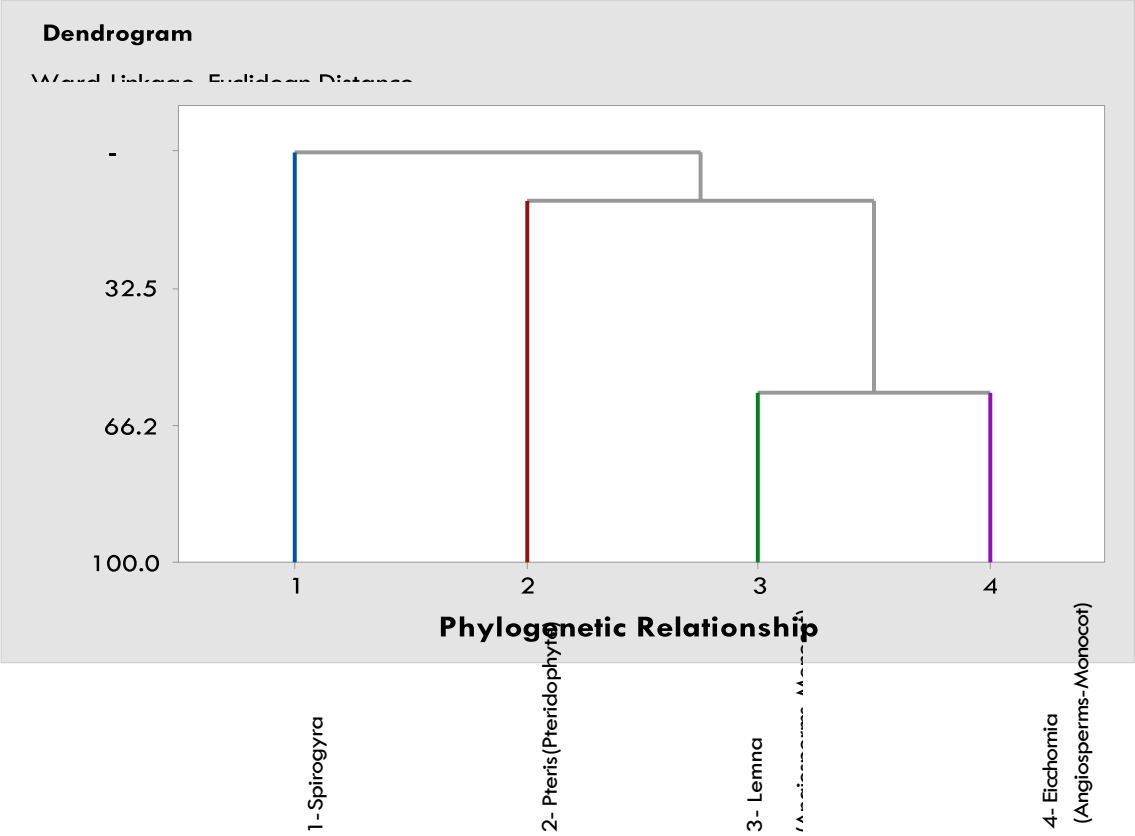


Fig (3) :phylogenetic analysis

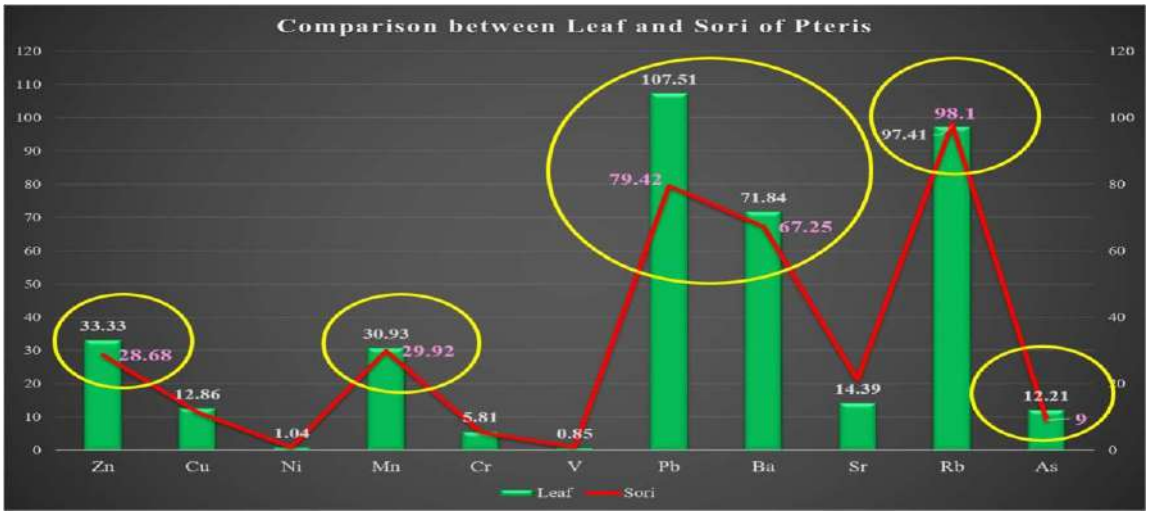


Fig (4): coMpaRison between leaF anD soRi

7. Conclusion

Selected plant specimens show efficient accumulation of heavy metals. They are bioindicators and hence they can be used as potential bioremediators as well. The algal mass showed greater accumultion of Mn and Fe, as compared to the angiosperms. This study also reflects phylogenetic relationship between the selected plant specimens on heavy metal accumulation.

It can be hence refered that even though the algal mass has thallus structure and lies at the base in the evolutionary hierchial lineage it is more potential in accumulation of heavy metals compared to more evolved plant groups.

8. Reference and acknowledgement

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I extend my heartiest gratitude to UGC-DAE-CSR-KOLKATA for giving me the opportunity to work in this project.

Article 15

Lockdown due to Covid19 - Healing changes over the Ganges

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1. Abstract:

Due to the sudden outbreak of Covid19 as a pandemic, originating from Wuhan, China, starting from the month of December 2019, and finally, India reporting the first case on 30thJanuary 2020, the government declared a nationwide complete lockdown from 24th March,2020. Leading to this, there was a sudden stop to all human activities and interactions. India is regarded as a land of rivers and Ganga being one of the majors originating from Gangotri, and finally flowing to the plains of Kolkata and the surrounding cities, ultimately emptying into the Bay of Bengal. Ganga is considered as one of the most sacred rivers, thus having a high socio-economic impact. Ganga is a lifeline to millions who live along its course, as it is the epicentre of tourism, agricultural, industrial and water- transportation activities. Therefore, Ganga is threatened by severe pollution, high acidification, heavy metal concentration, high level of dissolved carbon-dioxide and low oxygen due to various commercial activities. Along with this, accumulation of agricultural and biomedical wastes leads to high concentration of microbial load. Because of the sudden halt to all the activities over the river Ganga, a huge change in the river ecosystem is highly noticeable. In this study, we have compared the changes in the river before and after the lockdown period.

Keywords: pandemic, epicentre, pollution, acidification, ecosystem

2. Introduction:

India is referred to as the Land of Rivers.Ganges is one of the longest rivers flowing down the Himalayan plains across a wide area over the country. It originates at an altitude of 3100 metres, from the Gangotri glacier at Uttarkashi district of Uttarakhand, India. It covers a huge range of West Bengal, specifically Kolkata, the city of joy. Apart from notable mythological values, the river has a great socio-economic importance. It is also biologically significant due to its vast diversity of flora and fauna. Several parts and 'ghats' of the river are well decorated and maintained to increase the tourist attraction and commercialisation surrounding the riverbanks.

Kolkata being one of the major metropolitans of India, has several means of transportations and communication systems; the river hugely contributes to it. River Ganges serves as a huge waterway for trading as well. Besides mainstream occupations like fishing, it is also the source of income for a huge population living on both the sides of the river. It is the water inlet for many small to big scale industries. A lot of water sports and other entertainment arrangements are also based focusing on this holy river.

Unfortunately, the population dependent on the river, itself is one of the biggest reasons for its pollution. Starting from the chemical rich factory outlets, to untreated sewage depositions,

hospital effluents, as well as careless dumping of wastes by the people living around it, have become the major threat for the river ecosystem. These lead to increase of Carbon dioxide concentrations, decrease in dissolved Oxygen, decrease in good microbes and decrease in disease causing pathogens. Altogether the river has turned into the result of exploitations, becoming the source of water borne diseases across the cities on its banks.

Suddenly, the global pandemic and a long lockdown, turned up to be positive for the nature. The prolonged absence of high human interactions has resulted to restoration of wildlife and

pollution free air, across the Earth. Likewise, several positive changes were observed in India and around our area of interest, Kolkata. There was a notable decrease in the Carbon dioxide concentration in the air and also reduction in suspended particulate matter.

In this review paper, our objective was to study the different positive rather healing changes across the river Ganges, considering various parameters during the global lockdown period.

3. Process of study:

3.1*Difference in turbidity:*Based on the spectral response, the two important features studied were -difference in water turbidity andvisible reduction in solid waste. Depending on their spectral response to the interaction with electromagnetic radiation (EMR) these features of the water surface are identified in a satellite image. In regard to this, many factors affect the spectral response of the water such as time of the year, sun-elevation angle, the concentration of atmospheric constituents, roughness of the water, suspended matter, turbidity, depth of water, and submerged or emergent vegetation (Moore 1980).The concentration of a particular component can be qualitatively and quantitatively estimated using remote sensing data. Also, change in composition and reflectance can be simultaneously estimated. Minimal changes in the composition, changes the spectral properties. So, the studies are based on the estimation of turbidity, chlorophyll, dissolved organic matter (CDOM) concentration (Lim and Choi 2015; Gholizadeh *et al*. 2016; Trinh *et al*. 2017; Chander *et al*. 2019; Luis *et al*. 2019).

However, presence of suspended sediments is the most common problem in inland waters such as rivers, lakes, and estuaries (Ritchie *et al*. 1974). These suspended particles reduce the light reaching aquatic life (Ritchie *et al*. 1974; Doxaran *et al*. 2002; Garg *et al*. 2017), and is the indicator of eutrophication (Güttler *et al*. 2013; Sebastiá-Frasquet *et al*. 2019). Reduction in SPM during the lockdown period was measured visually through Secchi disk depth or directly using the light turbidimeters in the field (Pavelsky and Smith 2009; Quang *et al*. 2017).

Turbidity is directly proportional to the concentration of suspended solids or sediments in water (Ritchie *et al*. 1976) and hampers aquatic life by making water more opaque. Turbidity generally scatters the light particles along the water column. It was measured using the Sentinel-2A/B dataset. The Sentinel-2A and Sentinel-2B satellites were launched on 23 June 2015 and 07 March 2017, respectively. It is a Multi-spectral Instrument (MSI) which measures the data in 13 spectral bands ranging from visible and near-infrared to short wave infrared (443–2190 nm) regions, with a swath width of 290 km and a spatial resolution of 10 m (four visible and near-infrared bands), 20 m (six red edge and shortwave infrared bands) and 60 m (three atmospheric correction bands).

3.2 ***Measurement of Dissolved oxygen and near surface Carbon-dioxide level***: The change in Dissolved Oxygen (DO) level in the water of Ganga was studied. DO level is depleted constantly due to disposal of effluents and other organic wastes in the river leading to pollution. The DO level is a function of several factors. In the rivers, the physical factors include ripples, tides, wind generated waves etc. through which diffusion of atmospheric oxygen occurs at the air-water interface.The chemical factors depend on release of waste from various sources, oil spills, shipwrecks *etc*. The biological factor primarily includes the standing stock of phytoplankton community in the estuarine water. The rate of photosynthesis, respiration and decomposition by microbes regulate the DO level in the aquatic system. This further has an impact on the biotic community.

The change of DO due to less human interference, was carried out in six sites during the COVID-19 lockdown phase. For each observation, at least five samples were collected from the study site during high tide condition. Glass bottles of 125 ml were filled to overflow the collected water samples and Winkler titration was performed for the determination of DO.

Table 1: Sites with the coordinates

Serial No.	Area	Coordinates
1.	Ramkrishna Ghat	22°34'19.8"N 88°20'17.0"E
2.	ShibpurGhat	22°33'41.2"N 88°19'40.4"E
3.	PrinsepGhat	22°33'30.9"N 88°19'52.5"E
4.	Botanical Garden area	22°33'06.4"N 88°18'06.6"E
5.	Babughat	22°34'10.3"N 88°20'28.5"E
6.	Second Hoogly Bridge	22°33'31.4"N 88°19'38.5"E

Deposition of organic wastes results in the increase of Biological Oxygen Demand (BOD) in water. DO is inversely proportional to BOD.

The near surface atmospheric carbon dioxide concentrations was measured at three selected sites along the bank of the River Ganga for this study- Ramkrishna Ghat (22°34'19.8"N; 88°20'17.0"E), Botanical Garden (22°33'06.4"N; 88°18'06.6"E) and Babughat (22°34'10.3"N; 88°20'28.5"E). A portable CO2 analyzer (Lutron CO_2 meter, GCH-2018) was used for this study. During the afternoon hours, 10 readings were taken from each site at a distance of 8 meters apart and the mean values were considered for statistical analysis. The results obtained were subject to ANOVA using SYSTAT.

3.3 ***Changes in the acidification rate***: The pH of the surface water in the selected areas were measured during high tide condition with a portable pH meter (sensitivity = ±0.02). The measurement was carried out during pre-COVID-19 situation and COVID-19 lockdown phases respectively.NOVA was carried out to know whether significant variation of aquatic pH exists

between sites and time differences, pre-COVID-19 and COVID-19 lockdown phases. This also indicates the difference in pollution level in the river waters.

3.4 ***Changes in Nutrient Load:*** Surface waters for different nutrients like nitrate and phosphate analyses were collected in clean TARSON bottles and transported to the laboratory in ice-freeze condition. Triplicate samples were collected from the same sites and the nutrient concentration was estimated using the standard spectrophotometric method of Strickland and Parsons (1972) in surface water.

Nitrate was estimated by passing the sample with ammonium chloride buffer through a glass column packed with amalgamated cadmium filings and finally treating the solution with sulphanilamide. This reduced nitrate to nitrite. The resultant diazonium ion was coupled with N-(1-napthyl)-ethylene diamine to give an intensely pink azo dye. Determination of the phosphate was carried out by treating an aliquot of the sample with an acidic molybdate reagent containing ascorbic acid and a small proportion of potassium antimony tartarate. The samples were finally analysed through an UVspectrophotometer.

3.5 ***TotalColiform count:*** The total coliform count of the surface water was determined by multiple-tube fermentation technique (APHA,1998). It is a three-stage procedure in which the results are statistically expressed in terms of the Most Probable Number.The concentration of coliform bacteria in the water samples were determined using Lauryl tryptose broth as the culture medium and Brilliant Green Lactose Bile (BGLB) broth for the confirmatory test.

The first step in the technique requires inoculating the sample in a liquid medium of Lauryl tryptose broth which is a selective media used for the detection of *E. coli, Aerogenes* bacteria in water (Corry et al., 2003). The tubes are then incubated and examined for growth, acid and gas production by the coliform organisms. This test is known as presumptive test. Durham's

tube was placed in an inverted position to show the bacterial growth with emission of gas. Production of gas bubbles and acids with growth was shown in the tubes within 48 hours contributes a presumptive reaction. The density of bacteria was calculated based on positive and negative result combination of the tubes. The confirmatory test was done using tubes that were cultured with Brilliant Green Lactose Bile (BGLB) broth. The observations were expressed in MPN/100 ml (APHA, 1998) by comparing with the standard MPN table.

4. Results:

4.1*Difference in turbidity:*

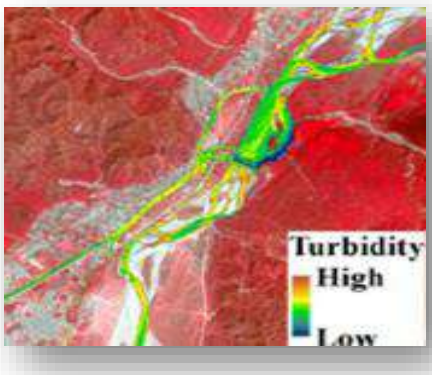

Fig. 1.a

Fig. 1.b

Figure 1.a and 1.b are the images taken by the multispectral remote sensor, before and during the Covid or lockdown situations respectively. The visible blue regions on the images, signify places with lower turbidity; whereas turbidity increases from green to yellow and finally red. So according to the images, the turbidity of the river in the pre-Covid situation was higher than during the lockdown. Therefore, turbidity of the river Ganges decreased during the lockdown.

4.2*Dissolved oxygen and near surface Carbon-dioxide level*

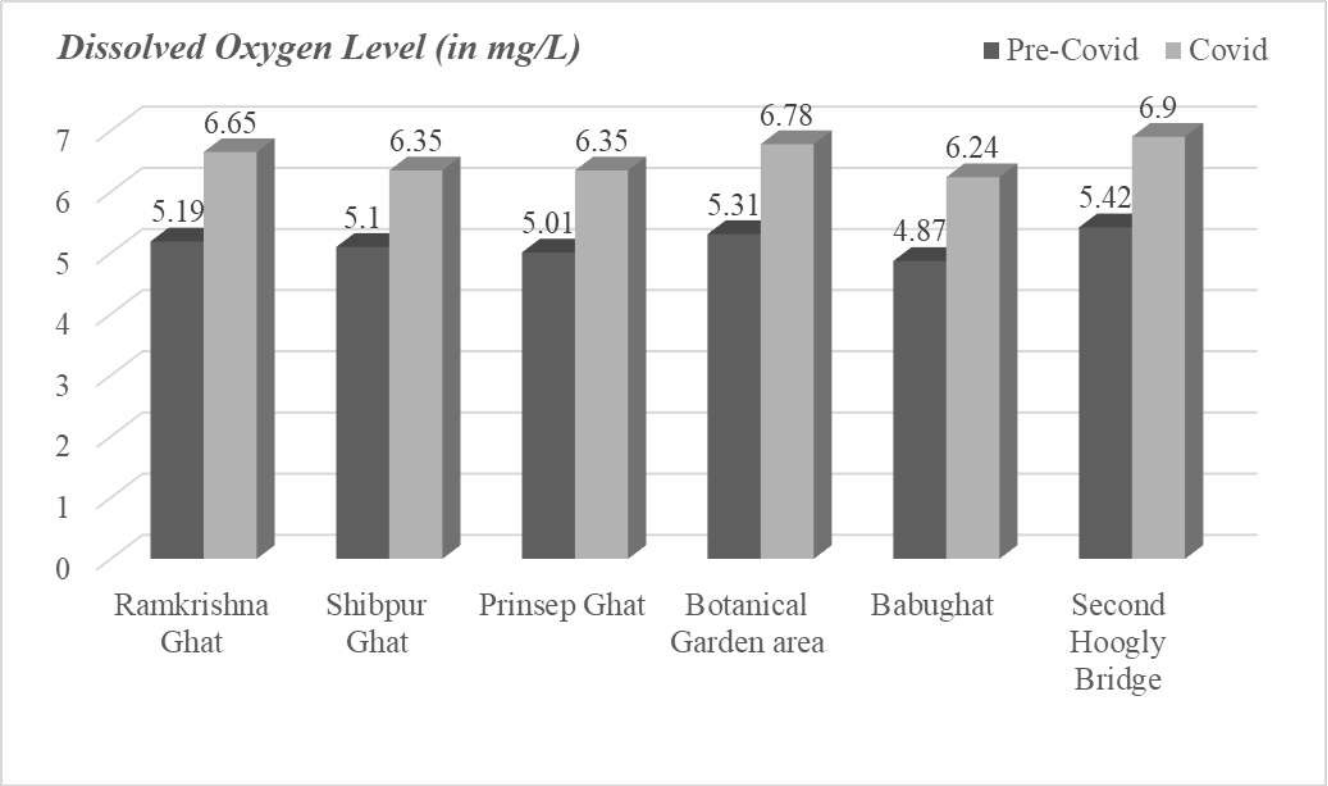

Fig. 2.a

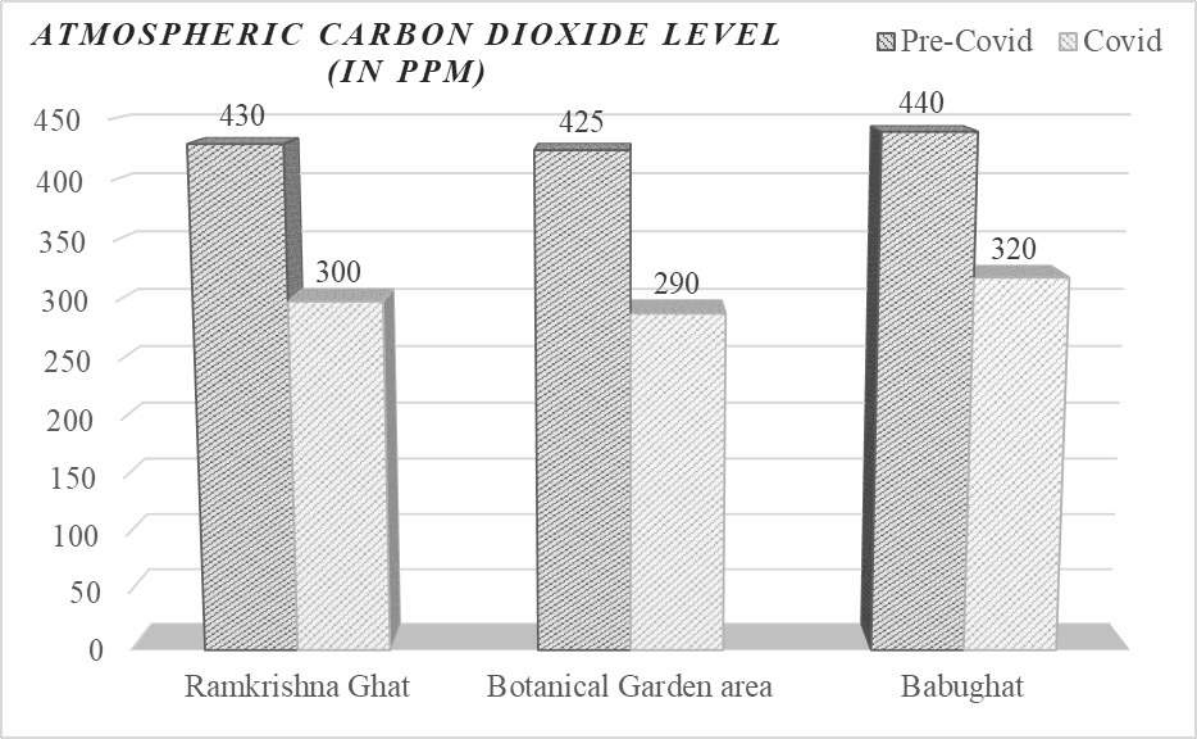

Fig. 2.b

Figure 2.a is the graphical representation of the difference in the dissolved oxygen levels (in mg/L) in the river water collected from the specified sites. Figure 2.b is the graphical representation of the change in the atmospheric carbon dioxide level (measured in ppm) in and around the three mentioned areas of Kolkata. It is observed that the dissolved oxygen concentration increased significantly during the lockdown period; the highest change was observed in the second Hooghly Bridge area, 5.42 mg/L during pre-Covid situation to 6.90 mg/L during the Covid lockdown situation. A sharp decrease in the atmospheric carbon dioxide level was also observed, the maximum decrease being 43.47% in the Botanical garden area.

4.3 *Changes in the acidification rate*:

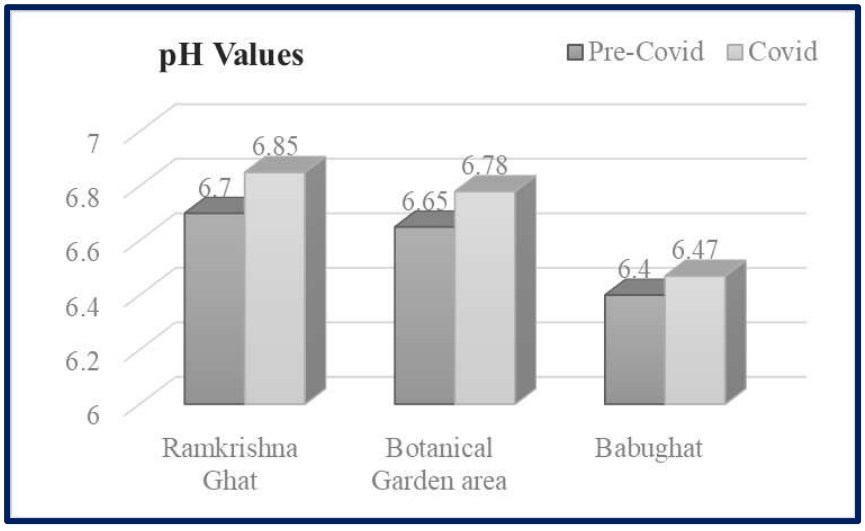

Fig. 3

The graph in the figure 3 represents the change in the acidification levels in the river water of the three mentioned locations. As we know, lesser pH value signifies more acidification and pH 7 being the neutral; it is clear from the observed data that the water of the river Ganges was

more acidic during the pre-Covid situation (with lower pH values) in comparison to the lockdown or Covid situation, when the pH values almost tends to be 7, that is, least acidic or neutral.

4.4 *Changes in Nutrient Load:*

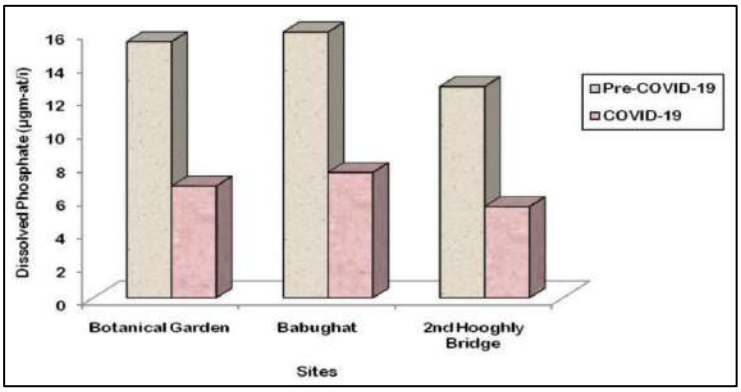

Fig. 4.a

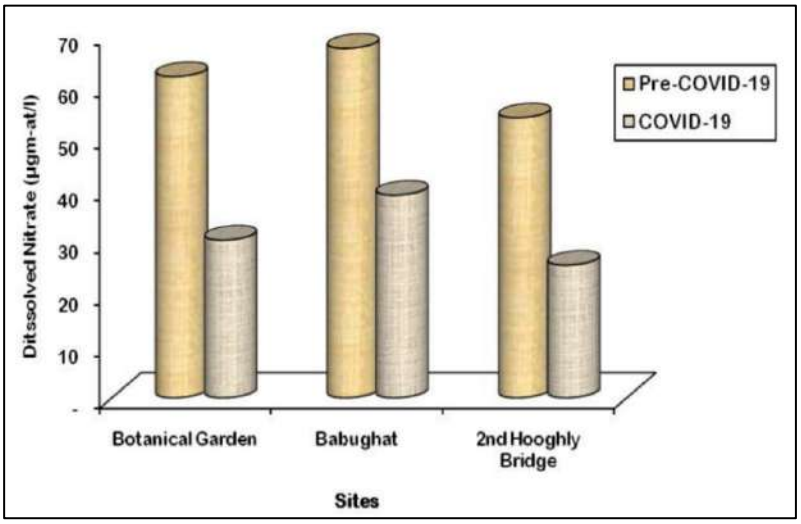

Fig. 4.b

Figure 4.a and 4.b taken from Sengupta *et. al* 2020, graphically represent the variation in dissolved phosphate level and dissolved nitrate level, respectively, (measured in µgm-at/I) in the three mentioned sites during pre-Covid 19 and Covid 19 or lockdown periods. A sharp decrease in the dissolved phosphate and nitrate levels were observed due to lesser anthropogenic pollution during the lockdown; the lowest being in the Second Hooghly Bridge location.

4.5 ***TotalColiform count:***

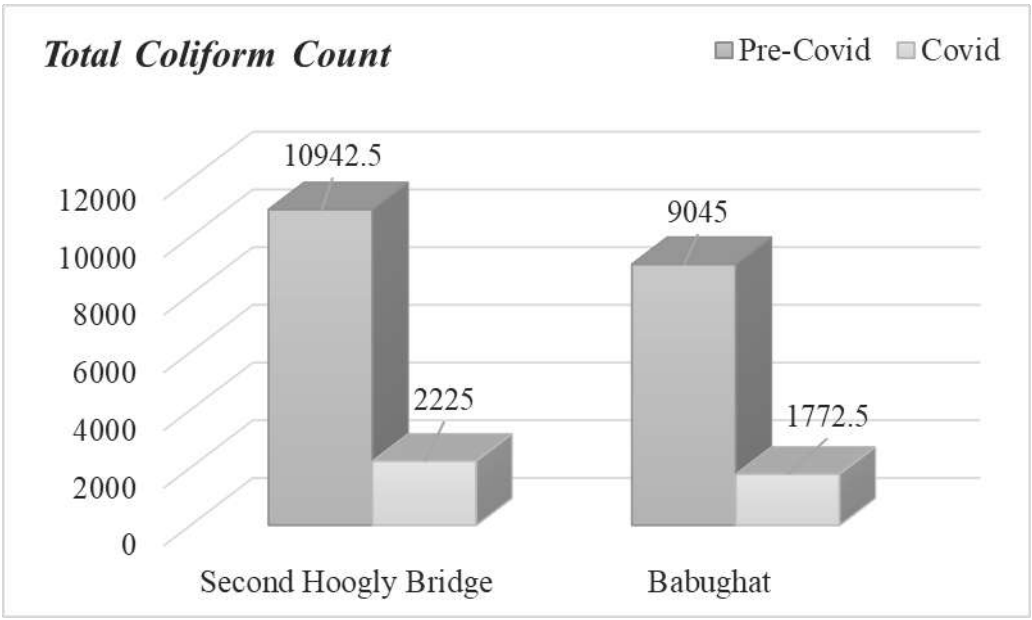

Fig. 5

Figure 5 is the graphical representation of the alteration in the total coliform count in the river water tested in the two above mentioned regions, which is observed to be decreased drastically during the Covid 19 lockdown period in comparison to the pre-Covid 19 period. Thus, this

lockdown lead to a significant decrease in pathogenic microbes and coliforms in the water of the river Ganges.

5. Conclusion:

Due to these changes that have happened during the Covid19 lockdown period, it has been observed that minimum pollution and human interference has led to a decrease in the nutrient load, which reduced eutrophication in the aquatic ecosystem of Ganga. There is an observable increase in the standing stock of the brackish water phytoplankton species and an increase in their growth rate, which acts as a potential energy source for the aquatic food web, being the primary producers and the initial source of oxygenic photosynthesis. There is also an increase in the diversity, population and growth rate of the native edible fishes of Ganga, which is beneficial both ecologically and economically. There is a much-reduced concentration of parasitic microorganisms found in these fish guts. It has also been observed that there is a greater availability of edible fishes for consumption, which reduces the price rate for the commercial fishes as well. This eventually contributes to the food security in the state of West Bengal. To conclude, all these changes happened due to the Covid19 outbreak and a standstill to all industrial and commercial activities, which is the basic source of livelihood for most sections of the society.

6. Discussion:

With the resuming activities and the lockdown being gradually lifted up, there will again be all the transportation, industrial and commercial activities happening in and around Ganga. These benefits will again be lost and there will be a drastic change in the observations we had

throughout this lockdown period. So, to ensure proper utilization of this boon and also to maintain Ganga in its purest form, there can many prospects. The enriched algal diversity which includes species like *Spirogyra, Ascophyllum, Fucus, Chlamydomonas* etc. can be used for biomonitoring the water pollution levels constantly and in commercial methods to be employed for phytoremediation of the lower Gangetic delta. The increased *ichthyoplankton* count can be aquacultured to further increase the edible fish production. Certain probiotic strains of bacteria or coliphages can be used to keep the coliform count of Ganga in check on a regular basis. Moreover, increased diatom colonies detected in the pollution free waters of Ganga during this lockdown, can be used for restoration work in the riverbanks utilising their siliceous deposition. Also, species like *Chaetoceros*, identified in the sample waters during this phase, can be modified in-vivo and in-vitro which source for the production of catalysts and zeolites by inducing silica leaching. They can even be used as natural water purifiers or as additives in sanitizers. Pertaining to the present pandemic situation, these changes can benefit humankind in ways manifold.

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CHAPTER 3

This section includes science articles by faculty of some colleges in Kolkata.

Article 16

Emerging gene-editing technologies: for therapies to the possibility of nightmares

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1. Introduction

In humans, the double-helical DNA comprises around $6x10^9$ building blocks in a specific sequence. These building blocks are four types of nitrogenous bases: adenosine (A), Guanine (G), cytosine (C), and thymine (T). Even a single error in the sequence of base pairs can turn fatal. More than ten thousand inherited diseases were identified that are monogenic like cystic fibrosis, sickle cell anemia, Huntington's diseases, Tay Sachs diseases, type I Myotonic dystrophy, Muscular dystrophy, Marfan syndrome, etc. Conditions like amyotrophic lateral sclerosis (ALS) are heterogeneous, genetic disorders.ALS can be monogenic or polygenic.[1, 2]

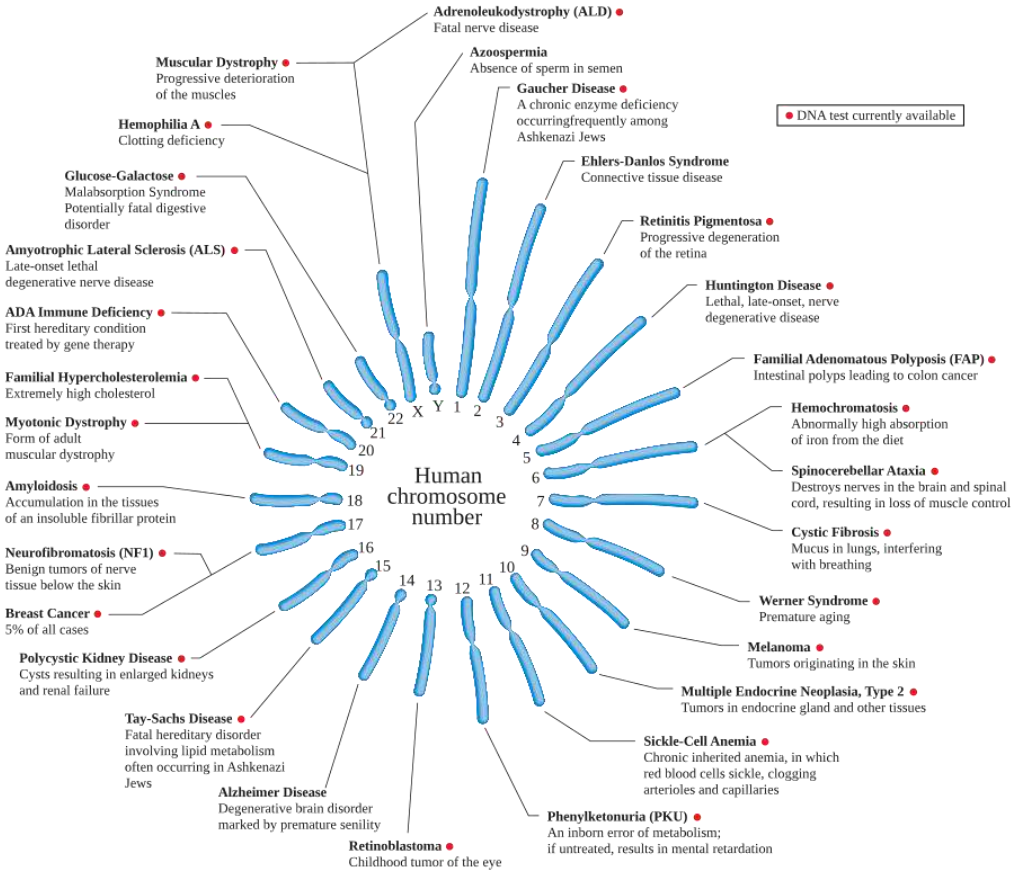

Figure1: Important human chromosomal diseases

(https://commons.wikimedia.org/wiki/File:Human_chromosome_diseases_set_en.svg)

Many of these monogenic diseases are incurable and affect body systems. Treatments or management of these ailments are mostly symptom-based. Take, for example, the treatment of the metabolic error might comprise dietary change or replacement of the specific missing enzyme. A genetic disorder causing heart defects can be corrected with surgical methods. People suffering from sickle cell anemia are often transplanted with bone marrow.[3] In a nutshell, very few treatment strategies do exist today. With the advancement of molecular biology, gene therapy, genome editing are emerging. This treatment involves replacing the defective genes that cause a particular disease.

Genome editing is a method where the DNA is altered. Such editing is not only restricted to humans but can be extended to all plants, animals, and microorganisms. Gene editing is not only used for the treatment of diseases but can also be potentially used for changing physical traits like eye color, etc. The first such editing techniques were invented in the 1990s, and with passing of time, newer and newer techniques are emerging. Many of the animals that bear genetic similarity with humans are experimented with. For example, mice and humans have a similarity in 85% of their genes. Experiments are carried out in zebrafish. Gene therapy involving genome editing is a subset that has a promising future for treating cystic fibrosis, diabetes, etc.[4]New technology shall obviously take some time to be wholly understood and be perfect. A lot more needs are to be unearthed, but out of all genome editing tools, CRISPR is undoubtedly one of the most valuable tools in research. Tracing back to 1987 when the Japanese discovered some unusual repeating sequences in the DNA while studying *E.coli*. Over the years, others have also found similar clusters of DNA in bacteria and Archaea. They were really a mystery unsolved until the scientists found these clusters to be a component of the immune system while studying Streptococcus in 2007. After a phage attack, the bacterial enzymes usually kill the invading phage. There are other enzymes that would interplay to cut the viral genes into pieces. Subsequently, the pieces are stored as pieces in the CRISPR spaces of the bacterial genome.CRISPR is the abbreviation of **"Clusters of regularly interspaced short palindromic repeats."** Bacteria use this genetic information to defend future attacks. One of the most important enzymesin bacteria is the Cas9.**Jennifer Doudna from California University** and **Emmanuelle Charpentier of Umea University** was working on the mechanism of CRISPR/Cas9 as to how the Cas9 enzyme matches the RNA in the mug shots with that in the viruses, and how the enzymes would know when to chop the gene in 2011. And in 2012, they showed the use of the CRISPR/Cas9 tool to chop up genes in any place they choose. It means the scientists could fool the Cas9 protein by feeding artificial RNA- a fake

mug shot. They found that the enzymes could chop anything with the same code and not just viral genes, and this finding was path-breaking. Later **Feng Zhang from Broad Institute in Boston** revealed its use in mouse and the human genome.[5] Another path-breaking finding! The selection and designing of the guide RNA serve to be crucial in targeting the gene of interest. In the absence of such gRNA, the chances of **multiple off-target effects** increase. There were many Biotech start-upsusing these tools for the benefit of mankind. Mammoth Biosciences, Inscripta, eGenesis, Synthetic Genomics, Plantedit, etc. are well-known companies. A critical issue regarding CRISPR is the question of technological diffusion and technology transfer. A lot more excitements depend on how to improve CRISPR/Cas9 precisionin the future.

Even if we assume that CRISPR/Cas9 technology is quite reliable and accurate to mutate a pathogen, it obviously does not indicate that one has a bioweapon automatically. More than mere access to the pathogen is requiredto create a mass casualty bioweapon. Various technical glitches need to be addressed first. Firstly, the acquiring of access to a virulent pathogen or toxin is important. CRISPR/Cas9 tool can afford to make one such variant but not without problems. Nations, like the former Soviet Union, had created a highly resistant pathogen, but it could not survive as it was environmentally sensitive. So the viability of bioweapons is not only dependent on the genetically engineered pathogen. The processing, scaling up, stabilizing, and delivery form and delivery means are key challenges to bioweapons.

Organisms have consistently used new means to eliminate each other since the origin and evolution of life, about 3.5 billion years ago. From bacteria to the snakes, all use toxins- and these are all forms of biowarfare. Humanity does participate in such warfare by taking the gain of these organisms capable of producing toxins. Starting from the throwing of cadavers to contaminate the water supply, to the use of botulinum, aflatoxin, abrin, smallpox, *Yersinia*, *Clostridium* for inflicting damage and destruction on the other side- are all forms of biowarfare.

The ease of its availability and the disruptions it causes led the scientists to call it **"Poor Man's Nuclear Arsenal."** These weapons have low conspicuousness, higher potency, easy accessibility, and easy transport and distribution. With advancement, today's bioweapons are more lethal and are able to trigger mass destruction even before suspected. The use of genetic engineering, along with information technology, has made it possible to design bioweapons of choice. The rampant progress of SARS-CoV-2, the helpless people, the desperation for the vaccine, left us clueless, paralyzed, and panicked. Hopefully, it shall teach us efficient response and handling of these bioagents in a sensible and ethical manner.

One of the fundamental principles of science is the notion that all researches are carried out with sound scientific methods that would add value to our civilization. But, of course, a country, a radical group or a terrorist organization could misuse such outcomes of research to harm innocents. In this article, I have focussed and discussed biowarfare, where biotechnology has been redirected to harm a group of individuals. The present article comprises 2 parts. The first part deals with genome editing, the various techniques with particular importance being given to the recent CRISPR technologies. The second part of this article deals with contraventions, especially how the CRISPR systems could be misused to make bioweapons that may lead to biowarfare.

2. Types of gene therapy

Gene therapies fall under two basic categories: germline and somatic therapy. A germline therapy alters the DNA in the reproductive cells, such as sperms and eggs. Such changes are inheritable, i.e., passed down from one generation to the next and subsequently. Thus they potentially prevent inheritance of diseases. Somatic cell therapy targets the non-reproductive cells (the somatic cells), and hence the changes made through gene therapy affect the person at the receiving end.[4] They are not passed on through generations. Such treatment is generally used to retard or converse the disease process.[6]

One of the successful somatic gene therapy was the treatment of leukemia in a one-year-old patient named Layla in the United Kingdom. They used a technology known as TALENs.[4] Somatic gene therapies are less controversial as compared to the germline therapies. Clinical trials have been conducted for Human immunodeficiency virus (HIV) and cancer patients. In the case of germline therapy, it is highly criticized as it might pave the way for genetic enhancement of characteristics that are medically irrelevant such as height, athletic capability, etc.[6]

3. Techniques of genome editing

The modern-day biochemical and molecular mechanisms used for editing DNAs modifies the downstream pathways. A summary of the various genome editing techniques are given below:

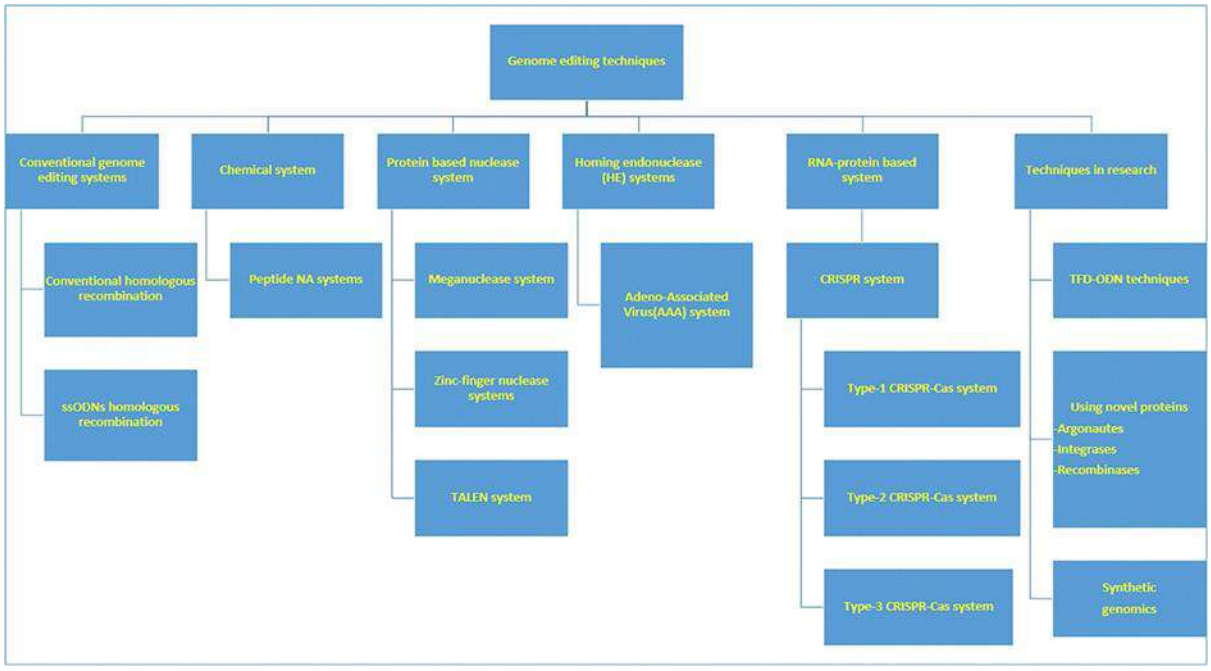

Figure2: Schematic representation of the various genome editing techniques.[7]

3.1. Conventional techniques for genome editing

This technique comprises of homologous recombination related to gene intervention. It involves a double-stranded (ds) repair system and is not usually practiced these days. The **RAD52** protein is said to be significant in mediating homologous recombination and hence the therapeutic target in the treatment of breast cancers like BRCA 1 and 2 repair that act as tumor suppressors.[7]

3.2. Chemical modalities of genome editing

Here **artificial restriction DNA cutter (ARCUT)** is used as a non-restriction methodology.**Pseudo-complementary peptide nucleic acid (pcPNA)** is used to identify the cleavage site in the chromosome or in the telomeric section. After the said nucleic acid determines the location, a mixture of cerium and EDTA is used to excise and conduct the splicing function. Then DNA ligase is used to seal the spliced nicks. The pluspoint of this process is it can be carried out in high salt concentration, but designing site-specificpseudo-complementary peptide nucleic acid is difficult, and so is the high turnaround time.[7]

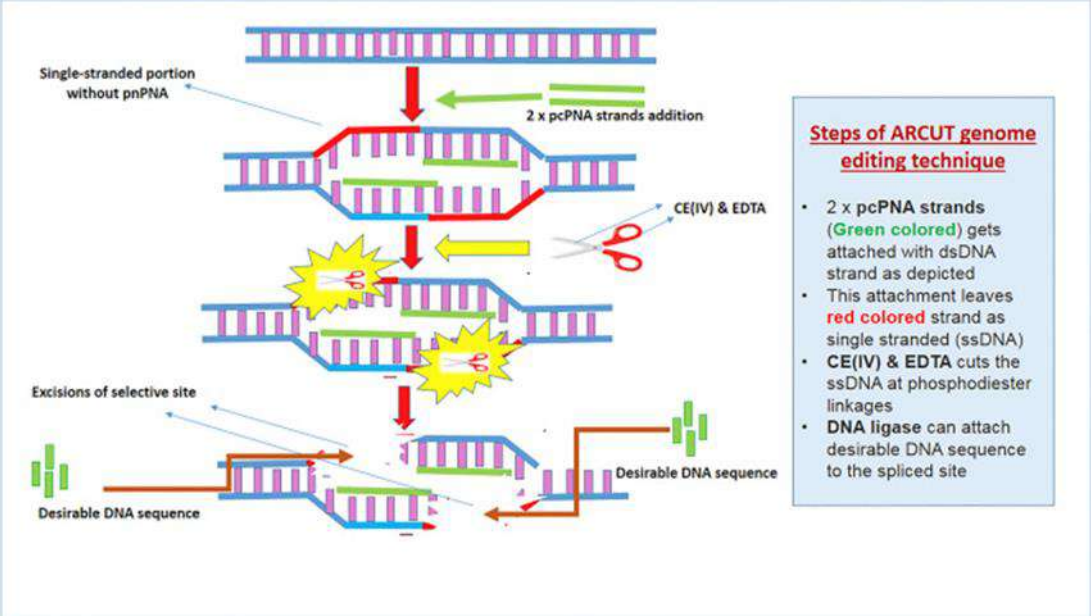

Figure 3: Mechanism ofexcision of the specific site of double-stranded DNA by ARCUT.[7]

3.3. Homing endonuclease systems:

The word ‘homing’ is interpreted as the lateral transmission of a genomic DNA sequence. Homing endonucleases are enzymes that are natural in origin. They are mostly around 14 bp in length. They are able to splice larger DNA sequences. The process comprises a DNA fragment in which a site is removed by the endonucleases, thus resulting in the two segmented DNA fragments. Of late, the **recombinant adeno-associated viruses (rAAVs)** are found to be competent vehicles to transfer the tools of genome editing inside the cell. The disadvantages of this technique lie in developing the nucleases, developing suitable vectors, and off-target effects.[7]

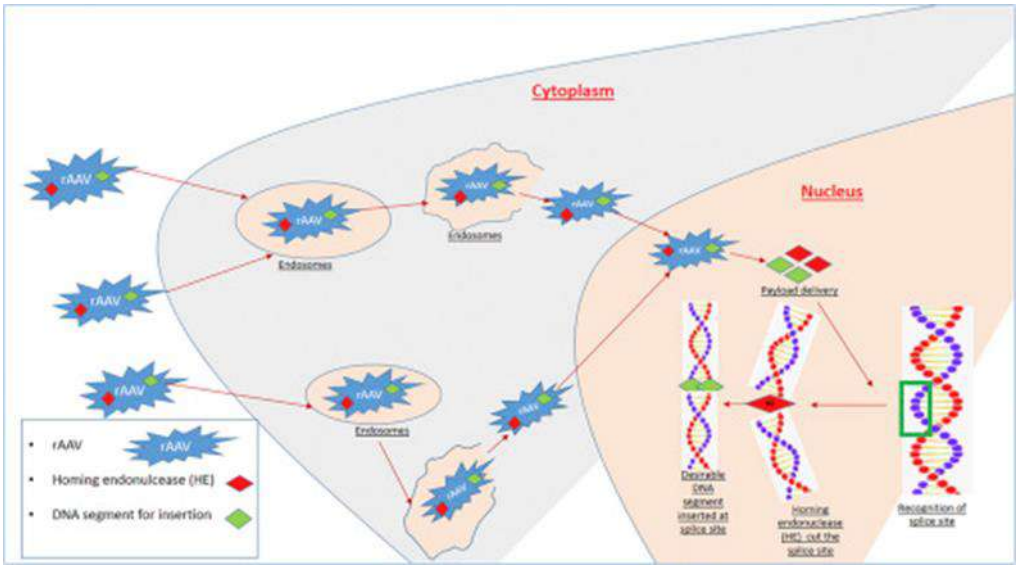

Figure 4: Mechanism of entry of adeno-associated viruses (rAAVs), cytoplasmic movement, attachment to DNA, and integration with DNA.[7]

3.4. Protein-based nuclease systems:

3.4.1. Meganucleases: These are large basepair (bp) structures occasionally found in the genomes. They act like molecular scissors and are tools to excise large sections of DNA. The enzymes can be coupled with proteins to form variants such as **DmoCre** and

E-Drel. These proteins can cleave nucleotide-specific site.[7] Chimeric meganucleases with a new recognition site could be produced. It is composed of the whole site of meganuclease A, and a half site of protein B. when I-Dmol and I-CreI are fused in such a way, it results in the formation of two chimeric meganucleases E-Drel and DmoCre.[8] the process comprises identification of the cleavage site and then splicing the region with endonucleases. This process has the advantage of being less toxic, natural meganucleases that can conduct cuts at specific locations.[7]

3.4.2. **Zinc finger nucleases (ZFNs):** Restriction endonucleases are joined with zinc finger binding protein to produce artificial structures. A binding protein domain recognizes and reaches the suitable location of splicing and restriction endonucleases called Fok1 cuts at the particular codon.[7] Fok1 is a IIS type of restriction endonuclease naturally occurring in *Flavobacterium okeanokoites.* The DNA binding area is at its N-terminal end, whereas the non-specific DNA cleavage area is at its C-terminal. The process is simple and quite specific but restricted because of its attachment with 3 codons.

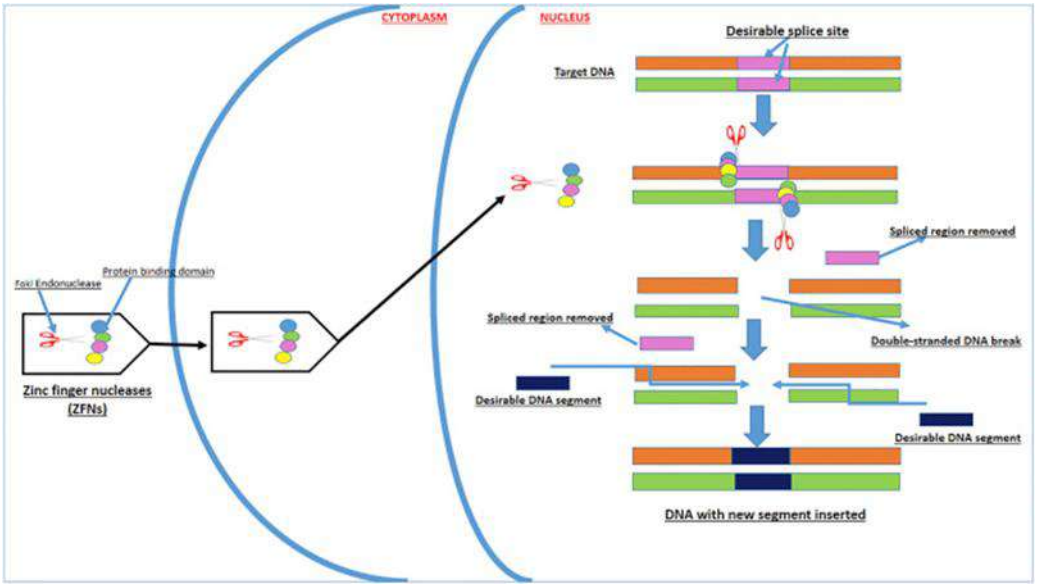

Figure 5: ZFN mediated genome editing.[7]

3.4.3. Transcription activator-like effector nucleases (TALENs): These are quite akin to the zinc finger nucleases in terms of development and method of action. In this case, a restriction endonuclease is coupled to a DNA binding region termed TAL effector. While ZFN could hit 3 nucleotides, the TALENs can hit 1 nucleotide. Hence the TALENs are more site-specific and have few off-target effects.[7]

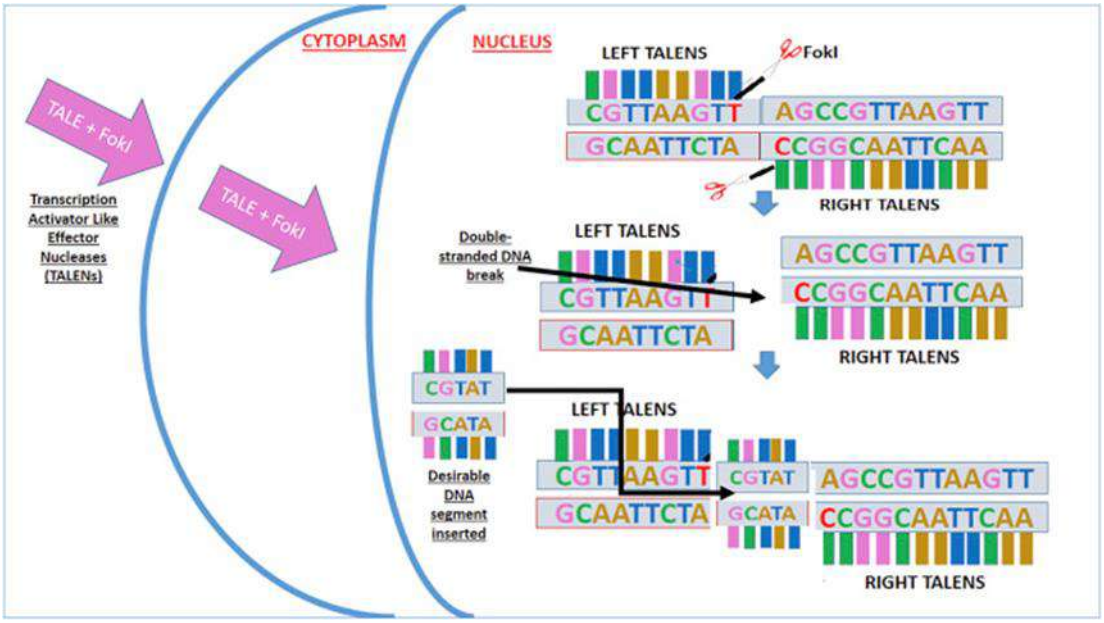

Figure 6: TALENs mediated genome editing.[7]

3.5. RNA-DNA systems:

3.5.1: Clusters of regularly interspaced short palindromic repeats (CRISPR) methods:

The method uses various types of **"Clusters of regularly interspaced short palindromic repeats" (CRISPR) methods**. It permits alteration of DNA sequences and thereby modify gene function. The concept is old and obtained from the earliest immunity system. This immune system was naturally present

in some bacteria and Archaea against viruses. CRISPR is a specialized region having 2 unique characteristics. It comprises **repeated sequences of nucleotides** throughout the CRISPR region. Bits of **spacer DNAs** remain interspersed among these repeat sequences. The spacers (SPR) are different nucleotide sequences. Each of these sequences signifies a past acquaintance to a foreign antigen like the viruses. These sequences serve as a reservoir of memories that enables the bacteria to identify the viruses in subsequent attacks. [9]

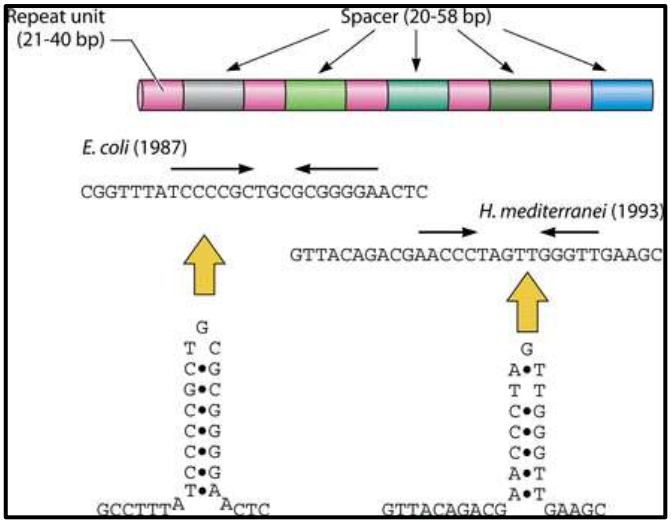

Figure 7: Structural features of CRISPR showing repeats and spacers. The two palindromic sequences of the CRISPR, identified from *E.coli* and *H. mediterranei* (Archaea).[10]

The first experimental demonstration was published in 2007 when the scientists could show that new spacers were incorporated in the CRISPR region of the *Streptococcus thermophilus* genome after a viral attack. Moreover, the sequence in these spacers was identical to the sequence of the viral genome.The evolution of gene editing could be traced back to the 1980s, but the most significant milestone was

reached in 2012. As mentioned earlier, a team led by **Jennifer Doudna**University of California, Berkley, and**Emmanuelle Charpentier**, from Umea University,reported that the CRISPR/ Cas9coordinationmight be used to perform edit genes outside bacteria.In 2014 they published in the Journal Science that the crRNA has a nucleotide repeat and a spacer component. After the viral attack, the spacer gets incorporated. In the subsequent phage attacks, a fragment of the CRISPR is transcribed and processed into CRISPR RNA (crRNA). The nucleotide sequence of the CRISPR serves as a template for producing a complementary ssRNA.[9]**Feng Zhang**, Broad Institute of MIT and Harvard, McGovern Institute of Brain Research at MIT, Massachusetts,was the first to adapt CRISPR/Cas9 genome editing in eukaryotic cells successfully.[11]

The protein gets bound to two RNA molecules- the crRNA and the tracrRNA (trans-activating crRNA). The two RNA guides the Cas9 protein to the target location for making the cut. The stretch of DNA is complementary to a 20 nucleotide stretch of crRNA. The Cas9 protein uses 2 separate regions in its structure and nicks both the strands of the DNA helix. To ensure precise cuts in the right place, short DNA sequences called **"protospacer adjacent motifs" or PAMs** place themselves next to the target DNA sequence to serve as tags. Cas9 complex will only initiate cuts if it recognizes the PAM adjacent to the target DNA sequence. This might be a possible explanation as to why the Cas9 protein never attacks the Bacterial CRISPR region.

The CRISPR/ Cas systems broadly fall under **3 major types**, which again can be subdivided into 10 types. The categorization is based on the core element and the sequences. Irrespective of its diversity, the immune activity follows **3 stages adaptation (procurement of spacer), CRISPR expression (crRNA synthesis),**and **interference (immunity).** In the first step, short foreign DNA spacer sequences are

incorporated into the CRISPR locus of the host/bacteria genome. Probably, such site selection and insertion requires the assistance of Cas1 and Cas2 proteins. In the second step, the transcription of CRISPR repeat-spacer arrays into precursor crRNAs (pre-crRNAs)takes place. Thepre-crRNAs is further processed into a set of crRNAs that contain a single spacer flanked by repeat segments.In type I and type III CRISPR systems, the pre-crRNA is cleaved within the repeat sequence by Cas6 endonucleases during RNA maturation. In type II CRISPR systems, a separate maturation pathway follows that involves a supplementary tracRNA molecule and RNase III.[12]

In the third step, the crRNAs join with the Cas proteins to form an effector complex that will identify the target site in the viral nucleic acid by means of base pairing to the complementary strand and induce sequence-specific cut. It is necessary to mention that the structure and function of the effector ribonucleoprotein (RNP) complexes participating in crRNA-mediated silencing of viral nucleic acids vary among the different CRISPR/Cas types. In the **type I-E** systems, the crRNAs are assimilated into a multi-subunit effector complex called Cascade. This unit then binds with the target DNA and, with assistance from Cas3 protein, induces degradation. In **type III** CRISPR/Cas systems, ribonucleoprotein (RNP) complexes of Cas RAMP (Cmr) proteins and crRNA identify and cutthe synthetic RNA in vitro.In **type II** CRISPR/Cas systems, a Cas9 protein is responsible for RNA silencing instead of Cmr complex.CRISPR1 and CRISPR3 both fall in type II CRISPR/Cas systems though the PAM characteristics for CRISPR1 and CRISPR3 vary.[12]

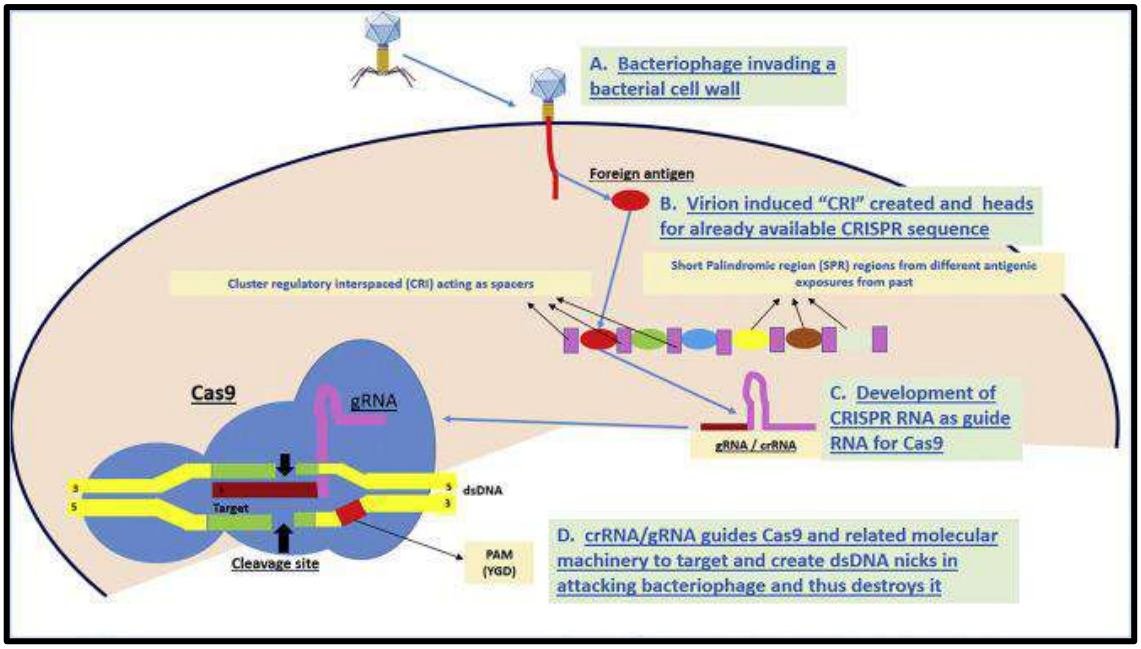

Figure 8: CRISPR/Cas9 mediated interaction to destruct phage genome.[7]

The strict necessity for tracrRNA is only for Cas9 mediated immunity in both *in vivo* and *in vitro*. Additionally, RNase III is needed to process the pre-crRNA. Such is not needed for maturation of crRNA that is obtained from CRISPR array with a single spacer. The Cas9 protein stimulates the annealing of a pre-crRNA: tracrRNA duplex in vitro and demonstrates the formation of a ternary complex of Cas9-crRNA-tracrRNA that severs the DNA.

Recently the invention of CRISPR technology along with its Cas9 enzyme has revolutionized the arena of "**genome editing**."The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), as is called, made small fragments of harmful DNA to be snipped off and be replaced with regular sequences.[13]In such editing, specific crRNA/gRNA has been designed that can be introduced into the nucleus of the cell and, subsequently, Cas9. The non-desirable double-stranded DNA gets associated with the Cas9 following the guidance provided by the particular crRNA/gRNA. Such a complementary pairing between the gRNA

and the undesirable DNA segment permits its Cas9 mediated destruction. There are three distinct types of CRISPR/Cas techniques- Type I system uses Cas5 or Cas6 for pre-processing the crRNA. Later the breakage needs Cas3, Cascade, and crRNA for interference. Type II system uses the Cas9 that works under the guidance of crRNA to hit the DNA. RNase III, tracrRNA and other proteins are involved in the processing of the 5' terminal. Type III system uses Cas6 for processing the 3' end of the crRNA. The uniqueness of this method is carried out by the type III Csm/Cmr complex.[14]

3.5.2. Steps for laboratory genome editing using CRISPR

1. The genes to be edited or modified are to be selected. Modification can be cutting, activation, or inhibition.The DNA sequence can be retrieved from the Genome Database.
2. The endonuclease protein to be used has to be decided upon. For example, Cpf1 is another type II endonuclease other than Cas9. Nuclease deficient Cas9 or Cpf1 could be used to activate or repress gene expression b fusing it to activation domain (like B42, VP16, etc.) or repression domain (like KRAB, Mxi1, etc.)
3. The next step involves designing the gRNA. The cells to be altered should produce a guideRNA (gRNA). Currently, designing gRNA has become exceedingly simple and is routinely practiced in the labs. Benching GenScript, E-CRISP, CRISPR Design, etc. websites can be used for assistance.
4. gRNA expression vector should be assembled, which may be optimized plasmids (such as pML107). Plasmid assembling requires expression vector (a plasmid that produces CAS9 and designed gRNA), designed gRNA, restriction endonucleases, ligases, buffers, etc.
5. Acquiring of the cells that are to be engineered.

6. The last step is engineering the acquired cell. The created expression vector that expresses both Cas9 (or Cpf1) and the designed gRNA needs to enter the living cells that are acquired and chosen to be engineered. The usual process for bacteria is transformation and are quitter easily transformed than eukaryotic cells. Once the gRNA is designed and expressed in the target cells, an endonuclease effector complex of Cas9 (or Cpfi) is allowed to bind by identifying a PAM sequence. The PAM sequence for Cas9 is NGG (N can be any of the nitrogenous bases).The Cas9 checks for the correct base pairing between the gRNA and the DNA. In the case of dsDNA, a blunt cut is initiated at the locus that lies 3bp upstream of the 3' end of the PAM sequence.[15]

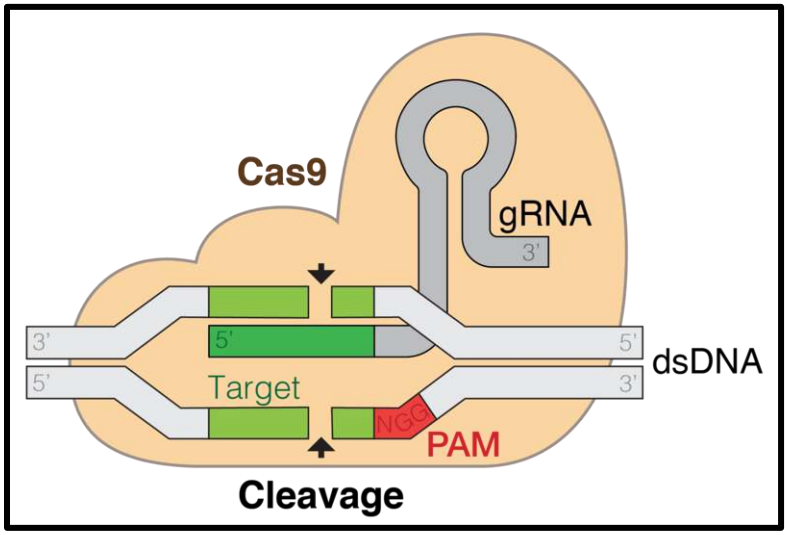

Figure 9: DsDNA cut by Cas9 /gRNA.[15]

7. There are two ways for cell repair after the dsDNA cut is made; **non-homologous end joining(NHEJ) and homology-directed repair (HDR).**The first one is fast but prone to errors. Using the NHEJ repair, nucleotides are often inserted in the DNA strand. This implies that few cells might have permanent damaged DNA that is responsible for the expression of a mutant, non-functional gene. So a large pool of cells undergoing CRISPR/Cas9 editing, some cells always misrepair and suffer a permanent

loss-of-function mutation in the target gene. This remains a crucial challenge for scientists to overcome.[15]

3.5.3. Applications of CRISPR

Metabolic engineering and synthesis of high-value compounds

The organism's metabolism could be rewired to make high-value chemicals and biofuels from cheaper raw ingredients like sugars. CRISPR can be applied for other purposes than genome editing. Amino acids modification in Cas9 proteins could turn them into mutants. The mutant enzyme can bind to the targeted DNA sequence but lacks the ability to induce nicks. The bound mutant enzyme can be programmed to be an activator or repressor of gene expression at that locus. These enzyme proteins are known as nuclease-deficient endonucleases. They can also be tagged with a fluorescent protein for better visualization of the process in the living cells.[15]

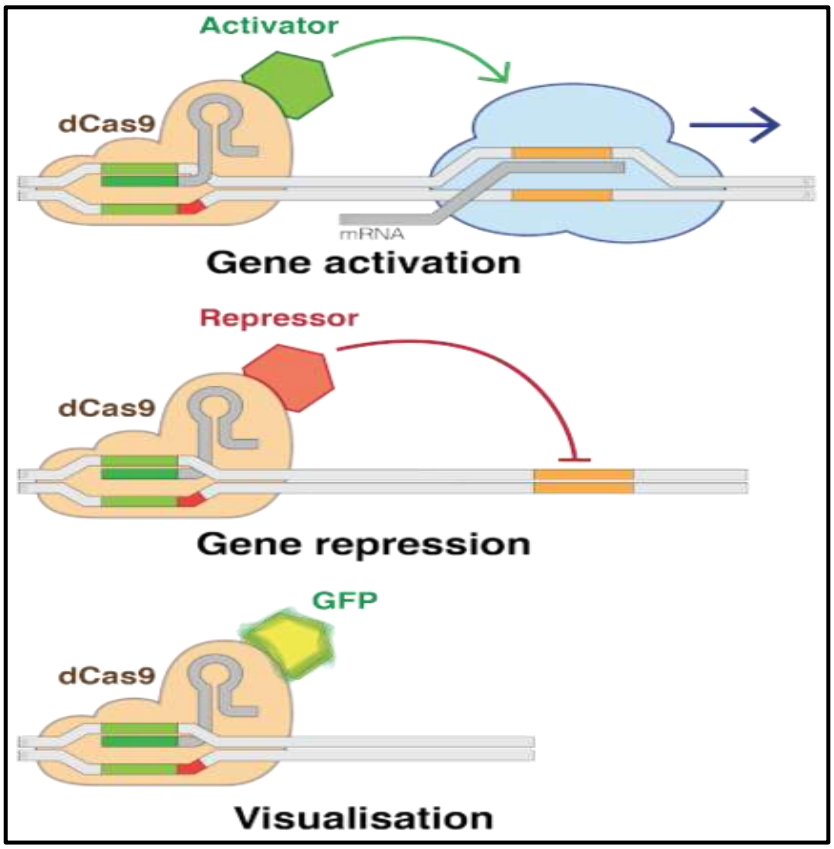

Figure 10:Use of dCas9 (deficient or mutant) mutants to activate (figure 10a), repress (figure 10b) gene expression, and fluorescent protein-tagged dCas9 for visualization of binding mechanism (figure 10c).[15]

Diagnosis of Pathogen: Itcan be fast and cheap. For example, Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK) are used for identification of virus and bacteria like the Zika virus.

Screening of cancer: Several thousands of genes could be screened that are related to tumor formation by the removal of fibroblasts and deleting every single gene of the genome one by one and using the designed gRNAs o hit each gene.

Mouse models: With CRISPR, gene knockouts have become much easier to create designer mice lacking specific genes. These animals serve to study diseases like Alzheimer's, ALS, etc.

Deleterious mutation correction: Though China was using CRISPR to edit human embryos since 2015, CRISPR was officially reported to edit Hunter syndrome in a 44-year-old patient in the USA.Lulu and Nana were claimed to genetically modified babies whose genome has been edited to induce HIV resistance.[14] Though CRISPR is a much more improved gene-editing technique, it is not perfect. Many times, the tools may cleave wrong locations, and this makes the scientists unsure about the impacts that these errors might do. Accessing safety in such therapy is crucial to its successful application in patients.[4]

Improved animal breeding: Though with prevailing ethical concerns, the CRISPR/Cas9 can precisely modify the DNA to breed livestock with desirable characteristics.

Malaria eradication: Since the female mosquitoes are responsible for causing malaria, these are first designed to carry the gene drive on one of its chromosomes. After their release in nature, they will breed to produce offsprings that will carry the **gene drive**. Through repeated breeding and after several generations, the gene drive will spread throughout the population

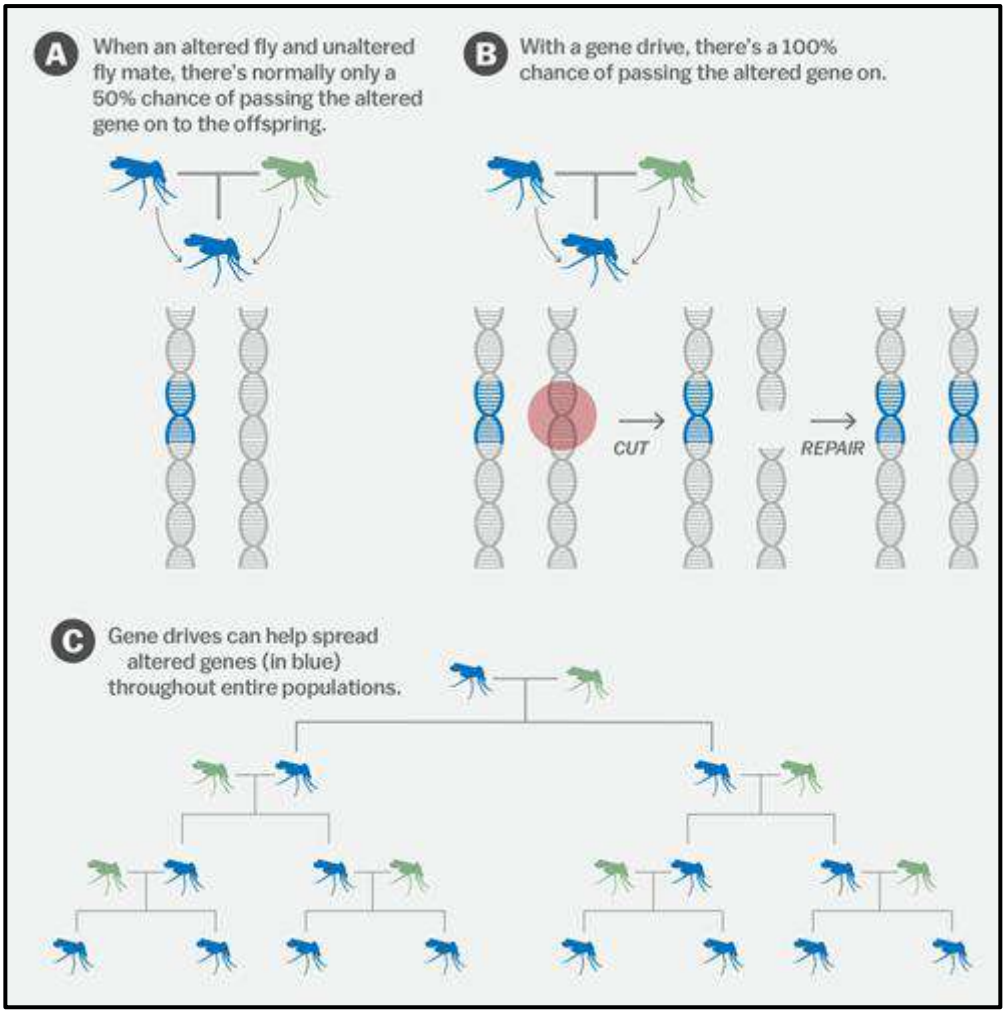

Figure 11: Mechanism to show how gene drive spreads throughout the population (https://www.vox.com/2018/7/23/17594864/crispr-cas9-gene-editing)

Modification of traits in living organisms: The CRISPR technique can be used to regulate gene expression as on an off at specific times during development to control the development of certain traits.

CAR-T cell therapy:T cells are extracted from cancer patients and then engineered to hunt down and attack tumors in patients.

Bringing Woolly Mammoth:DNA editing was used to bring back extinct lifeforms like the Pyrenean ibex and woolly mammoth. It seems practically tough because of the DNA

degradation over millions of years that needs prior repair before insertion into the viable embryo. CRISPR may possibly simplify such processes.

3.5.4. Issues related to CRISPR

'Off-target editing' seems to be a persistent issue amidst the hype of precise gene targeting that exists. Possibly in natural conditions, this has been created to work to protect the bacteria against invaders. But this is less valuable for scientists who want to control precise gene modification. A large number of unexpected deleterious mutations usually occur. Researchers were able to successfully correct the gene causing blindness in mice using the whole-genome sequencing method.There were around 1400 single-nucleotide mutations and over 100 larger insertions and deletions that could not be predicted by the computer algorithms.[16]Challenges remain in the delivery of the CRISPR system to the target tissue or organ. For cancer treatments involve ex vivo manipulation where the patients' cells are extracted, edited, and then reinjected back. There are no better ways to deliver the CRISPR system to the target organ. Scientists have tried with plasmids and viruses. Both of them trigger immune response resulting in the deactivation of the CRISPR system. In some cases, they persist in the patient's body and cause off-target effects. Moreover, the CRISPR system is too large to fit in 1 viral vector. Often 2 vectors are required to transport the components of CRISPR to the cell, making it complicated. The use of lipid or gold particles is currently under experimentation. Some individuals may possess immune proteins that can attack the Cas9 protein to deactivate the CRISPR system.[16]

CRISPR applications need not necessarily be fatal or harmful to change our lives. These tools are very useful in bringing about revolutions in **crop development, pathology**, etc. At the same time, in the wrong hands, it leads to abuse and misuse that includes manipulation in germline genetics that is unlawful. Other genetic alterations can involve **mortality, production of clones, superhumans,** etc.[14] With such equipment in our hands, we even talk of

"designer babies."Public anxiety heightened in 2015 when Chinese scientists edited the human embryo using CRISPR. However, these experimental embryos were nonviable. Concern aroused whether fertility clinics would use CRISPR to produce **genetically engineered children** with desired traits like tall, smart, strong, etc.[17]

The technologies can possibly make children with no biological parents, or bring back the extinct woolly mammoths, or might develop human organs in pigs or recreate the entire human genome! Biotechnology in doing good and marvelous may create **unequal societies**.It is for time to decide whether these technologies are a boon or bane.Revolutions in biotechnology are profoundly positive and negative across the globe. Man has been practicing changing plants and animals for ages, first through selective breeding and more recently through tools at the molecular level. It definitely lends the potential for progress in healthcare, agriculture, and environmental issues. Yet, the ever-increasing diversification presents new ethical challenges and risks, namely the **bioweapons and biowarfare**.

4. The use of bioweapons (BW)

There are innumerable instances of the use of biological agents and their toxins throughout human civilization. What is worth noticing is the evolution of these bioagents and the increasing threats that they pose to us.The French Nobel laureate in Medicine of 2008, Luc Montagnier, ignited controversy claiming SARS-CoV-2 producing COVID 19 to be created in the laboratory. Worth mentioning is Luc Montagnier co-discovered Human Immunodeficiency Virus (HIV) along with Francoise Barre-Sinoussi.[18] The WHO scientists studied around 15000 sequences of this virus. Subsequently, during the virtual briefing, they reported that this virus is natural in origin.[19] As of 7 August 2020, the SARS CoV-2 virus has infected over 19.3 million people, killing more than 7.18 lakh people worldwide. Studies were conducted by the Scrips Research Institute in the USA, which was subsequently published in Nature on 17

March 2020. According to them, SARS-CoV-2 binds perfectly to the ACE-2 receptors of humans, and such precision would not have been possible to design. Prior to this, another publication in Nature in February reported a 96.2% similarity between SARS-CoV-2 and BatCoV RaTG13. BatCoV RaTG13 virus is found to reside in the intermediate horseshoe bats. This 3.8% difference of about 800 nucleotides links the virus to its host as different strains.[20]

4.1 The early instances of bioweapons and biowarfare

As early as 184 BC, during the battle of Eurymedon,Hannibal of Carthage made a win over King Eumenes II of Pergamon by throwing vessels filled with venomous snakes into the ships. In the fourteenth century, the Tatar force threw plague-infected corpses in the enemy groups. In the French and Indian War of the eighteenth century, smallpox victim used blankets were given to the native Americans. Anthrax, glanders, cholera, etc. were used during the First World War. The Japanese army exposed three thousand prisoners to plague, anthrax, syphilis during the Second World War was one of the notorious examples. In 1944, the United States stockpiled enough quantities of botulinum toxin and anthrax for using if German forces would have used their bioagents in the first. The British carried out trial tests in 1952 that includes Operation Cauldron. The accidental release of anthrax from Sverfdlovsk, Soviet Union, killed a minimum of 66 people in 1979, denied earlier but confirmed in 1992. Iraq started the bioweapon program in 1985, making anthrax, aflatoxin, botulinum. They admitted the possession of Scud missiles, rockets, spray tanks as delivery means after the Persian Gulf War. Around 751 people were intentionally infected with*Salmonella* in 1984 by the followers of Rajneesh. A similar attempt was made in Tokyo to spray anthrax by the AumShinrikyo cult in 1994. Many of us could recall anthrax smeared letters delivery to the United States government offices in 2001.

4.2. Organized bioweapons program

Former USSR continued undercover and unlawful offensive bioweapons program in the early 1990s. Their massive army program called "**Biopreparat"** had a civilian cover in over 18 bioweapons (BW) facilities. The yearly allotment in such programs, in the eighties, was around tens of millions of US dollars.[21] Their research activities pursued the most contagious and lethal diseases like plague and smallpox. Unlikely, the USA primarily worked with organisms non-contagious to humanity like anthrax and tularemia.[22, 23] The operation was so secretive that it became known to the United States intelligence only in 1989 only after their one scientist being deserted to the United Kingdom.

It was Dr. Vladimir Pasechnik, was a top-ranking secret scientist and also the Director of the Institute for Ultra Pure Biological Preparations in Biopreparat. He reported about the wide-ranging research program in 1989 after he absconded to the United Kingdom. He revealed further information about the development of **novel bioweapons** through genetic engineering.[24] The topmost priority was given to enhance the lethality of plague and tularemia. They strived in developing successive generations of tularemia cultures by inserting engineered plasmids that would become resistant to all known western antibiotics. The USSR had stocked 20 tons dried, plague powder. Germs like plague, anthrax, and smallpox could be delivered through spray tanks, cluster bombs, intercontinental ballistic missiles.[25]

In 1992, another deserted scientist to the United Kindom, known by the code name **"Temple Fortune,"** corroborated the earlier statement. The USSR continued with clandestine operations even after the announcement of termination by Mikhail Gorbachev and subsequently by Boris Yeltsin.[25] The scientists reported the making of avirulent **super-plague** in a stored system that could be transformed into a lethal one before weaponization.

This is known as a binary biological weapon where the benign pathogens could be mixed with virulence augmenting plasmids just prior to the loading process.[24, 25] The third absconded scientist Kanatjan Alibekov, disclosed that the Russians by 1992 have a total of 52 pathogens or combined agents like Marburg, Ebola, smallpox, etc. They labeled the deadly infectious and easy to produce and transport pathogens as **"battled strains,"** anthrax being their most favorite.[24] Alibekov also disclosed the researches towards the creation of totally novel forms of life. The first **chimera** was made by the insertion of Venezuelan equine encephalitis viral DNA into the vaccinia gene. He also mentioned the transference of myelin toxin gene to *Yersinia pestis*. The accidental release of Bacillus anthracis spores in 1979 from the Sverdlovsk facility killed 66 people. [26]

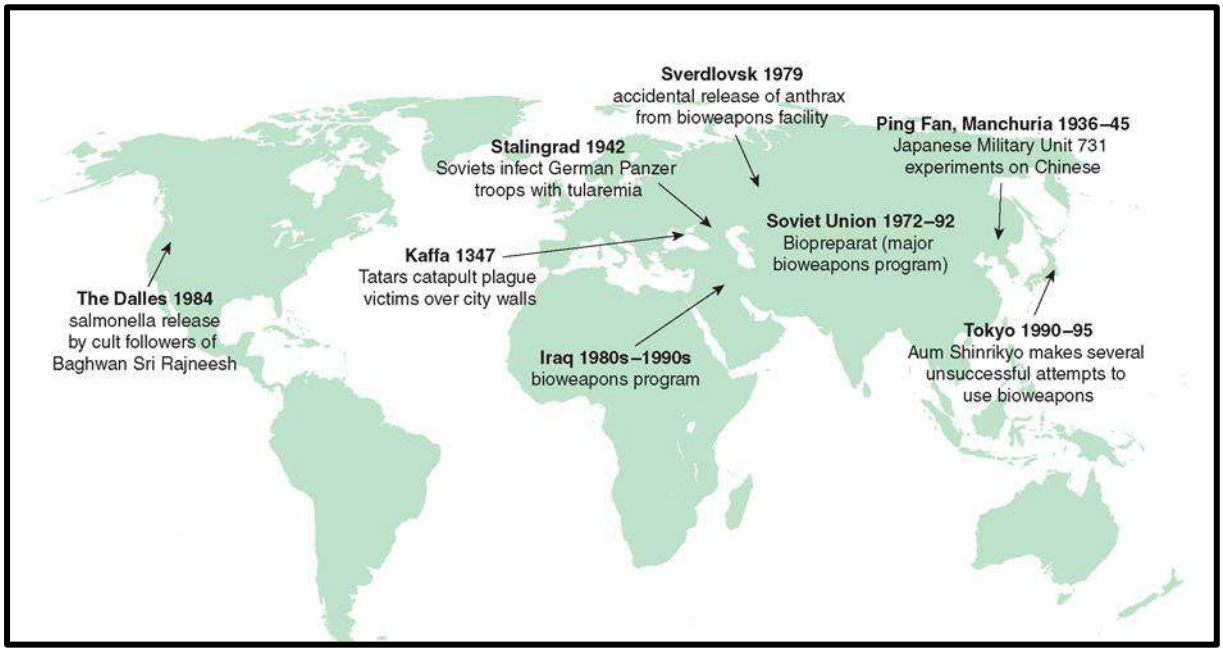

Figure 12: Some past incidents that involved bioweapons.[27]

4.3. The new generation of bioweapons

Bioweapons have been in use multiple times in the history of mankind, but what is trending is the **tailored making of pathogens** that would be more virulent and lethal. Recreation was first made possible by De. EckardWimmer in 2001 when he created the poliovirus.Dr. Jeffrey Taubenberger and Terrence Tumpey recreated the influenza virus of

1918 again in 2005. The application of genetic engineering allows phenomenal changes in the characteristics of the organisms. Such changes can be achieved by adding, altering, or deleting genetic sequences. With the advancement of knowledge, increased ability to synthesize DNA, and computation capability, the capacity of creating biological weapons increased manifold.[28] The United States evaluated the effectivity of *Fusarium* along with its genetically engineered version to be used against coca plantation from which cocaine is obtained.[29]Many of the well-known universities and institutions across the globe have both the expertise and technology that could produce such deadly strains of pathogens. It is not surprising that terrorist groups have easy access to this knowledge. Iraq possibly deterred from biological and chemical warfare during the Gulf war, fearing the overwhelming nuclear attack.[25] The possibility of genetically engineered bioweapon use by the terrorist group is squat, but the result of such a happening would be enormously great.

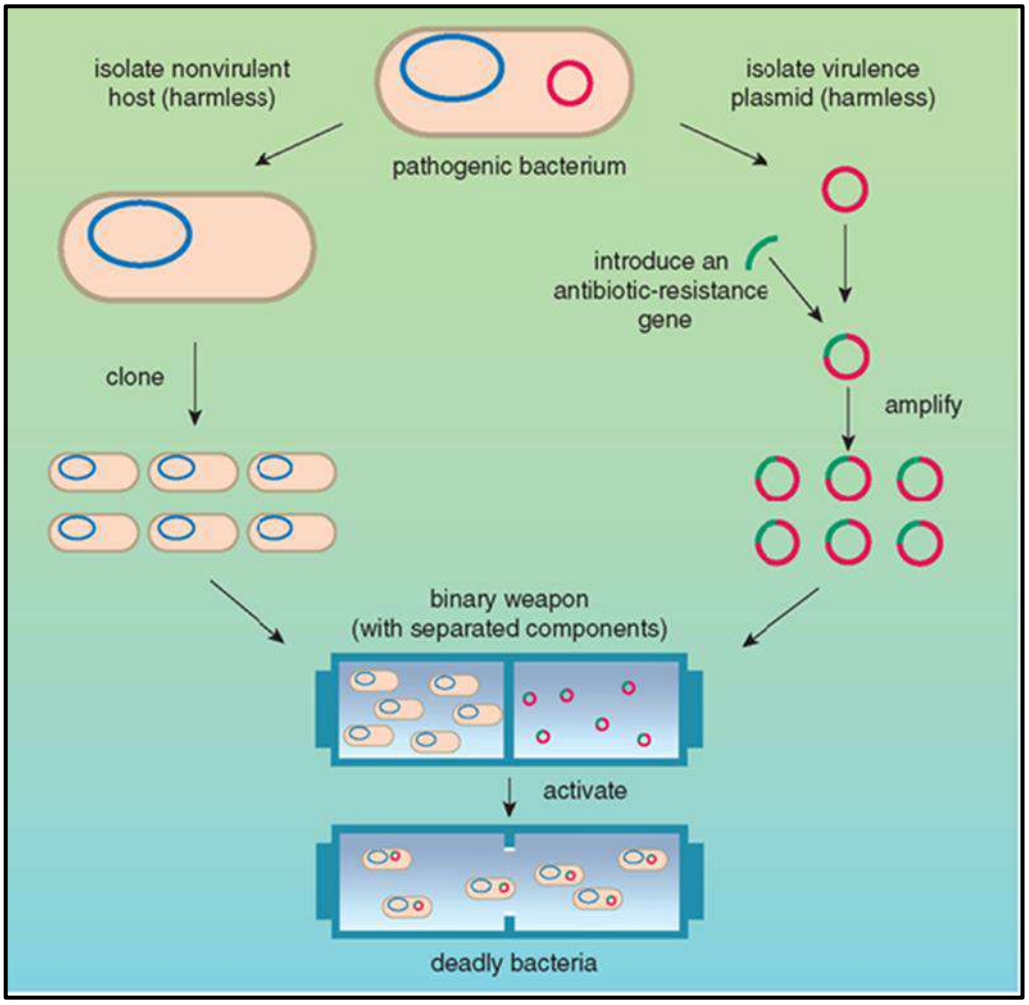

Figure 13: Modern technique to produce novel and desired strains of bioweapons.[27]

4.4. Biowarfare and its consequences

Biowarfare is the deliberate utilization of microorganisms and toxins to trigger diseases and/or kill cattle, crops, and humans. All the three categories of bioweapons, chemical weapons, and nuclear weapons, exhibit the shared property of inflicting mass annihilation; even though biowarfare is somewhat dissimilar. Biowarfare could be traced to hit economic targets like crops, livestock, and the environment. Further, they lead to a disease outbreak in the form of **endemics, epidemics, and pandemics**. Of all three lethal weapons, bioweapons are the most feared one. Biological warfare constantly threatens **human health, food security, and environmental resources**. Anticrop warfare agents like herbicides and defoliants may result in famines, malnutrition, and economic breakdown. The World Wars have witnessed the cases of late blight in potatoes, anthrax, wheat rusts, etc. Foodborne pathogens cause morbidity from six and a half to thirty-three million cases and up to nine thousand deaths in the United States every year. These incidents can be attributed to *Salmonella typhosa, Campylobacter jejuni, Escherichia coli, Listeria monocystogenes, Staphylococcus aureus,* and *Clostridium perfringens*. Whitefly transmitted viral disease of crops brought severe economic losses amounting to 50 million US dollars in the Dominican Republic in the mid-seventies. Even the use of cocaine, heroin, marijuana, derived naturally, or by genetic engineering is another form of biowarfare.

Table 1: Definition of Potential Bioterrorism Agent Categories based on threats, classified by the Centers for Disease Control and Prevention (CDC)and National Institute of Allergy and Infectious Diseases (NAID) component of the National Institutes of Health (NIH)https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/potential-bioterrorism-agents

Category A	Category B	Category C
Pose the highest risk to national security	Pose the second-highest risk to national security	Emerging pathogens that could be engineered for mass dissemination
Can be easily disseminated or transmitted from person to person	Are moderately easy to disseminate	Are easily produced and disseminated
		Have potential for high morbidity and mortality rates and major health impact
Require special public health preparedness actions	Require enhanced diagnostic capacity and disease surveillance	Are available
Have the potential to cause public panic and social disruption		

The biotechnology that promises to save lives can also be misused to **kill masses**. This is known as **black biology**. This revolution in biotechnology could be considered as a potential "Revolution in Military Affairs" (RMA) and requires 4 essentials- technology progression, integration of novel technology in army systems, army functioning invention, and structural adaptation. The human genome is sequenced. Gene therapy would permit replacement and mending of the defective genes. Gene therapy promises to be the holy grail of modern medicine. **Molecular genetics, genome sequencing, gene splicing** has the potential for dual-use. Paradoxically the biotechnology that is used to produce novel drugs or vaccines could be

utilized to develop dangerous virulent weapons. So science that could be employed to protect lives can also be utilized to kill people. Increased biotechnological knowledge results in the inclination of the terrorists to perpetrate mass fatalities and amplified desolation. [30]

President Clinton got highly sensitized after reading the 1997 fictional novel "The Cobra Event" by Richard Preston about a genetically engineered super-virus. This led him to issue 2 Presidential Decision Directives to address the national security deficiencies. It was in response to the 9/11 attacks and numerous anthrax smeared letters sent in various places that President Bush launched the Homeland Security Council to coordinate the various efforts of around forty institutions at a national level to have preparedness for the unexpected in the future.[31] The anthrax of 2001 was a known strain and not contagious, but they are quite stable and can remain viable for years. But genetically altered pathogens could prove a much more difficult challenge. The Atlanta based Centers for Disease Control and Prevention (CDC) leads in tracking epidemics globally. It had carried out investigations on the outbreak of Ebola, Marburg, Hantavirus, and others. The genomic sequence of smallpox was known and hence feared to be manipulated. The only authorized laboratories for smallpox globally are the American Centers for Disease Control and Prevention and the Russian State Research Center for Virology and biotechnology situated in Koltsovo. But it could be apprehended that such cultures might have been transferred elsewhere.[32]

The nations to upkeep the bioweapon capability of research facilities include Russia, China, Iran, Iraq, North Korea, Syria, Libya, India, Pakistan and Egypt, Israel, and Taiwan. Many developed nations have some sort of defense capability against **bioterrorism**. This generally includes deployment "military mission-oriented protective posture" (MOPP) kit and non-combatant "hazardous material" (HAZMAT) responder spacer outfits. Vaccines and antibiotics to such intimidations are stocked, which seemed to be very significant.

The bioweapons are unique in their indiscernibility and deferred impacts. It not only cause sickness but creates **panic and uncertainty**. It can **paralyze the government, military responses, and trigger a social and economic breakdown**. The choice of bioweapons usually determined by the technical, financial, and economic potential of the attackers.[33] The risks of infectious diseases emerge and re-emerge on a regular basis. For instance, consider the appearance of bird flu. Both the H5N1 avian influenza virus and the H1N1 swine flu virus resulted in mild pandemics in between 2009 and 2011. The bird flu virus emerging in 1997 and in ten years' time culled around three hundred million birds with over 200 connected human deaths. It appeared in Hong Kong but with time had spread across Asia, Europe, and Africa. Two small clustered sequence of H5N1 was thought to be responsible for a human to human transmission. The H5N1 virus underwent mutations and recombinations with the human-adapted influenza virus, which could make the possibility of easy transmission amongst humans.[34] Researchers have developed bio-armory with potent antibiotics, antiserum, toxoids, and vaccines to neutralize and evade a vast pool of these threats.[35]

Table 2: Few Examples of Potential Agents/Diseases by Category

https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/potential-bioterrorism-agents and https://emergency.cdc.gov/agent/agentlist-category.asp

Category A	**Category B**	**Category C**
Botulism (*Clostridium botulinum toxin*)	Food safety threats (*Salmonella* species, *Escherichia coli* O157:H7, *Shigella*)	Nipah virus
Anthrax (*Bacillus anthracis*)	Brucellosis (*Brucellaspecies*)	Hendra

Tularemia (*Francisellatularensis*)	Cholera(*Vibrio cholerae*)	Prions
Smallpox (variola major)	Glanders (*Burkholderia mallei*)	Rabies
Filoviruses (Ebola, Marburg)	Hepatitis A	Tickborne encephalitis
Arenaviruses (Lassa, Machupo)	Ricin toxin from Ricinuscommunis	
	Salmonella	
	Typhus fever(*Rickettsia prowazekii*)	
	Yellow fever	
	Psittacosis (*Chlamydia psittaci*)	
	Q fever (*Coxiellaburnetii*)	

5. Conclusion

The key dive in the genome editing tools and techniques over the years brought new urgency to the long-standing discussions regarding its ethical and social implications, especially concerning the application in humans and designing potential lethal bioweapons. The debate and dialogue relating such tools no longer remain in the papers and books with the advent of the CRISPR/Cas0 system since these tools have made the work really simple, fast, and affordable. The completion of the human genome project is an added fuel to it. From the earlier discussion we have seen than unlike the ZFNs and TALENs, the CRISPR system uses **RNA-DNA binding** rather than on protein-DNA binding. This binding system simplifies the

designing process and enables the application to a wide range of target sequences. Further, in 2015, Zhang reported the successful application of **Cpf1** instead of Cas9 that could be used in gene editing. Cpf1 is a microbial nuclease enzyme that has better advantages above the Cas9 system. Cpf1 requires only one gRNA for specificity and induce staggered cuts in the DNA rather than blunt cuts in the dsDNA. Hence it gives greater control on the insertion of the swapped DNA. By modifying the phage genomes with CRISPR tools, researchersassert to progress methods to terminate antibiotic-resistant bacteria. CRISPR has also empowered us to produce animal models for human diseases and the abstraction of HIV from the affected cells. In November 2018, a Chinese scientist, He Jiankui, declared the birth of the world's first **gene-edited human babies**-the twin girls that carry a gene to reduce the risk of HIV infection. He and his collaborators (Zhang Renli and Qin Jinzhou) were found guilty of conducting "illegal medical practices" by a court in Shenzhen and sentenced to 3-year prison life. He Jiankui was also fined with 3 million Chinese yuan.

The advent of genetic engineering and molecular biology since the eighties budded to dominant security imaginaries, more dystopian.All the possible discoveries in gene editing have their advantages and hidden challenges. Some believe that the remarkable progress in the last few years in biotechnology increases the chance of better and newer bioweapons that might violent the international norms to possess and use them. Modern-day bioweapons could become **designer biological weapons**. The recent tool CRISPR/Cas9 shows the easy guide to such deadly bioweapons when misused and hence the development of **dual-use technology**. It is a fast, more precise, and inexpensive tool to use that could quickly get in the hands of nations, radical groups, or terrorist organizations. Rapid scientific discoveries prompt the policymakers to ponder and analyze the security threats on such implications, like the possibility of making lethal and contagious pathogens, the possibility of introduction of antibiotic-resistant bioweapon agents, the possibility of chimeric bioweapons and undetectable bioweapons, etc. At the global level, the United Nations is struggling to control and predict such nefarious developments that would ruin the very existence of human civilization. The **Biological Weapons Convention {"Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on**

their Destruction"} was put to the signature on 10 April 1972 and was implemented since 26 March 1975 following the requisite ratification. As of 2019, the Convention has 183 Parties.**Technology never develops and diffuses in isolation- it is always fashioned by the variability of social forces and is the primary driver of the history of the contemporary world.** Genome editing tools can deliver miracles and either turn into boon or bane. It is absolutely in the hands, minds, and hearts of humanity to decide whether it should be used as a cure or to turn itself into a nightmare!

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Article 17

ARSENIC TOXICITY IN WEST BENGAL AND ITS POSSIBLE MODES OF PHYTOREMEDIATION

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Abstract:

Arsenic is found naturally in the soil. High levels of arsenic have been found in several parts if India especially West Bengal. Bihar, Jharkhand etc. Arsenic is a potent carcinogen and high levels of arsenic in the soil and groundwater is a source of menace for the living organisms like plants, animals and humans. Possible sources of bioremediation highlighting the use of natural plants can be used to curb these high levels of arsenic toxicity both in soil and groundwater.

Keywords:

Arsenic, West Bengal, toxicity, bioremediation, phytoremediation

Introduction:

Arsenic (As), a toxic compound that is found in many parts of the world both in soil and ground water. According to Nriagu et al (2007) Arsenic is found naturally in the Earth's crust. Levels of arsenic in the atmosphere usually ranges in between 1 to 3 ng/m^3 , varied concentration of arsenic in populated areas ranges between 20 to 100 ng/m^3 and concentrations of about (> 1000 ng/m^3) have been found in the industrial areas. Average levels of arsenic in the ground water are found to be in between 1–2 µg/litre .Background concentrations of arsenic in soil

varies from 1 to 40 mg/kg, and have mean values of around 5 mg/kg. High levels of naturally occurring arsenic in soils may be due to geological substrata such as sulfide ores.

According to Adhikary et al (2017) Arsenic contamination was first reported in the ground waters of West Bengal, India in 1978 . In some of the districts, the concentration of arsenic in the ground water was found to be more than 50µg/L. Many regions of North 24 Parganas, South 24 Parganas, Nadia, Murshidabad , Burdwan, Howrah, Hooghly and Maldah districts have been severely affected by this menace of arsenic pollution in the ground water. .

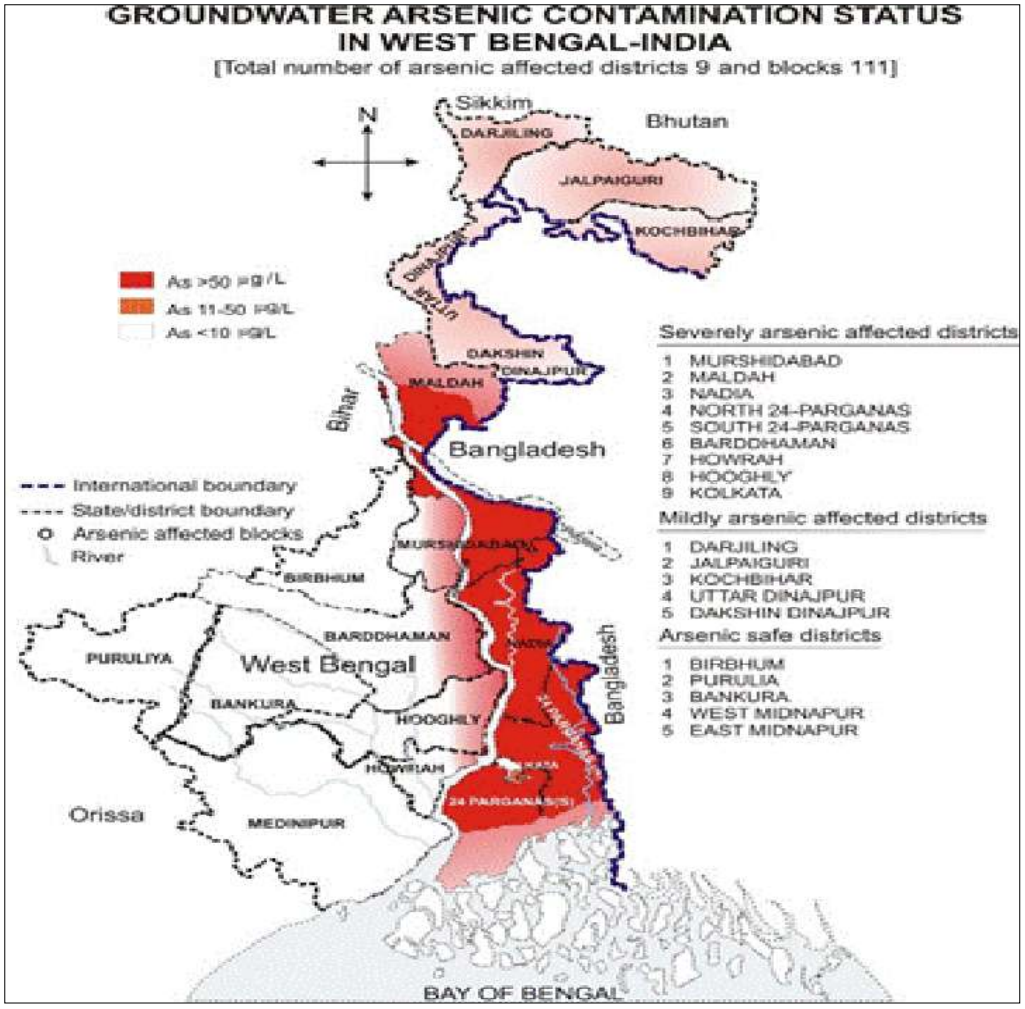

Fig 1: Map of West Bengal showing areas of arsenic pollution

Source : http://www.soesju.org/arsenic/wb.htm

Basu et al (2015) had said that the US Environmental Protection Agency (EPA) has regarded inorganic arsenic as the most potent source of “human carcinogen" (maximum contaminant level 0.01 ppm). People get exposed to these carcinogens via the groundwater which they use for drinking purposes. Ground water usually gets contaminated with inorganic arsenic salts. Water used for irrigating the crops also can contains high sources of arsenic that may ultimately percolate in the groundwater and soil , thereby would enhance the levels of arsenic toxicity in the fields and ground water.

Arsenic is highly toxic in nature and if it comes in contact with the human population , it can cause some serious diseases other than cancer like skin lesions which appear as "rain drop pigmentation" and keratosis that is commonly referred to as arsenicosis. Lung diseases like bronchitis, chronic obstructive pulmonary disease (COPD), liver diseases like non cirrhotic portal fibrosis, peripheral vascular disease, hypertension, edema, congestion in conjunctiva, feeling of weakness, anemia and certain forms of neurological disorders are some of the serious illness that arsenic pollution can cause among people. It has also resulted in an increased possibility of stillbirth.

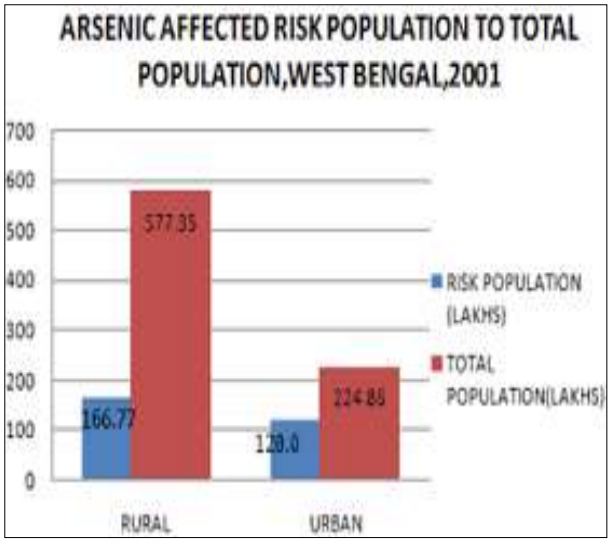

Fig 1A: Graph showing the arsenic affected risk population of people in West Bengal (2001). Source: PHED (Public Health Engineering Dept.) Data, 2001

1. Effects of Arsenic toxicity on Plants

According to Finnegan (2012) Arsenic, is not essential for the growth of the plants or certain living organisms except for some microbial organisms that usually uses arsenic in place of phosphorous for certain metabolic functions.

Generally, natural soils usually contain lower arsenic levels at about less than 3.6mg/kg. Concentrations of arsenic higher than 3.6mg/kg tend to affect the plants and creates toxicity in the. Many evidences have been found, whereby arsenic is taken by the roots from the soil or sometimes airborne arsenic is found to be settled upon the leaves (18)

The most prominent form of arsenic in soil is arsenate. It intervenes into the functions of plasmalemma of roots. It also hampers the growth of plants due to its high toxicity (15, 16)

Certain impacts of the toxic nature of arsenic upon plants results into reduction in the levels of seed germination[6], interrupted plant height (6,8,11,15,16)], hampers the root growth , causes necrosis[6], inhibits shoot growth [9] and also results in the decrease in the yield of fruits and grains[6,3,8 12.] High toxicity induced by arsenic upon the plants can also sometime lead to the formation of withered and yellow leaves [14] disfigured roots. There are significant reductions in the amount of proteins and chlorophyll content [15, 16] of the plants that are affected by the toxicity of arsenic present in soil and ground water. Majority of these effects are seen upon the rice plants in the paddy fields since there is greater possibility of the movement of arsenic in flood laden soils [13] and also the groundwater with which the plants are watered can contain high concentrations of arsenic**.** This groundwater is gradually been taken up by the roots and this cycle of arsenic toxicity progresses within the plant.

2. Biological Remediation to mitigate Arsenic toxicity

According to Shrivastava et al (2015), one of the methods to overcome the menace of arsenic toxicity is by using biological remediation. It can be achieved by using certain types of plants and microbes.

Metal hyper accumulation considers biomass of metal concentration in the vegetative portions that are located above the ground, and also the metal concentration within the soil. Bioaccumulation factor (BF) and translocation factor (TF) are taken into account before classifying a plant as a metal hyper accumulator (Ma et al., 2001). Bio accumulation factor (BF) usually refers to the ration of metal concentrations within the plant biomass to that of the metal concentrations present in the soil. Translocation factor (TF) refers to the metal concentrations present within the shoots to that of the roots (Tu & Ma, 2002). A plant can be considered as an arsenic hyperaccumulator if they have a ratio whereby the value of BF > 1 and TF > 1, and are able to have a total accumulation > 1,000 mg kg^{-1} arsenic in plant biomass.

Pteris vittata also commonly known as brake fern has been known to be hyperaccumulator for arsenic. It can absorb three to six times more amount of arsenic in soil. There are very few species of plants that are usually tolerant to arsenic naturally. Some of these plant species are *Pteris vittata* , some members are Pteridaceae that usually accumulate As (Ma et al., (13); Visoottiviseth et al., (21); Zhao et al., (22). Plants that are hyperaccumulators of arsenic allow arsenic movement to the shoots. They do not tend to affect the growth of plants.

Pteris vittata is usually found in parts of Nepal, Eastern Afghanistan, Bhutan, India (Arunachal Pradesh, Himachal Pradesh, Meghalaya, Sikkim, Uttarakhand, parts of West Bengal ,Jammu & Kashmir) , Pakistan, Myanmar , SW-China, parts of southern China, and Tibet

Phytoremediation of arsenic by green plants can be done by some methods like phytoextraction, phytostabilization, rhizofiltration , phytovolatilization

Phytoremediation technology is a modern concept. The plants and the microbial community of rhizosphere absorbs, removes, or transforms As along with other trace metals from the contaminated soils It is a cost effective mechanism and thus very advantageous, since it does not cause any additional harm to the plants and soil.

During the process of phytoremediation, plants remove the harmful components of the soils and transfer them to the vegetative portion, thereby relieving the soil of the toxicity. The effectiveness of phytoremediation of arsenic by Chinese brake (*Pteris vittata* L.) can be improved by increasing the quantity of phosphorous in the soil since higher amount of phosphorous helps in enhanced plant growth and simultaneous uptake of arsenic.

Some other species of plants that are capable of functioning as arsenic accumulators are *Pityrogramma calomelanos* [21], *Pteris cretica*, *P. longifolia* and *P. umbros*

Pteris sp can absorb around 1442–7526 mg/ kg arsenic in fronds from contaminated toxic soils, and about 27,000 mg/kg As in its fronds in hydroponics culture. It also transfers the arsenic to their vegetative structures that are situated above.

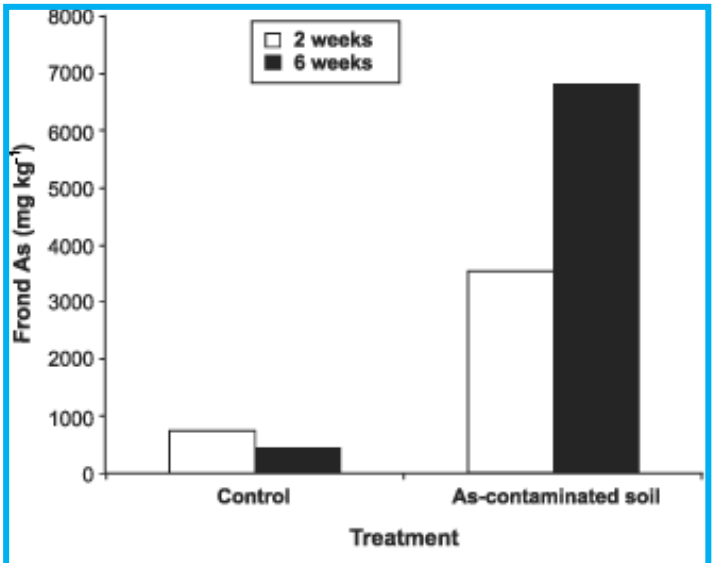

Figure 2 - Arsenic concentration in the fronds of *Pteris vittata* after growing for two and six weeks in uncontaminated soil (6 mg kg^{-1} As) and As-contaminated soil (400 mg kg^{-1} As). Adapted from Ma et al., (2001).

Source: Gonzaga, Maria Isidoria Silva, Santos, Jorge Antonio Gonzaga, & Ma, Lena Qiying. (2006). Arsenic phytoextraction and hyperaccumulation by fern species. *Scientia Agricola*, *63*(1), 90-101. https://doi.org/10.1590/S0103-90162006000100015

3. Phytoextraction

Shrivastava et al(2015) had stated that arsenic is been accumulated into the roots and transferred to the vegetative parts of the plant to the surface This is done by the plants that are growing on soils contaminated by high levels of arsenic toxicity. [7]. Some of the main limiting factors for phytoextraction are: Bioavailability of arsenic around the rhizoplane, arsenic uptake by roots ,time required for transfer of arsenic to the xylem and their subsequent transfer to the shoots ,level of arsenic that can be tolerated by the plant cells.

4. Phytostabilization

Some plants have the tendency to reduce the amount of water moving down through the sediment matrix and thus functions as a preventive measure. This method decreases the chances of soil contamination Phytostabilization can also occur through sorption, complexation, or metal valence reduction [17]. Plants with high arsenic tolerance and low translocation factors can be considered effective for arsenic phytostabilization . Plant families that can be considered suitable for As phytostabilization are members of Asteraceae and Chenopodiaceae (Mendez and Maier 2008b). Incorporation of iron and calcium in the soil can improve these abilities of the plants.

5. Rhizofiltration

During the process of rhizofiltration, along with the movement of capillary water through the root xylem, certain amounts of harmful contaminants like that of arsenic are also taken up. This process helps in relieving the soil from harmful contaminants like arsenic and thereby helps in reducing the toxicity of the soil.

6. Phytovolatilization

Arsenic along with other harmful chemicals are taken up by the plants from the groundwater with the help of roots. It gets transferred to the xylem vessels and gets volatilized and the harmful chemicals are usually released via transpiration [10]. *P. vittata* could absorb high amounts of inorganic As compounds, like arsenite and arsenate, in their tissues and released large amounts of As from the fronds(19)

Conclusion

Arsenic is one of the components that occurs naturally in the soil and groundwater of Earth's crust. Presence of industrial areas close to the civilazations tends to increase the arsenic toxicity of both soil and water. Although high levels of arsenic possess severe health hazards to the living organisms, it can be curbed down by using certain bioremediation measures like that of phytoremediation which are simple and cost effective. Use of plants like *Pteris vittata* ,*Pityrogramma calomelanos* , *Pteris cretica*, *P. longifolia* and *P. umbros* has been able to mitigate this menace of arsenic toxicity to certain extent. *P. vittata* is very effective for phytoremediation of As-contaminated soil since it is able to phytoextract As and release the bio accumulated As from the fronds. Use of transgenic plants in the near future can be done to treat this arsenic toxicity with higher improved abilities.

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Weblinks

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Article 18

Cucurbita maxima and *Momordica charantia*: Two members of the family cucurbitaceae with contrasting characters- A comparative study

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Abstract

Cucurbita and *Momordica* are the two commercially important genus of the family cucurbitaceae. The fruits of some species of these two genus are regarded as very popular vegetables in daily diet. In spite of belonging to the same family these two genus exhibits myriad contrasting characters those insisted to pursue a comparative study with these two genus. The most contrasting character that easily attracts the attention of all is the taste of the fruit of the two genus. *Cucurbita* fruit is extremely sweet in taste while *Momordica* fruit is extremely bitter in taste. Common name of *Cucurbita maxima* is pumpkin and common name of *Momordica charantia* is bitter melon. The food value of these two vegetables are quite contrasting. Diabetic patients are prohibited to consume pumpkin fruits whereas they are insisted to consume bitter melon fruits by medical practitioners of different systems of medicine as bitter melon fruit has hypoglycemic properties and pumpkin fruit's carbohydrate content is higher than that of bitter melon fruit. Food values of both the fruits is compared to analyze health benefits.

Introduction

Nutrition Facts	Bitter melon		Pumpkin	
Amount Per 100 grams				
Calories	17		26	
		% Daily Value*		% Daily Value*
Total Fat	0.2g	0%	0.1g	0%
Cholesterol	0mg	0%	0mg	0%
Sodium	5mg	0%	1mg	0%
Potassium	296mg	8%	340mg	9%
Total Carbohydrate	3.7g	1%	7g	2%
Dietary fiber	2.8g	11%	0.5g	2%
Protein	1g	2%	1g	2%
Vitamin A	9%		170%	
Vitamin C	140%		15%	
Vitamin D	0%		0%	
Vitamin B-6	0%		5%	
Calcium	1%		2%	
Iron	2%		4%	
Cobalamin	0%		0%	
Magnesium	4%		3%	

*Per cent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

The data on the above table shows that calorific value of bitter melon is quite lower than that of pumpkin. Diabetic patients are advised to consume foods having low calorific value.

Seed germination and further growth traits in these two genus are quite different. Pumpkin is more sensitive to different environmental factors like soil quality, light condition for seed germination. It needs well aerated soil and direct sunlight for seed germination. On the contrary less care is needed for bitter melon seed germination. Bitter melon grows faster than pumpkin. Bitter melons grow best in hot and humid climates. Bitter melons can tolerate less desirable sandy- or siltly-loam soil but good drainage is essential. Bitter melon seeds germinate in 8 to 10 days, though low and high temperatures and soil too dry or too wet can slow germination. Leaf growth starts after 11days of seed germination. Vines grow 13 to 16 feet long and commonly begin flowering about 35 to 42days after planting. Male flowers open first, followed in a week or so by female blossoms. Both flowers are yellow. Female flowers have a swelling (the ovary) at the base of the bloom resembling

a tiny melon. Bees and pollinating insects visit both blooms, transferring pollen from male to female flowers. Usually male blooms live only one day; they open in the morning and fall from the plant in the evening. Flower drop is not uncommon. The ovary of pollinated female flowers will begin to enlarge and fruit will mature in two to four months. Mature fruits will be ready to pick about 3 months after planting. They will be light green and juicy with white, bitter flesh.

A soil with sandy loam along with good drainage power and all essential organic matter is supposed to be the best soil for pumpkins**.** Seed germinates 5–7 days after sowing. The plants form an extensive fibrous root system. The growth habit is indeterminate; under suitable conditions, the trailing stems continue to grow indefinitely when they are permitted to root at the nodes. The stems may reach a length of more than 20 m, but they are usually no longer than about 5 m. Bushy cultivars with short internodes and semi-erect stems also exist. Flowering starts 35–60 days after germination and is more or less continuous. The ratio of male to female flowers is about 20:1. This ratio is influenced by the growing conditions, long days and high temperatures favouring male sex expression. Production of the sticky pollen is abundant. Anthesis and pollination take place early in the morning. Insects, mainly bees, effect pollination, so the flowers are predominantly cross-pollinated. The fruits mature 30–40 days after pollination. One or two fruits develop per stem. The fruit-harvesting period extends from 2–6 months after sowing.

Discusision

Cucurbita maxima originates **from temperate South America.** *Cucurbita andreana* Naudin is considered the wild progenitor; this species is native to warmer temperate zones in South America as well as humid lowland regions of Bolivia. Seeds of *Cucurbita maxima* excavated in Peru have been dated at 1800 BC. After 1492, when Columbus arrived in the Americas, it spread all over the tropics and subtropics, as well as temperate areas with warm summers. *Cucurbita maxima* has been reported from many countries in tropical Africa and probably occurs in all countries. It is most important in the cooler parts of southern Africa and the Sahel region, less important in more humid West and East Africa, where *Cucurbita moschata* Duchesne is more common.

Momordica charantia is **native to the Old World tropics,** but now pantropical. It was possibly domesticated in India and southern China and is now found naturalized in almost all tropical and subtropical regions. It is an important market vegetable in southern and eastern Asia, e.g. India, Sri Lanka, Vietnam, Thailand, Malaysia, the Philippines and southern China. Local cultivars originally from Asia are cultivated on a small scale in tropical America, and bitter gourd is also cultivated in the southern part of the United States for the Asiatic kitchen. It is a **common cucurbit in the wild flora of Africa, occurring almost throughout tropical Africa**. It is only occasionally collected from the wild as a vegetable or medicinal plant. It is occasionally cultivated in East Africa mostly by people of Asian origin using Asian cultivars.

Systematic Position	Pumpkin	Bitter melon
Kingdom:	Plantae	Plantae
Clade:	Tracheophytes	Tracheophytes
Calde:	Angiosperms	Angiosperms
Clade:	Eudicots	Eudicots
Clade:	Rosids	Rosids
Order:	Cucurbitales	Cucurbitales
Family:	Cucurbitaceae	Cucurbitaceae
Genus:	*Cucurbita*	*Momordica*
Species:	*C. maxima*	*M. charantia*

Conclusion

Though both genus are cultivated globally now a days they have different centre of origin and this may be the reason behind the difference in their germination, growth and development criteria. The effect of such differences in external features is manifested through the difference in their nutritional value. Both the genus have immense medicinal and economic importance.

Bitterness is the result of the alkaloid momordicine found in growing bitter melons. The darker the colour of a bitter melon the more bitter and intense the flavour of the fruit. Bitter melon helps in the entire digestion process. It stimulates appetite. It has emetic, purgative and anthelmentic properties. It is also anti-flatulent. It is known for its anti-lipolytic properties. It is anti-inflammatory and astringent. It also shows anti-cancer, anti-tumor, antibacterial, anti-viral, antidiabetic properties. It lowers blood pressure, body temperature and blood cholesterol.

Pumpkin has high carotenoid content. Lutein is the most abundant carotenoid in pumpkin.It is known to have diverse heath effects. Pumpkin with orange pulp have high content of beta-carotene and lutein both act as antioxidents. Pumpkin shows anti-tumor, hepatoprotective, diuretic properties. It acts as vermifuge and taenicide. It is a remedy for curbuncles. It reduces the symptoms of benign prostatic hyperplasia. Pumpkin flowers have antimicrobial activity. Pumpkin seed oil protects against genotoxicity caused by bisphenol A and azathioprine. Pumpkin seed oil also has antihypertensive and cardioprotective effects.

Both the genus have a broad range of health benefits and thus they have succeeded to acquire a place in the list of vegetables for daily consumption. Pumpkin has a high Glycemic Index (GI) at 75 but a low Glycemic Load (GL) at 3. Thus large amount of pumpkin consumption can drastically increase blood sugar. On the contrary bitter melon has hypoglycemic properties that acts like insulin. Therefore diabetic patients avoid pumpkin consumption and prefer regular consumption of bitter melon as a part of their daily diet.

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Article 19

Wood rotting fungus in a fossil wood of *Shorea* sp. collected from Garbeta,West Bengal..A Report

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Abstract

Plant fossils are one of the most reliable indicators of the past climate and petrified secondary wood elements act as highly conserved structure, which remain almost unchanged with time, and hence store valuable information in different geological ages.

In the present study fungal remains have been detected in the extinct woods of *Shorea* of Dipterocarpaceae family. The fossil woods were collected from the Tertiary exposures of Garbeta, West Bengal. Fungal hyphae were observed as a network within the wood vessels and ray cells. The hyphae were branched and aseptate with occasional bulges along the hyphal margins.

Wood is a rich organic substrate providing suitable source of nutrition for fungi. Fungal members draw nourishment from the cell wall material through a process of active enzymatic degradation. Wood comprises heartwood which is made up of dead xylem cells in the center of the tree trunk responsible for structural support. Heart wood is the most vulnerable part of the wood as it is attacked by various saprobic fungi. Hence wood decay by different fungal species is a common phenomenon in nature. Occurrence of fungal remains in timbre yielders reflect a hot and humid prevailing condition in the forest.

Fungi grow abundantly in present day moist tropical forests of Bengal Basin and *Shorea* wood often are found infested with fungal association. The presence of fungal remains in the fossil wood studied establishes similar association in the past and can also be referred to comment on past climatic condition.

Key Words: Fungal remains, Shorea ,Neogene, West Bengal

Introduction

Fungi are a diverse group of organism which play key roles in tropical forest ecosystems as mutualists, saprotrophs, pathogens and decomposers.

In particular, their interactions with trees, as the main structural component in forests, can influence carbon and nutrient cycling and the maintenance of biodiversity. Tropical forests flourish well under hot and humid conditions and hence act as a natural home for many fungal members which require similar climatic conditions.

West Bengal an eastern state of India has a wide cover of forests including temperate, evergreen, deciduous and tidal types. The districts of Bankura ,Birbhum and Midnapur reflect a dominace of tropical semi dry to moist deciduous to mixed evergreen forests. Western part of Midnapur is mostly covered by tropical moist deciduous forest bearing woody members viz.,Sal i.e ***Shorea*** along with *Lagerstroemia, Acacia, Dalbergia, Butea, Holarrhena Terminilia* Madhuka etc. Many of these timber yielders are infested with wood decaying fungi (K.R.Ranadive 2013; K.Acharya et.al 2012) .

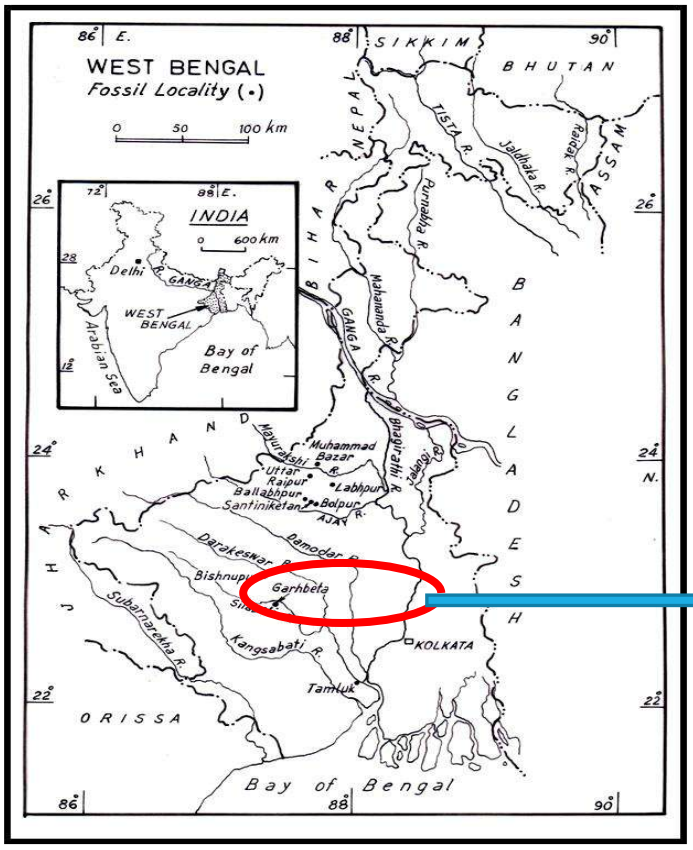
Shorea is a large, deciduous tree up to 50 m tall belonging to family Dipterocarpaceaea , abundantly distributed in tropical evergreen to moist and dry deciduous forests of West Bengal

SAL FOREST IN MIDNAPUR DISTRICT, WEST BENGAL

Wood is a rich organic substrate providing suitable source of nutrition for fungi. Fungal members draw nourishment from the cell wall material through a process of active enzymatic degradation. Wood comprises heartwood which is made up of dead xylem cells in the center of the tree trunk responsible for structural support. Heart wood is the most vulnerable part of the wood as it is attacked by various saprobic fungi. Hence wood decay by different fungal species is a common phenomenon in nature. Occurrence of fungal remains in timbre yielders reflect a hot and humid prevailing condition in the forest.

Material and Method

The petrified fossil wood for the current study was collected from Garbeta, (82^{o} 20 ′ 22^{o} 52′) lying in West Midnapur District of West Bengal. The fossil wood was sectioned along three standar planes for xylotomical data.



A FOSSILIFEROUS SITE

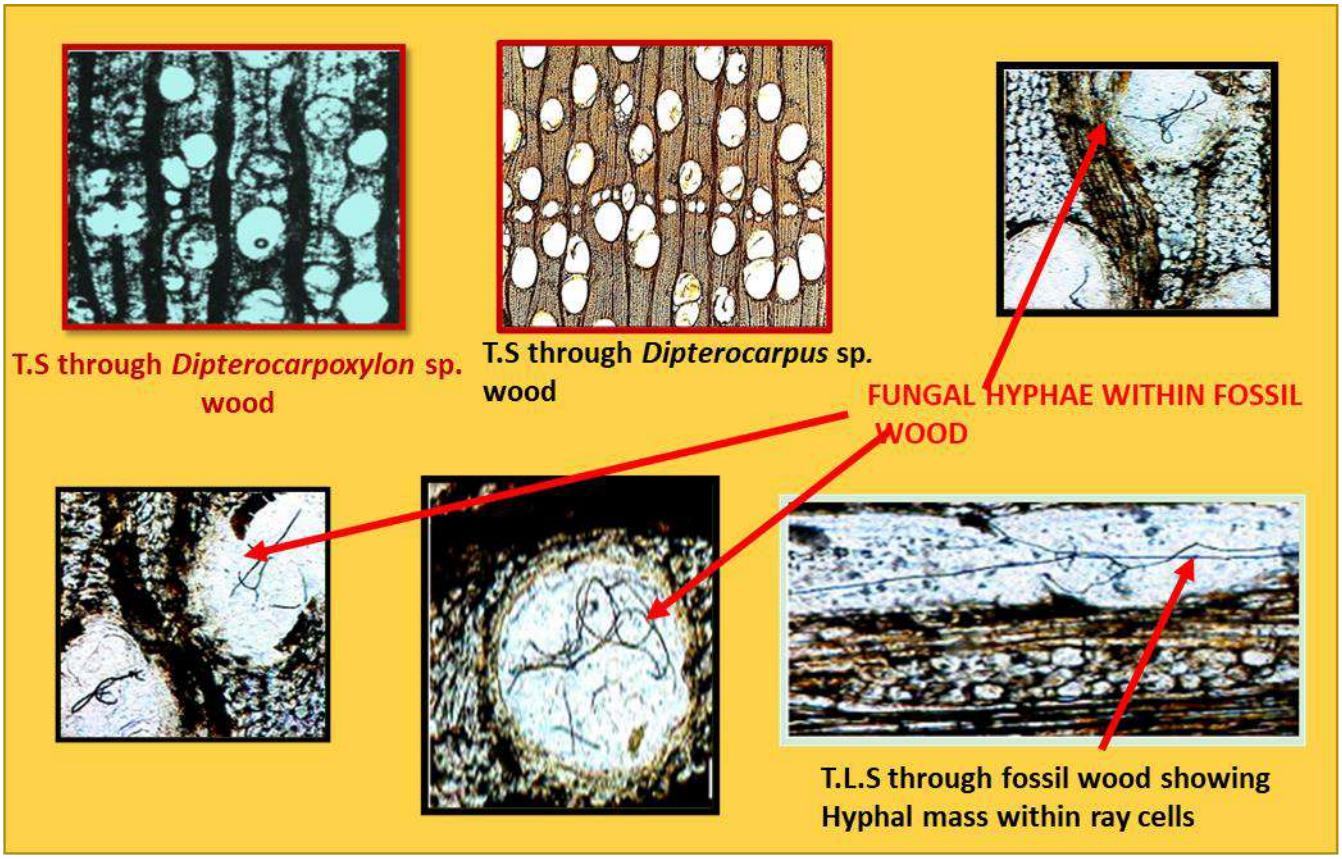
PETRIFIED WOOD FROM STUDY LOCALITY

Xylotomy is a branch of plant science which deals with the systematic study of different elements of secondary wood. Petrified secondary wood elements act as highly conserved structure, which remain almost unchanged with time and hence store valuable information regarding past environment and floral distribution pattern in different geological ages.

Observation :-

The anatomical features of the present wood suggest close resemblance with *Shoreoxylon robustoides* Roy and Ghosh of the family Dipterocarpaceae .The wood sections in different planes showed the following :

- Presence of abundant fungal hyphae in tracheids vessels and ray cells.
- Hyphae were flat , ribbon like with occasional twists in the filaments giving a spiral appearance
- The hyphae were , aseptate, with occasional bulges along the hyphal margins, 65.9-126.74µ to 1.64- 6.58µm in dimension
- Hyphae with ocassional knob like projections present on the hyphal wall



XYLOTOMY SLIDE PHOTOGRAPHS

Discussion

Wood being a rich organic substrate providing suitable source of nutrition for fungi is often invaded by diverse fungal communities that may differ even within a plant (Muller and Hallaksela 2000, Arnold et al 2003, Cordier et al 2012

Fungal members draw nourishment from the cell wall material through a process of active enzymatic degradation. Hence wood decay by different fungal species is a common phenomenon in nature. This mechanism is enhanced by supporting climatic conditions viz., warm environment with abundant precipitation. The wood rotting fungi mostly belong to class Basidiomycetes viz., species of *Fomes*, *Polyporus* and *Lenzites.*

Species composition on woody substratum are related to a range of controlling factors like trunk types, way of the tree died (Lindblad 1998;Pouska et al 2011;2016) the wound sites (Pearce 1996) and the microclimatic condition like moisture and temperature inside the trunks(Heilman).

Deadwood can also be considered as a highly heterogeneous chemical substrate harboring diverse community of fungi. (Baldrian et al 2016). For high lignin content and low nitrogen concentration woods are resistant to rapid penetration of microorganism. With the advantage of unique enzyme systems, fungi especiallyBasidiomycetes and Xylariaceous Ascomycetes (Rayner and Boddy 1988) can decompose the impermeable metabolites and colonize wood (Eichlerova et al 2015).

Earlier records of fossilified parasitic and saprophytic fungi in fruits, seeds and woods are from Deccan Intertrappean beds (Chitaley & Patil 1972; Chitaley & Sheikh 1971: Chitaley & Yavale 1978; Barlinge & Paradkar 1982; Kalgutkar et al 1993; Srivastava 2008,2009).Records of in-situ petrified fossil fungal remains from other parts of India especially from eastern India are meager except some documents on epiphyllous fungi on compressed leaves from eastern Himalaya Siwalikes.(Mitra and Banerjee 2000, Mitra et al 2002, Mandal et al 2009,2011, Bera & Mandal 2014).

Conclusion

Plant fossils are one of the most reliable indicators of the past climate and petrified secondary wood elements act as highly conserved structure, which remain almost unchanged with time, and hence store valuable information in different geological ages

In the present study occurrence of fungal hyphae within wood elements of extinct member of *Shorea* can be referred to as a significant data . However the fungus could not be identified due to lack fertile structures like spores or fruitbody.

Fungi occur abundantly in the present day moist tropical forests. Many fungal members grow within the timber yielders, under favourable environmental conditions. Fungi usually require optimum temperature of 60°-90° F and high moisture content in their surrounding for growth.

Recent day forest species of *Shorea* are commonly infested with wood rotting fungi causing considerable decay of sapwood (Heart rot disease).

Occurrence of possible wood rotting fungal hyphae within fossil *Shorea* woods indicate that a similar environmental set up existed in the present study area during the Neogene times and some of the tree members especially the Dipterocarps were attacked by the wood rotters

The presence also suggests that fungi were quite common in the woody forest elements of the past and played an important role as forest decomposer.

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CONCLUSION

“Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.” – Louis Pasteur

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