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Current Research in Life Sciences

First Edition

Editors

**Dr. R. B. Tripathi
Dr. Kena P. Anshuman
D.E. Nirman Kanna**

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Preface

Current Research in Life Sciences are more inclined toward interdisciplinary studies. The present book provides a balanced approach to higher levels of biological organization. It also serves in the emerging disciplines of conservation biology and natural resource management. Recent developments in the technologies have led to a better understanding of the living system and this has removed the demarcations between various disciplines of Life sciences.

This book discusses and interprets major issues in Entomology, Microbiology, Medical Sciences, Medical technology, Health sciences, Pharmacology, Pharmacy, Hematology, Immunology, Virology, Bioremediation, Sericulture, Aquaculture, Evolution, Ethnobotany, Plant biology, Phycology, Ecology and Food sciences.

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Editors

Dr. R. B. Tripathi

Dr. Kena P.Anshuman

D.E. Nirman Kanna

About Editors



Dr.R.B.Tripathi is currently working as Assistant Professor in P.G. Department of Zoology, M.L.K.P.G. College, Balrampur-271201, Uttar Pradesh, India. He has been completed his Ph.D.in Zoology from Dr. R.M.L. Avadh University, Faizabad (Ayodhya), Uttar Pradesh, India. He has 21 years teaching experience in U.G and 17 years teaching experience in P.G classes, published 12 book chapters, 41 research papers in international and national reputed journals, participated and presented papers in many international and national seminars, conferences and workshops. He is Indian Zoologist, published by Surya Scientist Unique Researchers Yare Association, 2015. He is Associate Editor in International Journal of Advanced Research in Biological Sciences (ISSN:2348-8069), Editorial board member in International Journal of Advanced Multidisciplinary Research (ISSN:2393-8870), 3 book Editor in Recent Trends in Life Sciences Research book (ISBN:978-81-947071-3-4),published by Darshan Publishers,Tamil Nadu, India, Recent Advancements and Research in Biological Sciences book (ISBN:978-81-952529-1-6) and Current Trends in Biological Sciences book (ISBN:978-93-94638-00-6) published by Thanuj International Publishers Tamil Nadu, India.



Dr. Kena P. Anshuman is working in Sir P. P. Insti. Of Sci., M. K. Bhavnagar Uni. Bhavnagar, Guj., India, since last nineteen years. She obtained her M.Sc., Ph. D. (Microbiology) and M. H. R. D. from Bhavnagar Uni., Guj., India. She has worked in various research projects. She has many international and national research papers published in leading research journals. She has written five book chapters and one book on "Textbook of Microbiology and biotechnology" 2012. She is an associate editor in "Current Trends In Biological Sciences, Thanuj international publishers, Tamil Nadu, India (ISBN: 978-93-94638-00-6). She has participated in many International and National workshops, Conferences, Seminars and presented research papers. She has also guided students for research. She has been awarded Life Membership of Int. Sci. Res. Org. for Sci., Eng. and Tech. (ISROSET) to promote research work. Her major area of research: Halophilic archaea, Biofertilizer, Water pollution and Diversity.



D.E. Nirman Kanna, Perfusionist, Department of Cardio Thoracic and Vascular Surgery, Faculty of Allied Health Sciences, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India. He is American Heart Association certified BLS (Basic Life Support) Provider and ACLS (Advanced Cardiovascular Life Support) Provider who was trained at JIPMER, Pondicherry. He is a young researcher and an author who works in the research areas of medicine, cardiology, cardio thoracic and vascular surgery, nanotechnology, ovarian cancer, prostate cancer, emergency medicine and infective diseases, who has published 7 research papers and 2 book chapters in national and international journals of repute. He has participated in many international and national workshops, conferences, seminars and presented research papers. He is also active in both clinical and research activities.

Current Research in LifeSciences

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Spiders as a helper of insect-pests Control in Agricultural fields of Eastern Uttar Pradesh, India

Dr.R.B.Tripathi

P.G.Department of Zoology, M.L.K.P.G. College, Balrampur (U.P.),
India

Email: *drrbtripathi.77@gmail.com*

Abstract

Spiders are among the most abundant insectivorous predators of terrestrial ecosystem and are the carnivorous creature which present in everywhere. Spiders are one of the most diverse animal groups in the world which plays an important role in regulating insects-pests in agricultural fields. Pesticides use is harmful and costly in agricultural fields so now a days Spiders are used as natural and safe insect-pests control agent in agricultural fields. During the present survey I have reported 141 species belonging to 21 families and 76 genera in agricultural fields of different districts of Eastern Uttar Pradesh were recorded during investigation. Some Spiders are among the most effective predators of leaf hoppers and other insect-pests, some Spiders are control agents of Aphids. Due to destroying the insect-pests or insects. Spiders are friends of farmers.

Key words: Spiders, insect-pests, agricultural fields and Eastern U.P.

Introduction

Spiders are among the most abundant insectivorous predators of terrestrial ecosystem. Spiders are the creature which present everywhere. Spiders play a major role as bio-control agents of insect-pests in all habitats. Spiders are one of the most diverse animal groups in the world. Spiders are carnivorous creature. Spiders play an important role in regulating insect-pests in the agricultural ecosystem. They mostly feed on insects, even though they may also feed on various other kinds of creatures. There are 41,218 Spiders species are found all over the world in almost every kind of habitat, Platnick (2009). Spiders are beneficial to human beings in the sense that they feed mostly on the insect-pests of agricultural fields. A particular Spiders as the giant Crab Spider has been known as an effective in controlling large insects

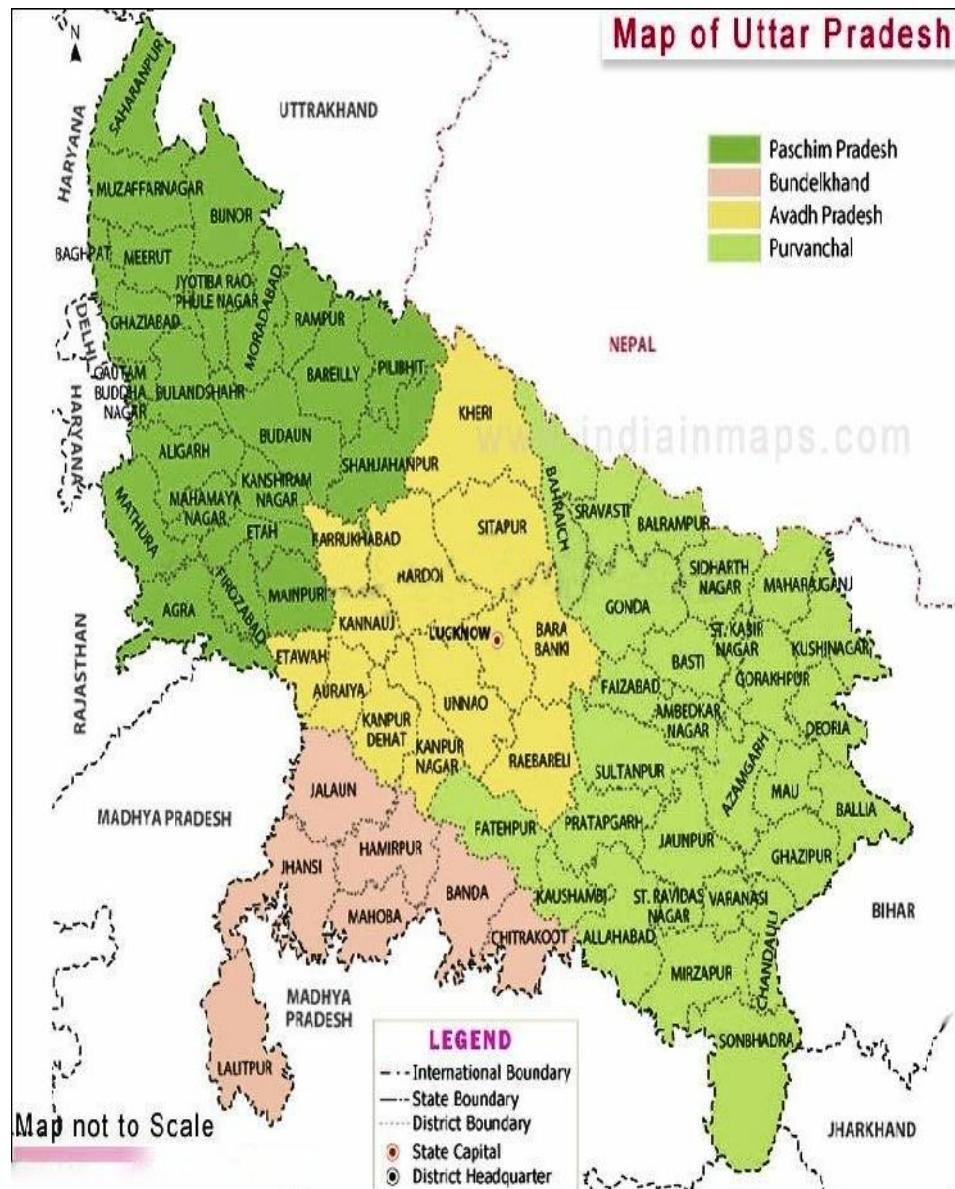
and other insect-pests found in the agricultural fields. Predatory arachnids such as Spiders are an important group of biological agents (Yadav et.al.2012).

The population densities and species abundance of Spider communities in agricultural fields can be as high as in natural ecosystem. Many uses of parasitic and predatory natural enemies to control agricultural insect-pests have been reported Greenstone (1999). They have usually been treated as an important biological control agent because there is ecological role of spiders in insect-pests control. Use of chemical pesticides has killed natural predators in the agricultural fields and also disturbing the natural fauna. Several toxic insecticides and pesticides are recommended to control pests in agricultural fields, Jeyaparvathi et.al. (2013). These chemicals insecticides and pesticides are destroying the vegetation so now a days Spiders are use as natural and safe biological control agent in agricultural fields.

The objective of the present survey was to documented Spiders as a helper of insect-pests controlin agricultural fields of different districts of Eastern Uttar Pradesh, India.

Material and Methods

A survey of Spiders was carried out in agricultural fields of different districts of Eastern Uttar Pradesh, India during January 2019 to December 2022. Spiders were collected from different areas of Agricultural fields in different districts. For collection of Spiders direct searching, collected Spiders by insect nets, pit fall trapping, beating steak and umbrella method were used. The Spiders specimens were identified according to Kaston Spider book (1970). The photographs were taken in different views, to get the clear eye position, shades of cephalothorax and abdomen, spines and hair pattern. Location of study areas shown in map.



Map: Location of study area in Uttar Pradesh

**Table: Number of Spiders species recorded in different districts of Eastern Uttar Pradesh
(Data January 2019 to December 2022)**

S.N.	Family	Genera	Species
1	Agelenidae	2	3
2	Araneidae	4	6
3	Barychelidae	2	2
4	Cheiracanthiidae	2	2
5	Clubionidae	1	5
6	Corinnidae	2	1
7	Dictynidae	2	3
8	Gnaphosidae	6	12
9	Hersiliidae	2	2
10	Linyphiidae	5	5
11	Lycosidae	4	14
12	Oonopidae	2	2
13	Oxyopidae	3	16
14	Pholcidae	4	4
15	Pisauridae	2	2
16	Salticidae	8	13
17	Sparassidae	4	10
18	Tetragnathidae	5	11
19	Theridiidae	7	14
20	Thomisidae	6	10
21	Uloboridae	3	4
	Total	76	141

Result and Discussion

During the present survey I have reported 141 species belonging to 21 families and 76 genera of Spiders in agricultural fields in different districts Eastern Uttar Pradesh state. Spiders of families-Agelenidae, Araneidae, Barychelidae, Cheiracanthiidae, Clubionidae, Corinnidae, Dictynidae, Gnaphosidae, Hersiliidae, Linyphiidae, Lycosidae, Oonopidae, Oxyopidae, Pholcidae, Pisauridae, Salticidae,

Sparassidae, Tetragnathidae, Theridiidae, Thomisidae and Uloboridae were recorded during the investigation (Table: 1).

Spiders are used to balance the effect of insecticides and pesticides. Spider's predatory capacity can have an effect in decreasing densities of insect-pests. Some Spiders are among the most effective predators of caterpillars and other insect-pests. Some Spiders and Spiderlings are main control agents of aphids. Due to destroying the insect-pests or insects, Spiders are friends of farmer. Most Spiders feed on insects that's why productivity of crops gets increased, hence spiders are important insect-pests control.

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Halophiles and its biofilm for hyper saline waste water treatment

Dr. Kena P. Anshuman

Microbiology Dept., Sir P. P. Insti. of Sci. , MK Bhavnagar Uni,
Bhavnagar, Guj., India,
E-mail: dr.kenaanshu@gmail.com

Abstract

Halophilic archaea are salt loving, lives in higher saline environments like Great salt lakes, Dead sea, Solar salterns, Salted food etc. Halophilic bacteria and archaea have ability to adopt fluctuation in extreme environment, maintain osmotic balance and osmotic pressure. They have unique adaptation to survive in extreme environments. compatible solutes, cell-wall with novel lipids, enzymes and biofilm work efficiently in such environment. This biofilm covered with extracellular polymeric substance (EPS), which protect cell from toxic substances, free radicals, low nutrition and high salinity and antimicrobial substances. Biofilm formation enhance by quorum sensing(QS) which helps in communication between the cells. Industries like pesticides, chemicals, pharmaceutical productions, oil and gas extraction, generates thousands of millions of litres of saline or hyper saline waste water. About 5% of industrial effluents are saline. Halophiles and it's biofilm helps in waste water treatment. Nonetreme halophiles could not work efficiently above that of sea water. While, halophiles and it's biofilm could be use to remediate hyper saline environment and treatment of waste effluents.

Key Words: Halophilic archaea, halophilic bacteria, biofilm, extracellular polymeric substance (EPS), hyper saline waste water treatment

Introduction

Halophiles:

Halophilic archaea are salt loving organism, lives in higher saline environment like Great salt lakes, Dead sea, Solar salterns, Salted food etc (Kushner and Kamekura, 1988; Larsen,1981). They are a group of extreme halophiles require at least 2.5M NaCl for growth. They are placed into order-*Halobacteriales* under family- *Halobacteriaceae* (Grant et al., 2001). They can survive extreme desiccation, starvation and radiation, sometimes apparently for

millions of years (Stan-Lotter and Fendrihan, 2015). Halophilic bacteria and halophilic archaea have ability to adapt to fluctuations in extreme osmotic pressure and maintain an osmotic balance between their cytoplasm and hypersaline extracellular environments. They have unique adaptation that allowssurvive in high salt concentration salt solution, these are:

1) Halophiles accumulate compatible solutes like K⁺, amine acids, betaine, actoene within cell to equalize ionic strength of cytoplasm and extreme environment. These solutes accumulation reduces osmotic forces and protect cell from desiccation.

2) Halophiles have proteins and cell - wall contains negative charged amino acids and polar lipids. These high concentrations of cations require toshield negative charges and stabilizes structure of macromolecules.

Finally, transport nutrients and growth factors are linked to Na⁺ gradients exist in hypersaline environment (Anshuman 2021, Hoschstein 1988; Ventosa, 1988; Woolard and Irvine, 1994).

Halophiles can form biofilm and accumulate Extracellular polymeric substance (EPS) at increasing salt stress. EPS enhance bacterial colonization of plant roots and soil particles. They can used to improve soil structure and plant growth. Thus, Halophiles produce compatible solutes, EPSs and halotolerant enzymes, these helps halophiles to cope up with saline environments (Banarjee et al., 2019).

Biofilm:

Biofilm is an aggregate of single or multiple microorganisms, attached to biotic or abiotic surfaces. It is covered with self produced extracellular polymeric substance (EPS) (Costerton et al., 1995). Biofilm formation is a sequential, multi-step process, governed by many physical, chemical and biological factors like adherence of microorganisms to surface, synthesis of EPS, interaction between microbes through signalling molecule, dessemination of microbial cell into planktonic form are necessary to form and enhance biofilm (Azeredo et al., 2017; Chattopadhyay et al, 2022).

EPS matrix is 0.2 -1.0mm thick share 50% of total biofilm so, it can protect bacteria from extreme environment like pH, presence of toxic substances, free radicals, low nutrition and high salinity and antimicrobial substances (Mosharaf et al., 2018; Davey and O'toole, 2000). It regulates structure and stability of biofilm through the presence of Ca²⁺ (Chattopadhyay et al, 2022). They are hydrophobic in nature due to the presence of surfactants (glycolipids, lipopeptides, ionic lipids and neutral lipids) and lipids.

Biosurfactants are nontoxic and biodegradable in nature. These surfactants used in enhancement of bioremediation of organic and inorganic pollutants. And also helps in the development of biofilm though nutrient transport in biofilm (Markande et al, 2021). This EPS plays important role in waste water treatment and bioremediation of soil (Sidharth et al, 2021).

Nutrients of wastewater induce the growth of microbe and microbe derived metabolites, which are used to remove the contaminants from the waste water (Chattopadhyay et al, 2022). Biofilm contain cell population 10^8 - 10^{11} per g wet weight (Flemming et al., 2016).

Quorum sensing (QS) is a communication procedure between bacterial cells through chemical mediators, which are known as "Autoinducer (AIs). It contributes to evolution of biofilm and secretion of EPS (Rajamanikandan et al., 2017). There are different types of IAs from different bacterial spp. helps in environmental remediation such as remediate toxic pollutants in soil and waste water (Feng et al., 2013; Varjani et al., 2020). Quorum sensing such as AHLs contribute efficiently to waste water treatment (Feng et al, 2013). AHLs enhance phenol degradation in waste water treatment by increase gene expression (Valle et al, 2004; Gao et al, 2018). Horizontal gene transfer (HGT) between bacterial cells in biofilm, more efficient to enhance bioremediation and waste water treatment (Chattopadhyay et al., 2022). Biofilm based environmental remediation is more ecofriendly and cost effective compare to chemical, physical and thermal approaches. Removal of xenobiotic substances in environment can also be removed by biofilm.

Moving bed bioreactor (MBBR) used in waste water treatment plant (WWTP), It has several advantages like, it reduce obstruction, resistance against pH, temperature, toxic substances and maintain biomass as a biofilm on carriers (Di Biase et al,2019). These carrier materials such as polyethylene, polypropylene and polyvinyl chloride are responsible for formation of biofilm. Membrane bioreactors (MBR) are also best for waste water treatment (Haung et al, 2020).

Waste Water Treatment:

Waste water contain high concentration of salts (3.5% W/V total dissolved solids), waste organics from industries. This waste brine water produced during production of pesticides/ herbicides, polyhydric chemicals, organic peroxides, pharmaceutical and other products, oil and gas recovery processes (Woolard and Irvine, 1994). Due to high salt content in waste brines makes treatment difficult because high salt disrupts cell membrane, denature

enzymes and desiccating osmotic force. This lyses organisms (Woolard and Irvine, 1994).

Industries like pesticides, chemicals, pharmaceutical productions, oil and gas extraction, generates thousands of millions of litres of saline or hyper saline waste water (Lefebvre and moletta, 2006). Petroleum industries also generates huge amount of oil and saline residual water with salinity up to 10% or more after separation of crude oil from reservoir water. Industrial and municipal effluents are often discharged into saline and hypersaline depression, especially in developing countries (Borgne et al., 2008).

About 5% of industrial effluents are saline and hyper saline. Halophiles are salt loving organisms, so they are good candidate for the bioremediation of hypersaline environment and treatment of saline effluents (Borgne et al., 2008).

Hypersaline wastewater is not possible with conventional microorganisms because these organisms couldn't operate efficiently at salinity above that of sea water (Pieper and Reineke, 2000; Oren, 2002). As such high salinity conventional organisms loss their cell-wall integrity, protein denature and changes in osmotic pressure (Pernetti and Di palma, 2005). Halophilic microorganisms have metabolically versatility and are adapted to extreme salinity. But research on halophiles for waste treatment has been limited and less information available on biodegradation.

Halophilic archaea classified based on their salt requirements i.e. Slight halophiles (2-5% W/V NaCl), Moderate halophiles (5-20% W/V NaCl) and Extreme halophiles (20-30% W/V NaCl (Anshuman 2022; Kushner, 1978; Kushner and Kamekura, 1988, Kanekar et al, 2012). So, they could be easily tolerate and degrade pollutants at different level of saline environments.

Phenol and phenolic compounds are major pollutants in industrial waste water like oil refining, coke conversion, pharmaceutical and resin manufacturing plants. Woollard and Irving, 1994,1995 used halophilic bacterial biofilm to degrade phenol in hyper saline waste water, and more than 99% of the phenol was removed from synthetic waste water contain 15% W/V NaCl. Hinteregger and Streischsberg, 1997, also found *Halomonas Spp.* strain isolated from Great saltlake and it degrade phenol in industrial saline water. It degraded 0.1g/L of phenol in 13hr at 3-5 % W/V NaCl concentrations. While, Alva and Peyton, 2003, reported that haloalkaliphilic bacteria, *Halomonas campisalis* degrade phenol at 8-11 pH and 0-150 g/L NaCl. These bacteria use phenol as sole source of carbon and energy.

Halogenated Hydrocarbons are covers wide group of aliphatic and aromatic compounds. These compounds used in petrochemicals, food industries and agricultural applications. Chlorinated hydrocarbons are widely used as their fungicidal, herbicidal and insecticidal properties. Chlorinated phenols have been used as wood preservative and agricultural biocides (Borgne et al., 2008). Maltseva and Oriel, 1997, found alkaliphilic and slight halophiles can degrade these chlorinated phenol.

Azo dye generally release in waste water generated both by dye producing and dye consuming industries. Asad et al, 2007 isolated three *Halomonas* strain from textile effluents, were able to decolorize azo dye at pH (5-11) and NaCl (up to 20%) under anaerobic condition.

Moreover, several saccharolytic enzymes are produce from these microbial communities of biofilm. These enzymes are involved in the detachment of microbes from surface to colonize in a new location (Feng et al, 2013). Halophilic enzymes are more stable in varying concentration of hypersaline conditions. Enzymes are nontoxic, biodegradable and great catalysts. So, halophiles and its enzyme system could be preferred efficiently in hyper saline waste water treatment. Still more research require to check halophilic true potential for hyper saline environments.

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Arrhythmia Heart Syndrome- A silent killer

^{1*}D.E. Nirman Kanna,

Perfusionist, Department of Cardio Thoracic Surgery, Faculty of Allied Health Science, Meenakshi Academy of Higher Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

²M. Mohammed Eliyas,

Anesthesia Technologist, Department of Anesthesia, Faculty of Allied Health Science, Meenakshi Academy of Higher Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

Corresponding Author:

^{1*}D.E. Nirman Kanna, Perfusionist, Department of Cardio-Thoracic and Vascular Surgery, Faculty of Allied Health Sciences, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

Email ID: *nirmankanna.d.e@gmail.com*

Abstract

Arrhythmia heart syndrome or cardiac arrhythmias can be defined as an irregular heart rhythm or heartbeat, which occur when the electrical signals that coordinate the heart's beats don't work properly. Due to changes in heart tissue, cardiac activity, or in the heart's electrical impulses during an arrhythmia, can cause the heartbeat to be too fast (Tachycardia), too slow (Bradycardia) or erratic. This irregular heartbeat known as arrhythmia can cause a sudden death in individual. Arrhythmias can be classified into different types but it commonly divided into two types; one is Bradycardia and another is tachycardia. Bradycardia is a type of arrhythmia when the heart rate is too slow (Heart rate less than 60 beats per minute). Tachycardia is a type of arrhythmia when the heart rate is too fast (Heart rate more than 100 beats per minute). When arrhythmias are severe or last long (treatment should be initiated within 3-4 mins) it will affect function of heart which the heart can't be able to pump enough blood to the body. It can affect any age group of peoples at any time without any warning signs. This can cause victim to feel tired, lightheaded or even it leads to sudden death, so it is called as a "silent killer". Identifying arrhythmia and managing it at correct time can save the precious life of a

victim. In this review we focused on current updates on arrhythmia heart syndrome, etiology, classification, identification and its management.

Keywords: Arrhythmia heart syndrome, Arrhythmia, Bradycardia, Tachycardia, cardiac arrest, Sudden cardiac death, Types of arrhythmias, identification and management of arrhythmias.

Introduction

Arrhythmia heart syndrome or cardiac arrhythmias can be defined as an irregular heart rhythm or heartbeat, which occur when the electrical signals that coordinate the heart's beats don't work properly. Due to changes in heart tissue, cardiac activity, or in the heart's electrical impulses during an arrhythmia, can cause the heartbeat to be too fast (Tachycardia), too slow (Bradycardia) or erratic. This irregular heartbeat known as arrhythmia can cause a sudden dead in individual^[1].

An arrhythmia is an abnormal heart rhythm, which may feel like fluttering or a brief pause and it may be so brief that it doesn't change the overall heart rate or it can cause the heart to beat too fast or even too slow which reduces the cardiac output and impacts the systemic perfusion. Some arrhythmias are asymptomatic but some arrhythmia can make the victim to feel lightheaded or dizzy^[1].

Arrhythmias can be commonly divided into two types; one is Bradycardia and another is tachycardia. Bradycardia is a type of arrhythmia when the heart rate is too slow (Heart rate less than 60 beats per minute). Tachycardia is a type of arrhythmia when the heart rate is too fast (Heart rate more than 100 beats per minute). When arrhythmias are severe or last long (treatment should be initiated within 3-4 mins) it will affect function of heart which the heart can't be able to pump enough blood to the body. This can cause victim to feel tired, lightheaded or even it leads to sudden dead, so it is called as a "silent killer"^[2].

Classification

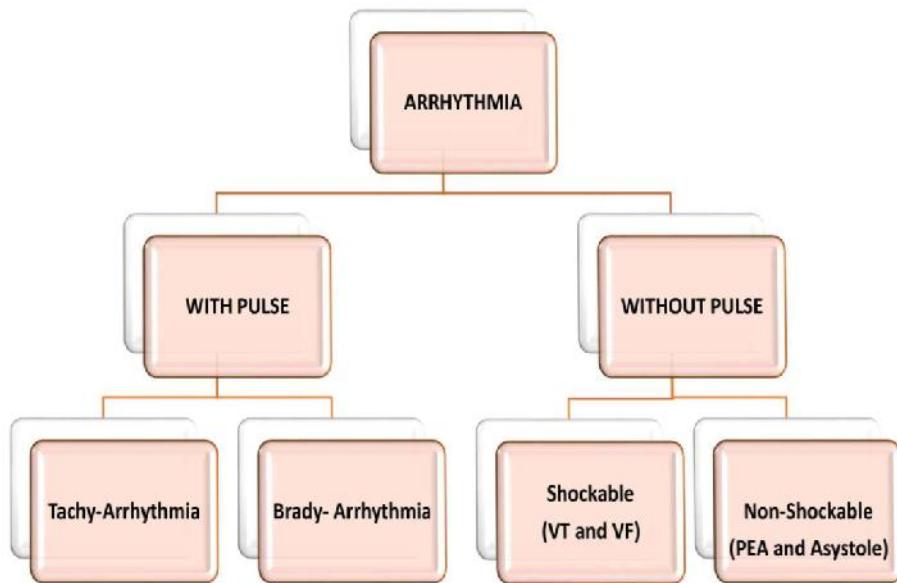


Figure 1

Arrhythmia with pulse

Tachy-arrhythmia

Tachycardia arrhythmia is also referred to as tachycardia, it is an abnormal heart rhythm which the heart beats faster than the normal (heart rate > 100 beats per minute). If left untreated Tachycardia can cause serious complication including blood clots, heart failure, frequent fainting spells or Sudden death^[3].

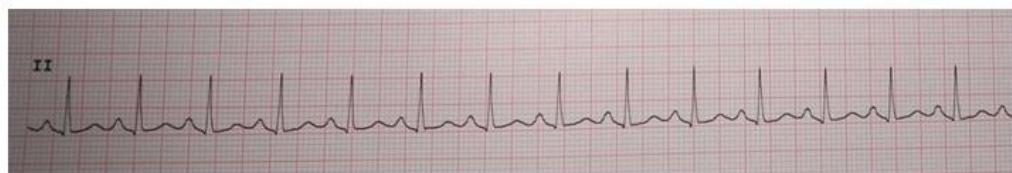


Figure 2-Sinus Tachycardia

Types of tachy-arrhythmias

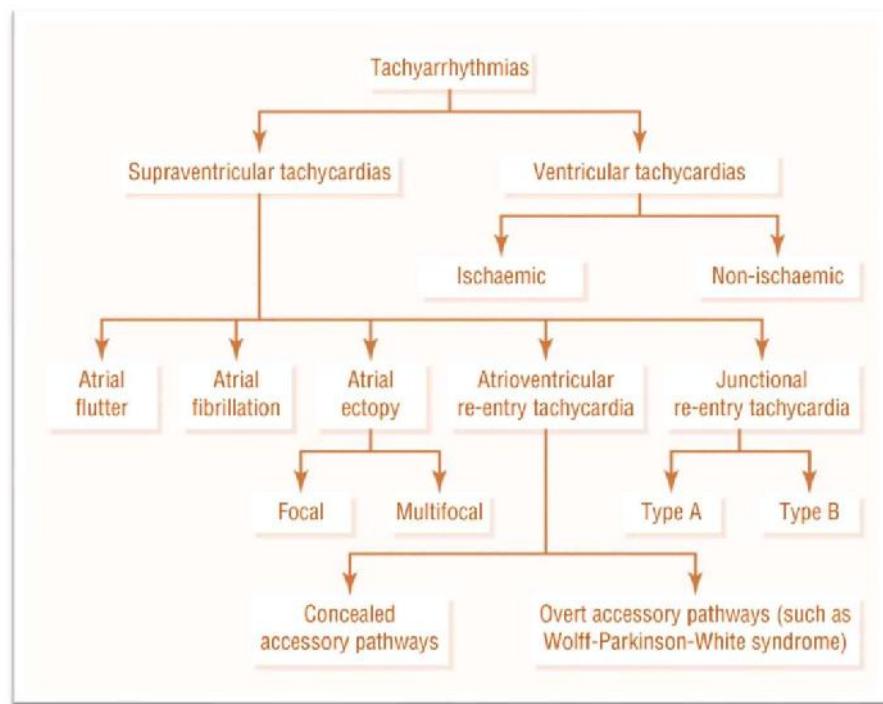


Figure 3

Supraventricular tachycardia

Supraventricular tachycardia (SVT) is defined as a dysrhythmia or a irregular heart rhythm which originating at or above the atrioventricular (AV) node and is described by a narrow complex (QRS < 120 milliseconds) at a rate > 100 beats per minute (bpm). Atrioventricular nodal reentrant tachycardia (AVNRT) is also known as paroxysmal SVT, which is an intermittent SVT without provoking factors, and typically presents with a ventricular rhythm of 160 bpm. During supraventricular tachycardia (SVT), the heart rate become high, which begins in the upper chambers of heart (Right atrium and Left Atrium). This is due to abnormalities in the electrical signals and circuitry in the heart. When the heart starts beating too fast, heart can't fill with blood between beats, making it hard to get sufficient blood to the systemic circulation (i.e., there is not enough time to fill with blood before the heart chambers contract)^[4].

Treatment

Initial management for SVT includes administering adenosine 6mg in IV (flush the IV line using 20cc NS within 1-3 seconds), If the victim still demonstrates an SVT rhythm after 1-2 minutes administer 12 mg IV bolus (flush the IV line using 20cc NS within 1-3 seconds). According to latest ACLS guidance IV route is preferred than IO, still if SVT persist consider synchronized cardioversion^[5].

Carotid sinus massage can be performed to manage SVT by applying gentle pressure on the neck where the carotid artery splits into two branches. During this type of massage, the body releases chemical compounds that slow the heart rate^[6]. Vagal maneuvers also help to manage SVT by instructing the patient to hold nose, close his mouth and try to blow the air out in a syringe or a plastic bag for 10-20 seconds. This creates pressure in the chest that may activate the vagus nerve, which helps control the heartbeat^[7].



Figure 4- Supraventricular Tachycardia (SVT)

Atrial flutter

Atrial flutter is one of a type of supraventricular tachycardia caused by a re-entry circuit within the right atrium. The size of the right atrium corresponds to the length of the re-entry circuit, resulting in a fairly predictable atrial rate of around 300 bpm (range 200-400). It has the special characteristic of Narrow complex tachycardia with regular atrial activity at ~300 bpm and Loss of the isoelectric baseline, with “Saw-tooth” pattern of inverted flutter waves in leads II, III, aVF^[8].

The AV conduction ratio determines the ventricular rate (“degree of AV block”). The most common AV ratio is 2:1, resulting in a ventricular rate of ~150 bpm. Higher-degree blocks can occur — usually due to medications or underlying heart disease — resulting in lower rates of ventricular conduction, e.g., 3:1 or 4:1 block, Atrial flutter with 1:1 conduction can occur due to sympathetic stimulation, or in the presence of an accessory pathway^[9].

The administration of AV-nodal blocking agents to a patient with Wolff-Parkinson-White syndrome can precipitate this Atrial flutter with 1:1

conduction, which is associated with severe hemodynamic instability and progression to ventricular fibrillation^[10].



Figure 5-Atrial flutter

Treatment

Treatment method for Atrial flutter is similar to the management protocol of SVT and identifying and reversing the underlining cause should be followed. If the patient become unstable electrical cardioversion should initiated^[11].

Atrial fibrillation

Atrial fibrillation (AF) is a type of tachyarrhythmia in which abnormal heart rhythm originates from upper chambers of heart (right atrium and left atrium), this is due to malfunction of signaling pathway in upper chamber of heart and it is similar to supraventricular tachycardia (SVT), but it resembles the special characteristics of absence of P waves with irregular ventricular rate and it resembles high frequency of 300-600 waves per minute^[12].

Symptoms of atrial fibrillation are sudden unexpected palpitation, dyspnea, fatigue, chest discomfort, syncope, dizziness and impaired excersie capacity^[13].



Figure 6-Atrial Fibrillation

Treatment

Commonly atrial fibrillation doesn't require any treatment, it reveres by its own in stable patients. If it persists more than 48hrs or patient become symptomatic and unstable, the treatment should be initiated. The method of management of Atrial fibrillation is similar to the management protocol of SVT and identifying and reversing the underlining cause should be followed.

Drugs such as beta blockers, Calcium channelblockers, anticoagulants and anti-arrhythmic can be used to manage AF^[14].

Atrial ectopy

An atrial ectopic beat is a type of tachy-arrhythmia in which the irregular rhythm is due to premature atrial contraction by the electrical system of the heart. Due to abnormal electrical focus signal from the upper chambers of the heart (the atria) causes the extra heart beat which leads to decreased cardiac output. This abnormal rhythm is also called an atrial premature beat or a premature atrial contraction^[15].

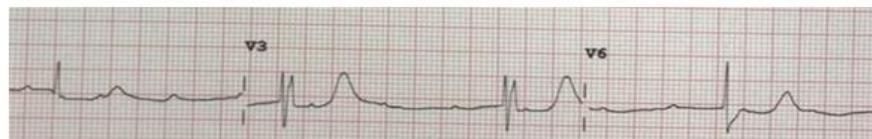


Figure 7-Atrial Ectopic Beat

Treatment

Treatment for Atrial Ectopic beat includes administering anti-arrhythmic, radiofrequency ablation, cardiac pacing (transcutaneous or transvenous) and placement of permanent pacemaker^[16].

Atrioventricular re-entry tachycardia

Atrioventricular re-entry tachycardia (AVRT), or atrioventricular reciprocating tachycardia, is a type of abnormal fast heart rhythm which is classified as a type of supraventricular tachycardia (SVT). Most commonly AVRT is associated with Wolff–Parkinson–White syndrome (WPWS), but is also noticed in permanent junctional reentrant tachycardia (PJRT). In AVRT, an accessory pathway allows electrical signals from the right and left ventricles to enter the atria and it causes earlier contraction than the normal contraction, which leads to repeated stimulation of the atrioventricular node^[17].

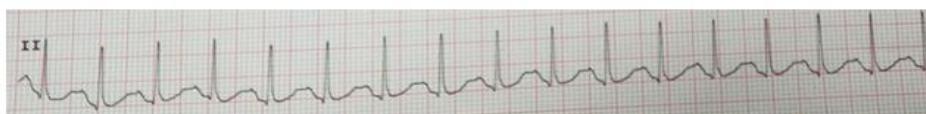


Figure 8- Atrioventricular re-entry tachycardia (AVRT)

Treatment

Treatment for Atrioventricular re-entry tachycardia (AVRT) includes administering anti-arrhythmic, Vagal maneuvers, radiofrequency ablation,

cardiac pacing (transcutaneous or transvenous) and if it persists too long, not responds to medical management, placement of permanent pacemaker is recommended^[18].

Ventricular tachycardia with pulse

VT with pulse is classified by duration as non-sustained or sustained. Non-sustained ventricular tachycardia is defined as more than 3 beats of ventricular origin at a rate greater than 100 beats per minute that lasts less than 30 seconds in duration. When the rhythm lasts longer than 30 seconds or hemodynamic instability occurs in less than 30 seconds, it is considered sustained ventricular tachycardia. Identifying and reversing the underlying etiology helps to manage the VT with pulse. If VT become pulseless, it is a medical emergency, ACLS protocol should be initiated as soon as possible^[19].

Brady-arrhythmia

Bradycardia arrhythmia is also referred to as bradycardia, it is an abnormal heart rhythm which the heart beats slower than the normal (heart rate of < 60 beats per minute). If symptomatic bradycardia is left untreated. It can lead to Fainting, Seizures or even leads to death^{[20][21]}.

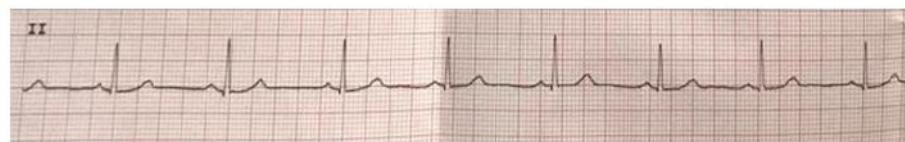


Figure 9- Sinus Bradycardia

Types

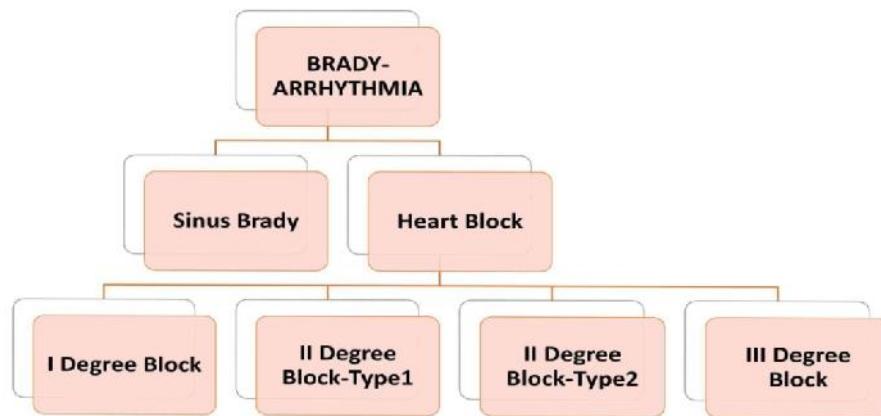


Figure 10

Heart Block

Heart block is a type of brady-arrhythmia which is highly lethal compared to sinus bradycardia, so it should be identified and managed as soon as possible. Heart block can be classified into three types, I-degree, II-degree (Type 1 and 2) and III-degree heart block^[22].

I- Degree heart block

First-degree atrioventricular (AV) block is defined as a condition of abnormal slow rhythm of heart which is due to slow conduction of electrical signals through the AV node. The ECG findings of I degree heart block includes PR interval of greater than 0.20 without disruption of atrial to ventricular conduction. This condition is generally asymptomatic and diagnosed only on routine ECG^[23].

Fibrotic changes in heart, coronary heart disease, myocardial infarction, electrolyte abnormalities (particularly hypokalemia and hypomagnesemia), inflammation, infections (endocarditis, rheumatic fever, Chagas disease, Lyme disease, diphtheria) drugs (antiarrhythmics Ia, Ic, II, III, IV and digoxin), infiltrative diseases (sarcoidosis), collagen vascular diseases (SLE, rheumatoid arthritis, and scleroderma), idiopathic degenerative diseases (Lenegre and Lev diseases) and neuromuscular disorders are the common etiologies of I degree heart block^[24].



Figure 11- I- Degree Heart Block

Treatment

There is no specific indication for antiarrhythmic medication for first-degree AV block. Permanent pacemaker placement is recommended for patients with the PR interval greater than 0.30 seconds who are experiencing symptoms believed to be due to the AV block^[24].

II- Degree heart block type 1

II-degree heart block type 1 is also called as Mobitz type I or Wenckebach which is a progressive prolongation of the PR interval (AV conduction) until eventually an atrial impulse is completely blocked. When an atrial impulse is completely blocked there will be a P wave without a QRS

complex. This pattern is often referred to as a “dropped beat.” Mobitz type I occurs because each depolarization results in the prolongation of the refractory period of the atrioventricular (AV) node^[25].

When an atrial impulse arises via the AV node during the relative refractory period, the impulse will be conducted more slowly, resulting in a prolongation of the PR interval. Eventually, an impulse arises when the AV node is in its absolute refractory period and will not be conducted. This will manifest on the ECG as a P wave that is not followed by a QRS complex. This non-conducted impulse allows time for the AV node to reset and the cycle continues. This phenomenon leads to a grouped beating^[26].



Figure 12- II- Degree Heart Block Type-1

Treatment

Treatment is often not necessary, because it occasionally results in bradycardia leading to hypotension. If hypotension and bradycardia occur prolongs, Administer 0.5 mg atropine via IV route for every 3-5 minutes with a maximum dose of 3 mg (6 doses). If unresponsive to atropine, transcutaneous or transvenous pacing should be initiated for stabilization. If the patient is on any beta blockers, calcium channel blockers or digoxin, the dose of these medications should be reduced or the medication discontinued. All patients with Mobitz 1 block should be admitted and monitored^[27].

II- Degree heart block type 2

Type 2 second degree heart block is also called as Mobitz type II which is a constant PR interval across the rhythm strip both before and after the non-conducted atrial beat and each P wave is associated with a QRS complex until there is one atrial conduction or P wave that is not followed by a QRS. Mobitz type II is often a problem in the infra-nodal conduction system. So, it is associated with a widened QRS complex, bundle-branch block, or fascicular block. If more than one P wave is not conducted this is no longer a Mobitz type II and it should be considered as a high degree AV block^[28].



Figure 13- II- Degree Heart Block Type-2

Treatment

Transvenous pacing should be initiated as soon as this rhythm is identified, if it persists too longer, pacing should be continued until a permanent pacemaker is placed^[29].

III- Degree heart block

The III-degree heart block is also known as complete heart block, which has no impulses from the SA node get conducted to the ventricles and this leads to a complete atrioventricular dissociation. The SA node continues its activity at a fixed regular rhythm but the ventricles activate via an escape rhythm that can be mediated by either the AV node (junctional escape) one of the fascicles (fascicular escape) or by ventricular myocytes (ventricular escape rhythm). During III-degree heart block, heart rate will be less than 45-50 beats/min, which results in hemodynamic instability of patients^[30].



Figure 14- III- Degree Heart Block

Treatment

Transvenous pacing should be initiated as soon as this rhythm is identified, if it not responds to pacing, transvenous pacemaker should be placed to save the patient. Drug therapy such as administering atropine, epinephrine, dobutamine will not be effective to restore the normal rhythm, by increasing the heart rate^{[31][32]}.

Arrhythmia without pulse

Ventricular tachycardia (without pulse)

Ventricular tachycardia (VT) is a shockable arrhythmia which characterized as a wide **QRS** complex (QRS duration greater than 120 milli

seconds) at a heart rate greater than 100 beats per minute. The most common etiology of VT is ischemic heart disease^[33].

Pulse less VT is classified into monomorphic and polymorphic on the basis of QRS morphology. Monomorphic ventricular tachycardia demonstrates a stable QRS morphology from beat to beat while polymorphic ventricular tachycardia has changing or multiform QRS variance from beat to beat. Torsades de pointes is a polymorphic ventricular tachycardia that occurs in the setting of a long QT interval and appears as waxing and waxing QRS amplitude on ECG^[34].

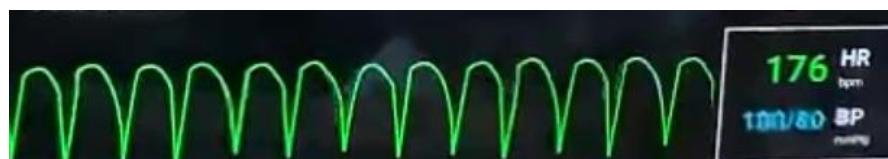


Figure 15- Monomorphic Ventricular Tachycardia



Figure 16- Polymorphic Ventricular Tachycardia

Ventricular fibrillation

Ventricular fibrillation (VF) is one of the lethal shockable arrhythmia which the lower heart chambers of heart contracts too rapid in uncoordinated manner. As the result, the heart doesn't pump enough blood to body (reduced cardiac output). The QRS morphology in VF varies in shape, amplitude, and duration with a prominent irregular rhythm. Ventricular fibrillation is a medical emergency that requires immediate medical attention, because It's the most frequent cause of sudden cardiac death, if the VF untreated immediately, it will cause death within few minutes^[35].



Figure 17- Ventricular Fibrillation

Treatment for shockable arrhythmia

The initial management is to begin chest compressions (High quality CPR) according to the advanced cardiac life support (ACLS) protocol followed by administrating epinephrine 1mg IV for every 3 to 5 minutes, while simultaneously looking for any reversible causes and treat it immediately. By using defibrillator or an AED provide shock (360J in monophasic defibrillator and 120 J to 200 J in biphasic defibrillator for adults and 2 J per Kg in pediatrics). Followed by first shock continue high quality CPR for 2 mins, check for return of spontaneous circulation (ROSC), if not the rhythm reversed to normal, shot second shock, followed by it administer anti-arrhythmic drugs such as amiodarone (300mg IV) or Lignocaine 2% (1-1.5mg per Kg). Continue ACLS protocol until rhythm become normal or ROSC achieved^[36].

Pulseless electrical activity

Pulseless electrical activity (PEA), also known as electromechanical dissociation, is a clinical condition characterized by unresponsiveness and impalpable pulse in the presence of sufficient electrical discharge. A lack of ventricular impulse often points to the absence of ventricular contraction, but the contrary is not always true. It means that the electrical activity is pertinent, but not sufficient, condition for contraction. In the case of cardiac arrest, the organized ventricular electrical activity does not usually follow sufficient ventricular response. The word “sufficient” is being used to describe a degree of ventricular mechanical activity that is adequate to generate a palpable pulse^[37].

The ventricular contractions and detectable pressures in the aorta during PEA are known as pseudo-PEA. True pulseless electrical activity is a state in which cardiac contractions are lacking in the presence of coordinated electrical impulses. Pulseless electrical activity can include a number of organized cardiac rhythms that may be supraventricular in origin, sinus versus non sinus, or ventricular in origin such as accelerated idioventricular or escape. An impalpable pulse should not always be taken as a pulseless electrical activity because it may be due to severe peripheral vascular abnormality^[37].

Asystole

Asystole, colloquially referred to as flatline, which represents the cessation of electrical and mechanical activity of the heart. Asystole typically occurs as a deterioration of the initial non-perfusing ventricular rhythms: ventricular fibrillation (VF) or pulseless ventricular tachycardia (VT). It is too

difficult to resuscitate compared to other arrhythmias, Prognosis is highly poor. it is one of the major lethal arrhythmic rhythms causes sudden cardiac death^[38].

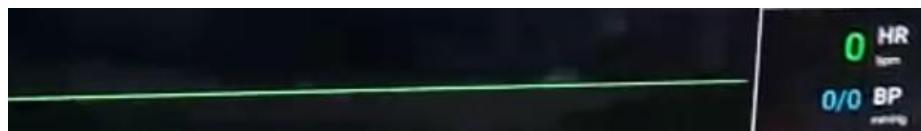


Figure 18- Asystole

Treatment for non-shockable arrhythmia

The initial management is to begin chest compressions (High quality CPR) according to the advanced cardiac life support (ACLS) protocol followed by administrating epinephrine 1mg IV for every 3 to 5 minutes, while simultaneously looking for any reversible causes and treat it immediately. Identify the underlining cause and reversing it increases the prognosis and chance of survival of the patient^{[37][39][40]}.

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Current updates on angina pectoris- A deadly oxygen thirst of the heart

^{1*}**D.E. Nirman Kanna**, Perfusionist, Department of Cardio-Thoracic and Vascular Surgery, Faculty of Allied Health Sciences, Meenakshi Academy of Higher Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

²**Subbulakshmi Packirisamy, MSc., (Ph.D.)**, Senior Lecturer, Department of Pharmacology, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

³**Dr Deepa Rajendiran PhD.**, Associate Professor, Department of Biochemistry, Madha Dental College and Hospital, Kundrathur, Chennai, Tamil Nadu, India.

Corresponding Author,

^{1*}**D.E. Nirman Kanna**, Perfusionist,

Department of Cardio-Thoracic and Vascular Surgery,
Faculty of Allied Health Sciences,
Meenakshi Academy of Higher Education and Research,
Chennai, Tamil Nadu, India.

Email ID: nirmankanna.d.e@gmail.com

Abstract

Background: Angina pectoris is defined as a chest pain or discomfort due to coronary heart disease, which occurs when the heart muscle doesn't get sufficient blood as it needs. The heart muscles get degenerated due to lack of sufficient blood supply. Angina usually causes uncomfortable pressure, fullness, tightness, squeezing or pain in the center of the chest which radiates to left arm, shoulder, neck, jaw. Emotional stress, Exposure to very hot or cold temperatures, Obesity, Smoking, drug abuse, genetics, diabetics, extreme weight lifting are the most common triggers of Angina pectoris. Medications such as beta-blockers, calcium channel blockers, Nitroglycerines (NTG), Angiotensin-converting enzyme (ACE) inhibitors, statins and antiplatelet drugs are the first line treatment of Angina pectoris. Avoiding smoking and drug abuse, regular physical exercise, good mental health, low fat diet, adequate rest can be helpful to prevent angina pectoris.

Conclusion: In this review, we focused on the latest findings in the etiology, Types of Angina pectoris, Printzmetal angina, pathophysiology, clinical manifestations in male vs female, diagnosis, prevention and treatment of angina pectoris, Intra-aortic balloon pump (IABP) in Angina and the incidence and severity profile of angina pectoris in Indian populations.

Keywords: Angina pectoris, Nitroglycerines (NTG), Angiotensin-converting enzyme (ACE) inhibitors, statins, Chest pain, Printzmetal angina, Diagnosis, Intra-aortic balloon pump (IABP), Prevention and treatment of angina pectoris.

Introduction

Angina pectoris is defined as a chest pain or discomfort due to coronary heart disease, which occurs when the heart muscle doesn't get sufficient blood as it needs. Angina usually causes uncomfortable pressure, fullness, tightness, squeezing or pain in the center of the chest which radiates to left arm, shoulder, neck, jaw. This commonly happens due to one or more coronary arteries narrowed (atherosclerosis) or blocked, which leads to ischemic damage of heart muscles^[1].

Globally, Angina pectoris is the major cause of morbidity and mortality, which is one of the major signs of acute coronary syndrome (ACS) and can further subdivide into stable angina and unstable angina. Stable angina can be defined as the occurrence of symptoms with exertion only, but in unstable angina, the symptoms occurring at rest requires more prompt evaluation and management^[2].

The heart muscles get degenerated due to lack of sufficient blood supply. Angina usually causes uncomfortable pressure, fullness, tightness, squeezing or pain in the center of the chest which radiates to left arm, shoulder, neck, jaw. Emotional stress, Exposure to very hot or cold temperatures, Obesity, Smoking, drug abuse, genetics, diabetics, extreme weight lifting are the most common triggers of Angina pectoris^[3].

Medications such as beta-blockers, calcium channel blockers, Nitroglycerines (NTG), Angiotensin-converting enzyme (ACE) inhibitors, statins and antiplatelet drugs are the first line treatment of Angina pectoris. Nowadays, Intra-aortic balloon pump (IABP) a mechanical invasive pump, used to improve the coronary perfusion and decreases the workload of heart which prevents further myocardial damage and eliminates lethal Myocardial infarction (MI). Avoiding smoking and drug abuse, regular physical exercise, good mental health, low fat diet, adequate rest can be helpful to prevent angina pectoris^[4].

Etiology:



Figure 1

Other than the mentioned cause (Figure 1), common etiologies of Angina pectoris are obesity, mental stress, inadequate physical activity, genetics, smoking, drug abuse like cocaine, ischemic heart diseases, blood coagulation disorders, cardiotoxins, etc...^[5].

Types of angina pectoris

Stable angina:

It is the most common form of angina, which commonly happens during exertion and relieved with rest or angina medication administration. Stable angina pain is predictable, short duration and usually similar to previous episodes of chest pain, which lasts less than 5-10 mins^[6].

Unstable angina:

It is a medical emergency, which is unpredictable and occurs at rest or even in less physical effort. It's typically severe and lasts longer than stable angina, it may occur more than 20 mins. The pain doesn't relieve with rest or the usual angina medications. If the blood flow doesn't improve, the heart is starved of oxygen and MI occurs, which is dangerous and requires emergency treatment^[7].

Prinzmetal angina:

Variant angina or Prinzmetal angina, is a type of angina caused due to spasm in the coronary arteries but not a coronary artery disease that temporarily reduces blood flow. The main symptom of variant angina will be severe chest pain, which commonly occurs in cycles, usually at rest during overnight^[8].

Refractory angina:

It is type of angina, in which the angina episodes will be frequent and lethal. it can be managed by administering a combination of medications and lifestyle modifications^[9].



Figure 2

Pathophysiology

The heart is dependent on adequate oxygen supply for energy production to support contractility. At the cellular level, ischemia causes an increase in anaerobic glycolysis. This increases the levels of hydrogen, potassium, and lactate in the venous return of the ischemic or affected area of the myocardium. The hydrogen ions compete with calcium ions causing hypokinesia/akinesia of the affected area. For stable angina, this change in

oxygen supply requires a trigger that would cause metabolic mismatch—exercise, stress, and low temperature^[10].

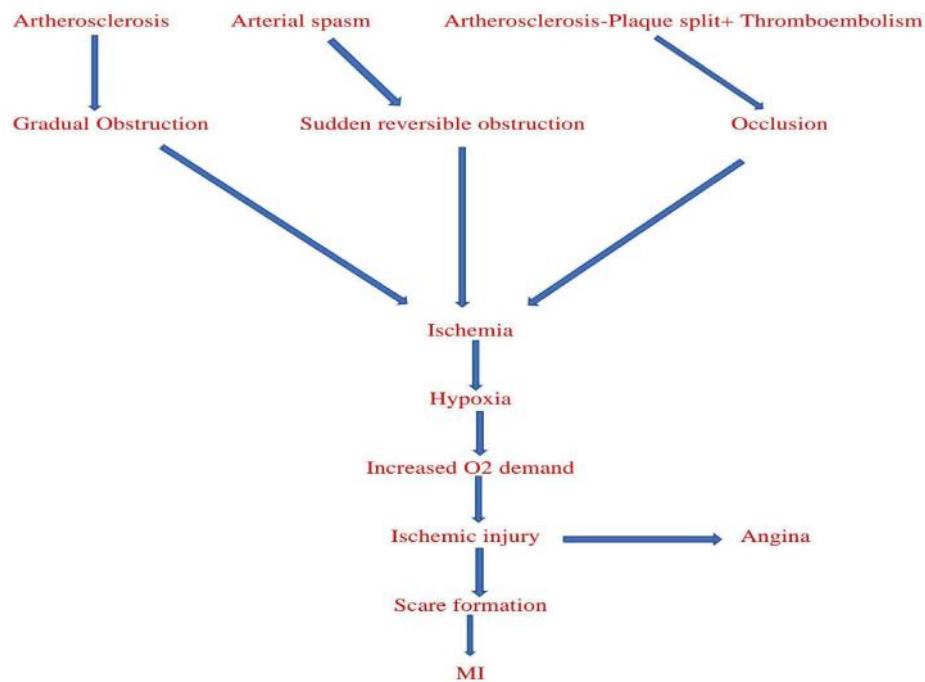


Figure 3

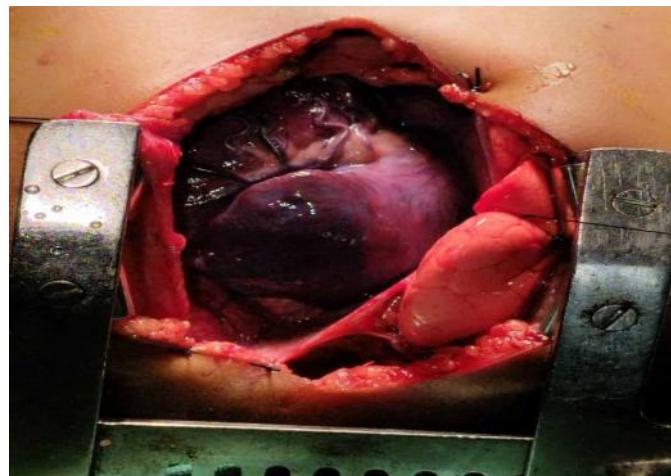


Figure 4

Figure 4 represents the ischemic damage of heart, which turned into blue due to poor organ perfusion (Increased oxygen demand)

Clinical manifestations angina pectoris in male vs female

Most common etiology of angina pectoris in men are due to blockages in their coronary arteries, which is known as obstructive coronary artery disease (CAD), but most common etiology of angina pectoris in women are due to obstruction within the very small arteries that branch out from the coronary arteries, called as microvascular disease (MVD) and it occurs particularly in younger women. Up to 50% of women with anginal symptoms who undergo cardiac catheterization don't have the obstructive type of CAD^[11].

Symptoms in women

- ❖ Chest pain (mostly asymptomatic)
- ❖ Pain or pressure in the lower chest or upper abdomen
- ❖ Pain radiates to Jaw, neck or upper back
- ❖ Nausea or vomiting
- ❖ Shortness of breath (Dyspnea)
- ❖ Fainting
- ❖ Indigestion
- ❖ Extreme fatigue

Symptoms in men

- ❖ Squeezing chest pressure or pain
- ❖ Pain radiates to Jaw, neck or back
- ❖ Nausea or vomiting
- ❖ Shortness of breath (Dyspnea)

Diagnosis

The diagnostic test for angina pectoris includes a 12-lead electrocardiogram (ECG), chest X-ray, and evaluation of lab findings, which includes complete blood count (CBC), basic metabolic profile (BMP), along with serial troponin-T levels if ACS is suspected. ECG may not show any abnormalities in the cases of stable angina, unstable angina, or NSTEMI. ECG findings of myocardial ischemia include T-wave flattening or inversions or ST-segment depressions^[12]. Further testing may include exercise or pharmacologic stress testing with or without nuclear perfusion imaging and diagnostic heart catheterization. ECG changes will appear in STEMIs and prompt immediate need for coronary revascularization^[13].

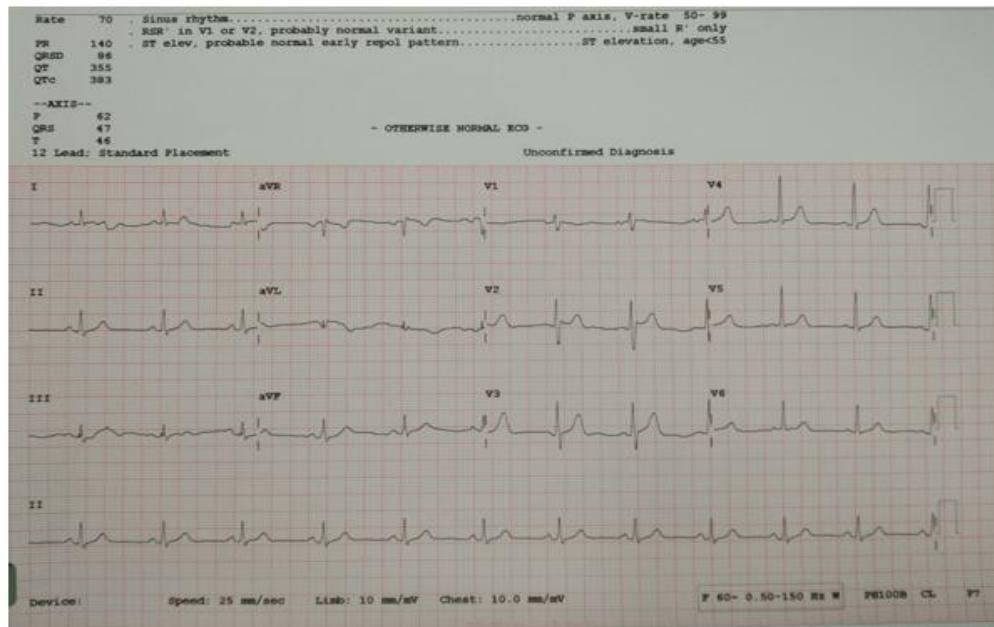


Figure 5

Figure 5 represents ST elevation in 12 lead ECG

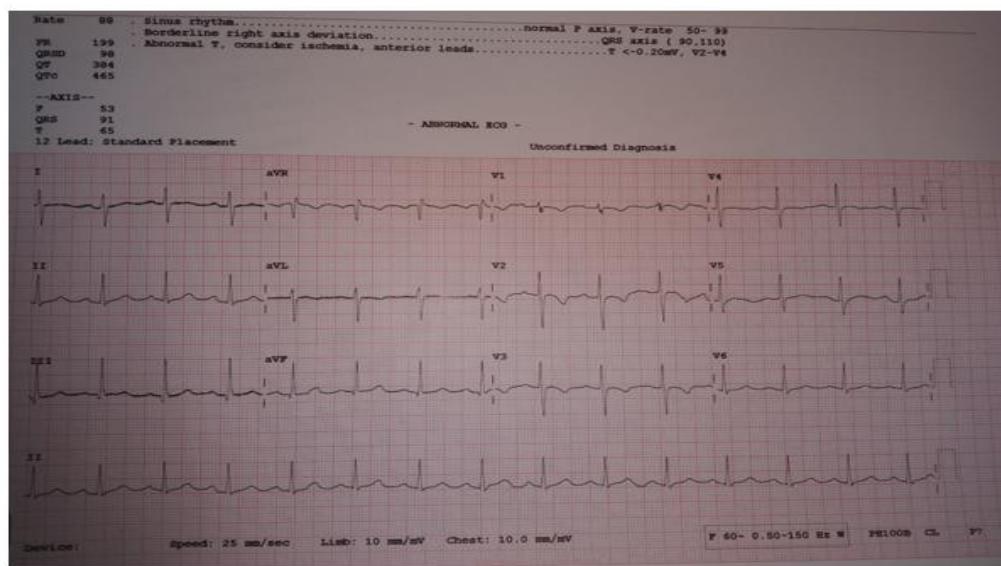


Figure 6

Figure 6 represents T wave inversion in 12 lead ECG, which indicates ischemic damage of heart

Prevention

Avoiding smoking and drug abuse, regular physical exercise, good mental health, low fat diet, intake of high fiber content in regular diet, adequate rest, maintaining regular sleep cycle can be helpful to prevent angina pectoris^[14].

People more than 50 years or with co morbidities such as Diabetes mellitus should undergo regular health checkup at least a year, Early detection of stable angina can be reversible which has a good prognosis^[15].

Treatment

Treatment for angina pectoris is a multifactorial management which involves lifestyle modifications, risk factor modification, and medical therapy. Medical management is considered as essential components of treating angina, unless in which symptoms are refractory to medical therapy, revascularization may be attempted^[16].

Lifestyle modifications include regular physical exercise, weight control, and smoking cessation and good mental health. Risk factor modification includes controlling blood pressure, cholesterol, and blood glucose levels. Drugs used in risk factor modification and to prevent disease progression includes aspirin, statins, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers^[17].

Medical therapy can be used to control symptoms as well as help mitigate the risk of progression of atherosclerosis and cardiac events. Antianginal drugs can be classified based on the mechanism of symptom relief in angina. In general, symptomatic control is achieved by way of decreasing myocardial oxygen consumption, which reduces myocardial oxygen demand and prevents the heart from ischemic damage^[18].

As heart rate is the main influencer of oxygen consumption, most anginal events are initiated by an increase in heart rate, if it persists it may end in cardiac arrest. Increased in calcium concentration in blood causes systolic arrest of heart. First line antianginal drugs used in angina to reduce symptoms by reducing heart rate, the drug includes beta-blockers, ivabradine, non-dihydropyridine and calcium channel blockers. Calcium channel blockers should be avoided in patients with left ventricular dysfunction and decreased ejection fraction^[18].

Nitroglycerine (NTG) cause vasodilation, which decreases preload and left ventricular end-diastolic volume and reduces myocardial oxygen consumption. They should not be administrated in patients with hypotension and previous use of phosphodiesterase inhibitors within the past 48 hours. Beta-blockers are used to reduce heart rate, contractility, and blood pressure, which reduces myocardial oxygen demand and prevents Ischemic damage. Antiplatelet agents can be used as dual therapy with aspirin and either clopidogrel, ticagrelor, or prasugrel decreases the risk of cardiovascular events in patients with acute coronary syndrome, acute myocardial infarction, cardiovascular death, and stroke. Anticoagulants such as heparin are used to decreasing re-infarction rates in combination with antiplatelet agents, which reduce mortality rate. Combination of three lifesaving drugs should be given orally as initial loading dose to adult patient with angina, which include Aspirin (325mg), Atorvastatin (8mg), Clopidogrel (300mg). This loading dose reduces the work load of heart, reduces myocardial oxygen demand and improves the tissue perfusion to heart muscles. This loading dose was contraindicated in patients with coagulation disorders, hypotension, 48 hours prior to the surgery^[19].

In recent days along with medical support, mechanical like IABP is initiated to improve coronary perfusion which reduces the mortality and increases the chance of recovery^[20].

Intra-Aortic Balloon Pump (IABP)

An intra-aortic balloon pump (IABP) is a invasive mechanical device that helps the heart to pump more amount blood and improves coronary perfusion, on the same hands it reduces the work load of heart. An intra-aortic balloon pump connects to a machine which controls it when to inflate and deflate^[20].

IABP works on the principle of counter pulsation, balloon deflates at systolic phase (when your heart pumps blood out), then inflates during diastolic phase (when your heart relaxes), This phenomenon is known as counter pulsation. Deflation helps in pumping the blood throughout the body and inflation helps in improving both the systemic circulation and coronary circulation of heart^[21].



Figure 7 - IABP

Conclusion

In this review, we focused on the latest findings in the etiology, Types of Angina pectoris, Printzmetal angina, pathophysiology, clinical manifestations in male vs female, diagnosis, prevention and treatment of angina pectoris, Intra-aortic balloon pump (IABP) in Angina and the incidence and severity profile of angina pectoris in Indian populations.

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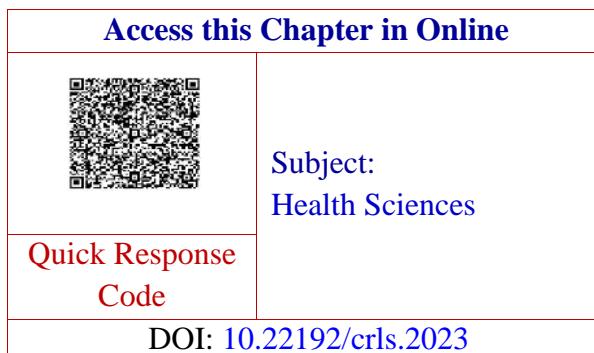
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Holiday Heart Syndrome- A Nightmare of Alcoholics on Holiday

^{1*}**V. Dilip,**

Perfusionist, Department of Cardio Thoracic and Vascular Surgery,
Faculty of Allied Health Science, Meenakshi Academy of Higher
Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

²**D.E. Nirman Kanna,**

Perfusionist, Department of Cardio Thoracic and Vascular Surgery,
Faculty of Allied Health Science, Meenakshi Academy of Higher
Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

Corresponding Author: ^{1*}**V. DILIP**, Perfusionist,

Department of Cardio-Thoracic and Vascular Surgery, Faculty of Allied
Health Sciences, Meenakshi Academy of Higher Education and
Research, Chennai, Tamil Nadu, India.

Email ID: v.dilip2000@gmail.com

Abstract

Background: As the name suggests, the nightmare deadly arrhythmias occur during holidays to people, who consume more alcohol during the holidays and the alcohol is directly toxic to the heart. Drinking excessive amount of alcohol can lead to cause cardiomyopathy. Holiday heart syndrome is not just a beginning condition which is actually a very potentially serious condition that causes people to develop heart failure. Cardiac arrhythmias presenting during weekend or holiday drinking episodes are associated with conduction delays and depressed cardiac performance indicative of early cardiomyopathy. The typical presentation, usually described as “holiday heart syndrome” is characterized as an acute conduction impairment associated with heavy (>600 mg/100 ml or 130 mmol/L) ethanol consumption in a person without any underlying cardiac disorder and normalization of the rhythm with avoidance of alcohol. Usually, holiday heart syndrome or alcohol induced arrhythmia can be controlled by medical management but sometimes there is risk of collapse and even lead to death. In this review, we focused on current updates on holiday heart syndrome, its aetiology, epidemiology, pathophysiology, clinical manifestations, risk factors, complications, ECG and histological changes and its management.

Keywords: Holiday heart syndrome, Alcohol, Arrhythmias, Atrial fibrillation, Atrial flutter.

Introduction

Holiday Heart Syndrome or alcohol induced atrial arrhythmia syndrome can be defined as an acute cardiac rhythm (irregular heart beat) associated with excessive alcohol consumption or binge drinking in a person without other clinical evidence of heart disease. In 1978, Philip Ettinger discovered the connection between the arrhythmia and alcohol consumption^[1]. The typical presentation, usually described as “holiday heart syndrome” is characterized as an acute conduction impairment associated with heavy (>600 mg/100 ml or 130 mmol/L) ethanol consumption in a person without any underlying cardiac disorder and normalization of the rhythm with avoidance of alcohol. Usually, holiday heart syndrome or alcohol induced arrhythmia can be controlled by medical management and can be reversed but sometimes there is risk of collapse and even lead to death^[2].

Epidemiology

In developing countries like India, alcohol addiction is the major issue which causes a serious impact, this problems are due to various socio-cultural practices across the nation, different alcohol policies and practices across the various states, lack of awareness of alcohol-related problems among the community, false mass media propaganda about alcohol use, various alcohol drinking patterns among the alcohol consumers and the emergence of social drinking as a habit because of the widespread urbanization across the country^[3]. Many research study confirms that intake of excessive alcohol act as causative agent in most onset of atrial Fibrillation. These studies conclude that “holiday heart syndrome” is characterized as an acute conduction impairment associated with heavy (>600 mg/100 ml or 130 mmol/L) ethanol consumption in a person without any underlying cardiac disorder and normalization of the rhythm with avoidance of alcohol^[4].

Etiology

Too much or heavy alcohol consumption, elevated stress levels and dehydration are the most common causes of holiday heart syndrome. Other aetiologies include deranged plasma electrolytes, particularly low potassium levels (hypokalaemia) and low levels of magnesium in blood which leads to causes cardiac arrhythmias^[5]. High serum alcohol levels may interfere with sodium, potassium and calcium ion channels in the heart. Moreover, alcohol

may also lead to instabilities in autonomic regulation of cardiac rhythm, which results in arrhythmias^[6].

Pathophysiology

Chronic ethanol use leads to build-up of ethanol and its metabolites and causes cardiac structural and cellular changes. Acetaldehyde is the most commonly researched metabolite in holiday heart syndrome^[7]. This acetaldehyde is produced by the liver by a chemical reaction with alcohol dehydrogenase. Acetaldehyde and along with other substances, it is possible to cause Oxidative damage, cell death, Mitochondrial dysfunction, reducing the effects of cardioprotective molecules, alteration in protein synthesis and calcium transport^[8]. Ethanol consumption might be associated with intra myocardial changes well as adrenal release of catechol amines abnormal autonomic nervous system discharges or electrophysiological consequences of acetaldehyde (the metabolite of ethanol)^[9].

Risk factors

- ❖ Heavy alcohol consumption
- ❖ Binge drinking
- ❖ Intake of high sodium content in regular diet
- ❖ Intake of high lipid foods
- ❖ Low fibre diet
- ❖ high levels of stress
- ❖ Dehydration^[10].

Differential diagnosis

- ❖ Alcohol use disorder
- ❖ Arrhythmia
- ❖ Cirrhotic cardiomyopathy
- ❖ Dilated cardiomyopathy
- ❖ Psychiatric problems^{[10][7]}.

ECG changes observed in Holiday Heart syndrome

Alcohol intoxication can cause prolongation of the PR, QRS and QT-intervals and sensitize the myocardium to atrial arrhythmias as well as life threatening ventricular arrhythmias^[8]. The sudden cessation of alcohol intake results in beta-adrenergic stimulation which increase the levels of

catecholamine. So, the patients may be prone to several arrhythmias during alcohol detoxification^[9].

ECG changes are observed when serum concentration of alcohol is >600 mg/100 mL (130 mmol/L). A decrease in the rate of rise of phase “O” of the action potential and the amplitude of the action potential is observed with intoxication. Bradycardia and atrioventricular (AV) block have been occasionally reported with acute alcohol intoxication^[9].

It is possible that decreased calcium as well as sodium currents are related with AV block after alcohol consumption. A research study results that among 8 cases of AV block following acute alcohol consumption, five patients had first-degree AV block and 3 patients presented with second-degree AV block, the 5 patients with first-degree AV block, 1 evolved into a third-degree AV block, in 7 patients, complete recovery occurred, while in 1 patient first-degree AV block persisted during follow-up and may have been present previously, which concludes that Alcohol intoxication will cause atrial fibrillation^[10].

Till now, there is no proper studies that defines P-wave prolongation with alcohol intoxication which is either related to atrial fibrillation or not. As reported in the AFFIRM study, P wave duration > 135 m sec in lead II was a risk factor for atrial fibrillationrecurrence after cardioversion^[11].

The atrial fibrillation with acute alcohol intoxication may be more common than what is reported in the literature but transient in nature, so not necessarily detected by a single ECG. It is more commonly associated with chronic alcohol consumption which can lead to dilated cardiomyopathy, hence making the heart prone to atrial fibrillation^[12].

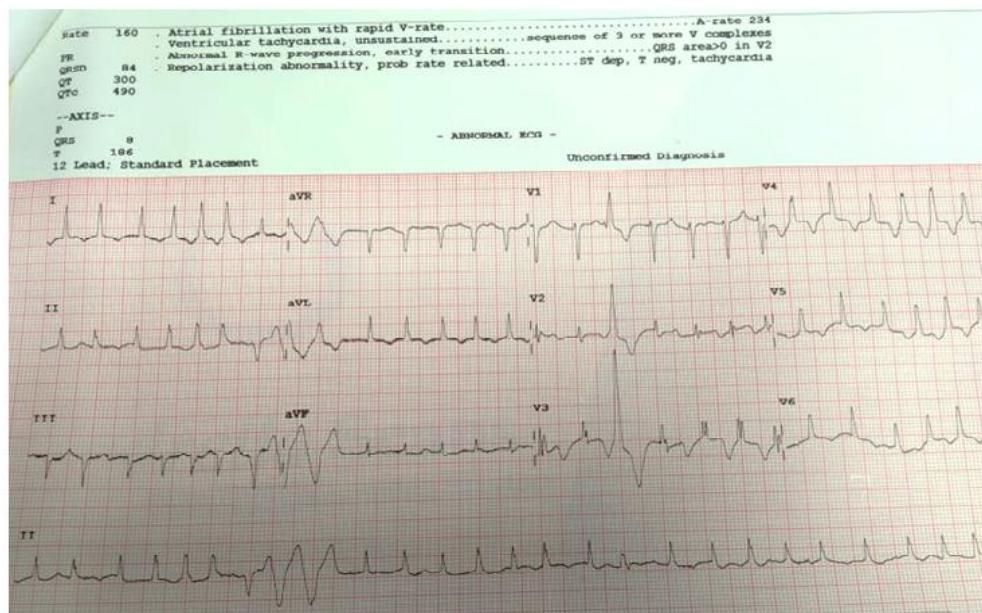


Figure 1

Figure 1 represents the ECG findings of patient with alcohol induced atrial fibrillation

Histological changes of myocardium in patients with holiday heart syndrome

Examination of myocardium obtained by biopsy under the electron microscope revealed destruction and fragmentation of muscle elements into short segments arranged in more or less haphazard fashion, widely spaced apart, and large numbers of swollen, damaged mitochondria. Evacuation of mitochondrial contents also was observed, leaving mitochondrial ghosts. Nuclei were enlarged and sarcoplasmic reticulum was dilated, sometimes to cystic proportions. Lipid was not characteristically increased in the material examined but glycogen was abundant. Inflammatory cells were absent. These changes appear to be characteristic of alcoholic heart disease, although similar to those described by others in experimental potassium and magnesium deficiency^{[13][14]}.

Symptoms

Symptoms like shortness of breath, sweating, anxiety, general fatigue, rapid and irregular heartbeat, fluttering, dizziness, Faintness or confusion, excessive sweating at rest, chest pain and pressure over the chest palpitations oftentimes associated with Atrial Fibrillation which is associated with higher risk of stroke^{[15][16]}.

Complications

- ❖ If holiday heart syndrome is left untreated, the complications are thrombosis, community- acquired pneumonia, cirrhosis and heart failure.
- ❖ The duration of Holiday Heart Syndrome lasts 24 hours in most of the Holiday Heart Syndrome patients^[17].

Other complications of the holiday heart syndrome include^[18].

- ❖ Severe or worsening heart failure
- ❖ Life-threatening arrhythmias
- ❖ Community-acquired pneumonia
- ❖ Thromboembolism
- ❖ Death

Management

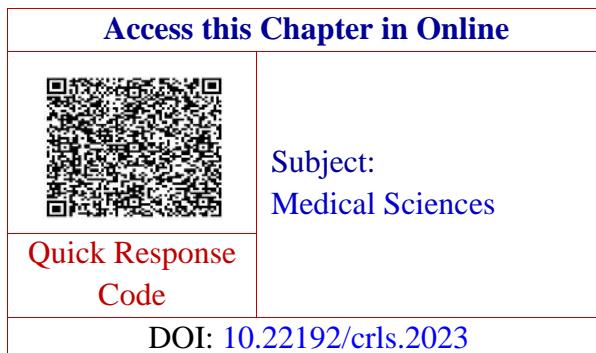
Cardioversion is the treatment of choice, if the patient is unstable and has atrial fibrillation. If the patient is stable, the therapeutic indication is recommended for arrhythmia treatment. If the symptoms persist too long, medical management is required which includes administering anti-arrhythmic drugs such as amiodarone initial dose of 300mg IV bolus, followed by administering 150 mg IV bolus 3-5 minutes after the initial dose given. It should not exceed more than 2.2grams per day. Also, radiofrequency ablation, carotid sinus massage can be performed to manage Atrial Fibrillation (AF) by applying gentle pressure on the neck where the carotid artery splits into two branches. During this type of massage, the body releases chemical compounds that slow the heart rate. Vagal maneuvers also help to manage AF by instructing the patient to hold nose, close his mouth and try to blow the air out in a syringe or a plastic bag for 10-20 seconds. This creates pressure in the chest that may activate the vagus nerve, which helps control the heartbeat^{[19][20]}.

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Pharmagonosy and Health benefits of Clove

N. Uma Maheswari and L.Deepika

PG and Research Department of Microbiology
STET Women's (Autonomous) college, Sundarakkottai,
Mannargudi, Thiruvarur, Tamil Nadu, India.
Email id: umasamyamf@gmail.com

Introduction

Clove, is a popular spice often used in Indian, African, Mexican and Middle Eastern cuisine, and has useful medicinal properties. Clove, historically for a certain period of time (around 17th century) were grown only on the Spice Islands or the Malaku Islands by the Dutch to gain monopoly. But the French succeeded in introducing the clove trees in Mauritius followed by many other countries. Before this in around third century BC clove was used in China and then by the fourth century had gained popularity in Europe too. Due to its medicinal properties its use had started as an expectorant, anti-emetic and analgesic which still continues today. Eugenol is the active ingredient which is mainly responsible for the pharmacological properties of the drug. It is safely used in foods, beverages and toothpastes. Because of its analgesic (pain-killing) and antiseptic (bactericidal) activity it has gained wide acceptance in dentistry. Its analgesic and expectorant (cough-suppressing) activity makes it a very good herbal treatment for symptomatic relief of sore throat. It is also known as lavang in India.



Figure 1: Clove

History

As early as 200 BCE, envoys from Java to the Han-dynasty court of China brought cloves that were customarily held in the mouth to perfume the breath during audiences with the emperor. During the late Middle Ages, cloves were used in Europe to preserve, flavour, and garnish food. Clove cultivation was almost entirely confined to Indonesia, and in the early 17th century the Dutch eradicated cloves on all islands except Amboina and Ternate in order to create scarcity and sustain high prices. In the latter half of the 18th century the French smuggled cloves from the East Indies to Indian Ocean islands and the New World, breaking the Dutch monopoly. In the early 21st century, Indonesia was the world's largest producer of cloves, followed by Madagascar, Tanzania, and Sri Lanka.

Analytical profile of Clove

Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Myrales
Family	Myrtaceae
Genes	Syzygium
Species	Aromatisum
Common Name	Laung

Cultivation of clove

Clove tree is evergreen and 10 to 20 m in height. The plant requires moist, warm and equable climate with well-distributed rainfall. It is propagated by means of seeds. The seeds are sown in well-drained suitable soil at a distance of about 25 cm. The plants should be protected against pests and plant diseases. Initially it has to be protected from sunlight by growing inside a green house or by constructing frames about 1 m high and covering them with banana leaves. As the banana leaves decay gradually more and more sunlight falls on the young seedlings and the seeds are able to bear full sunlight when they are about 9 months old. The seedlings when become 1 m high, they are transplanted into open spaces at a distance of 6 m just before the rainy season. The young clove trees are protected from sun even for a longer period by

planting banana trees in between. The drug can be collected every year starting from 6 years old till they are 70 years old.

Collection of Clove

Clove buds change the colour as they mature. At the start of the rainy season long greenish buds appear which change to a lovely rosy peach colour and as the corolla fades the calyx turns yellow and then red. The buds are collected during dry weather in the month of August to December. The collection is done either by climbing on the tree or by using some ladders or with the help of mobile platforms. In some places the trees are even beaten using bamboo sticks for the collection of the bud. The drugs which are collected are then separated from the stalks and then placed on coconut mats for drying under sun. The buds lose about 70% of its weight, whereas drying and change their colour to dark reddish-brown. The dried clove is graded and packed.

Description

The clove tree is an evergreen that grows to about 8 to 12 metres (25 to 40 feet) in height. Its gland-dotted leaves are small, simple, and opposite. The trees are usually propagated from seeds that are planted in shaded areas. Flowering begins about the fifth year; a tree may annually yield up to 34 kg (75 pounds) of dried buds. The buds are hand-picked in late summer and again in winter and are then sun-dried. Cloves vary in length from about 13 to 19 mm (0.5 to 0.75 inch). The buds contain 14 to 20 percent essential oil, the principal component of which is the aromatic oil eugenol

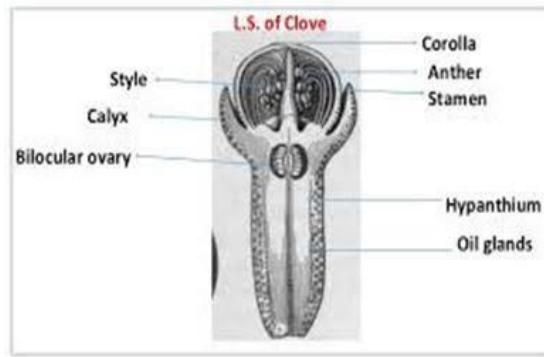


Figure 2: L.S.Clove

Characteristics

Clove is reddish-brown in colour, with an upper crown and a hypanthium. The hypanthium is sub-cylindrical and tapering at the end. The hypanthium is 10 to 13 mm long, 4 mm wide, and 2 mm thick and has schizolysigenous oil glands and an ovary which is bilocular. The Crown region consists of the calyx, corolla, style and stamens. Calyx has four thick sepals. Corolla is also known as head, crown or cap; it is doineshaped and has four pale yellow coloured petals which are imbricate, immature, and membranous. The ovary consists of abundant ovules. Clove has strong spicy, aromatic odour, and pungent and aromatic taste

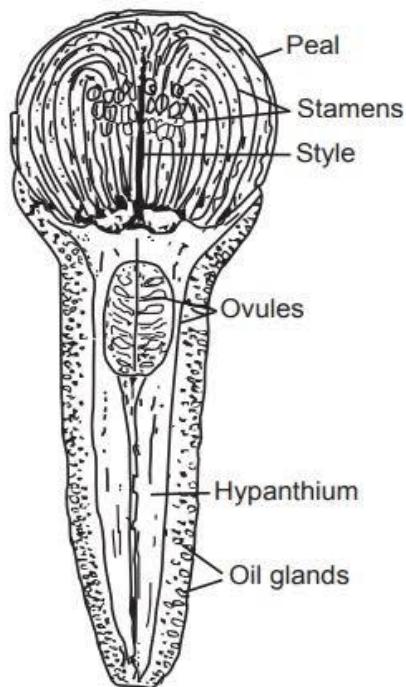


Figure 3: Structure of clove

Chemical Constituents

Clove contains 14–21% of volatile oil. The other constituents present are the eugenol, acetyl eugenol, gallotannic acid, and two crystalline principles; - and - caryophyllenes, methyl furfural, gum, resin, and fibre. Caryophyllin is odourless component and appears to be a phytosterol, whereas eugenol is a colourless liquid. Clove oil has 60–90% eugenol, which is the cause of its anesthetic and antiseptic properties.

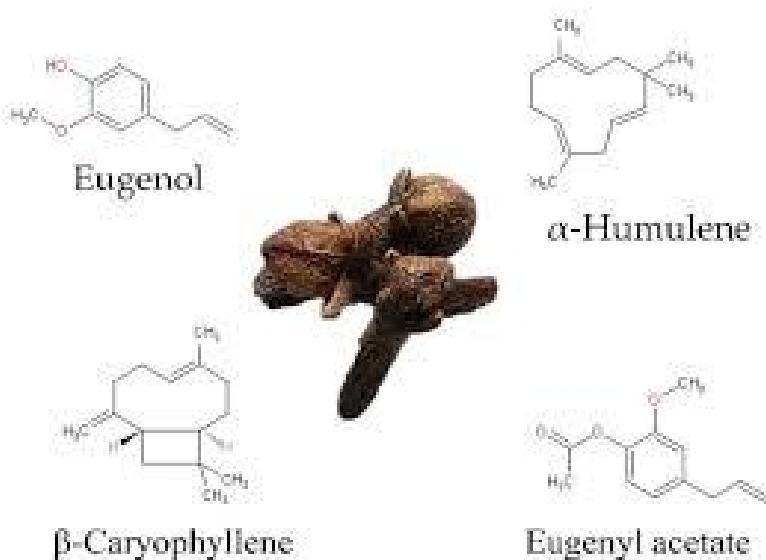


Figure 4: Chemical constituents of clove

Popular Clove Varieties in India

There is no specific cultivar or a variety of clove defined in India, but in the trading context, its cultivars are distinguished based on the native regions of their cultivation.

1. Penang

Penang cloves are plumpy and large in size and come in a dark red-brown hue. They're commercially exported all over the east, but the demand is very high all across the globe. This spicy clove has uses in pomanders and culinary, both.



Figure 5: penang

2. Zanzibar

Zanzibar clove is smaller and leaner in shape as compared to the Penang cloves and has a black-brown color. This high-quality clove works as a flavorful spice in savory and sautéed dishes. It's popular among the growers because of its high productivity rate and better adaptability.



Figure 6: Zanzibar

3. Amboyan

Amboyan cultivar produces large-sized and plumpy cloves like the Penang. These pungent and aromatic cloves are popular in Indian savory recipes, toothpaste, cosmetics, and pomanders. But, this clove variety has slightly low productivity as compared to Zanzibar and they're also not very adaptable.



Figure 7: Amboyan

Uses of cloves

Clove is used as an antiseptic, stimulant, carminative, aromatic, and as a flavouring agent. It is also used as anodyne, antiemetic. Dentists use clove oil as an oral anesthetic and to disinfect the root canals. Clove kills intestinal parasites and exhibits broad antimicrobial properties against fungi and bacteria and so it is used in the treatment of diarrhea, intestinal worms, and other digestive ailments. Clove oil can stop toothache. A few drops of the oil in water will stop vomiting, eating cloves is said to be aphrodisiac. Eugenol is also used as local anaesthetic in small doses. The oil stimulates peristalsis; it is a strong germicide, also a stimulating expectorant in bronchial problems. The infusion and Clove water are good vehicles for alkalies and aromatics.

Health benefits

-) Cloves are a great source of beta-carotene, which helps give them their rich brown color. The carotene family of pigments are important antioxidants and provitamins. Carotene pigments can convert into vitamin A, an important nutrient for keeping your eyes healthy.
-) Cloves include multiple compounds that are linked to anti-inflammatory properties. Eugenol is the most important of these compounds. Eugenol has been shown to reduce the inflammatory response in the body, reducing the risk of diseases such as arthritis and helping to manage symptoms.
-) Eugenol is also a potent antioxidant. Cloves are full of antioxidants. These compounds help your body to fight free radicals, which damage your cells and can lead to disease. By removing free radicals from your system, the antioxidants found in cloves can help reduce your risk of developing heart disease, diabetes, and certain cancers.
-) Cloves can help protect your stomach from ulcers. Most ulcers are caused by thinning in the layers of mucus that protect your stomach lining. Preliminary studies show that cloves can thicken this mucus, lowering your risk of developing ulcers and helping existing ulcers heal.



Figure 8: Health benefits of clove

Conclusion

Based on the information presented, it could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant compounds.

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Pharmacology (Drug disposition in new born baby)

M. Kannahi and V. Desika

PG and Research Department of Microbiology,

STET women's college, (Autonomous)

Sundarakottai, Mannargudi, Thiruvarur, Tamilnadu, India.

Phone number 9894101402, 6369512389

Email.id: kannahiamf@gmail.com and 2004011desika@gmail.com

Abstract

During the neonatal period, there is physiological immaturity of organs, systems and metabolic pathways that influences the pharmacokinetics and pharmacodynamics of administered drugs, the dosage of which should be constantly amended, considering the progressive increase in weight and the maturation of the elimination pathways. In this article, we analyse the main pharmacokinetic aspects (absorption, distribution, metabolism and excretion) that exist during the neonatal period, to offer a description of the physiological background for variability in pharmacological dosing.

Keywords: infant, newborn, pharmacology

Introduction

The correct dosage of drugs is crucial for newborns, whose disposition is significantly influenced by the route of administration, the metabolic capacity and the elimination pathways, which are significantly different in newborns compared with adults.

The neonatal period, which includes the first 28 days of life from the moment of birth in term infants and a period up to 44 weeks of postmenstrual age in former preterms, is characterized by a physiological immaturity of organs and apparatuses. This affects the pharmacokinetics and pharmacodynamics of the administered drug, as well as the tolerance of the newborn to it.

It should be considered that developmental and maturational changes are complex processes, and simplified methods of administering a drug may result in subtherapeutic doses and lack of effect, or adverse toxic events. For this reason, dosages must be constantly modified on the basis of progressive

increase in weight and the maturation of the metabolic pathways. The investigation and understanding of the response mechanisms of the neonatal organism to administered drugs is extremely useful to determine the correct dosage. However, many of the drugs for neonatal use remain poorly studied, and their dosage is often based on information that is extrapolated from their use in adults or in older children. This is because conducting clinical trials during the neonatal period is problematic for ethical and logistical reasons.

Little information exists about the effect of human ontogeny on interactions between drugs and receptors and the consequence of these interactions (i.e. the pharmacodynamics).

This article, we analyse the main aspects of pharmacokinetics (absorption, distribution, metabolism and excretion) that are typical of the neonatal period.

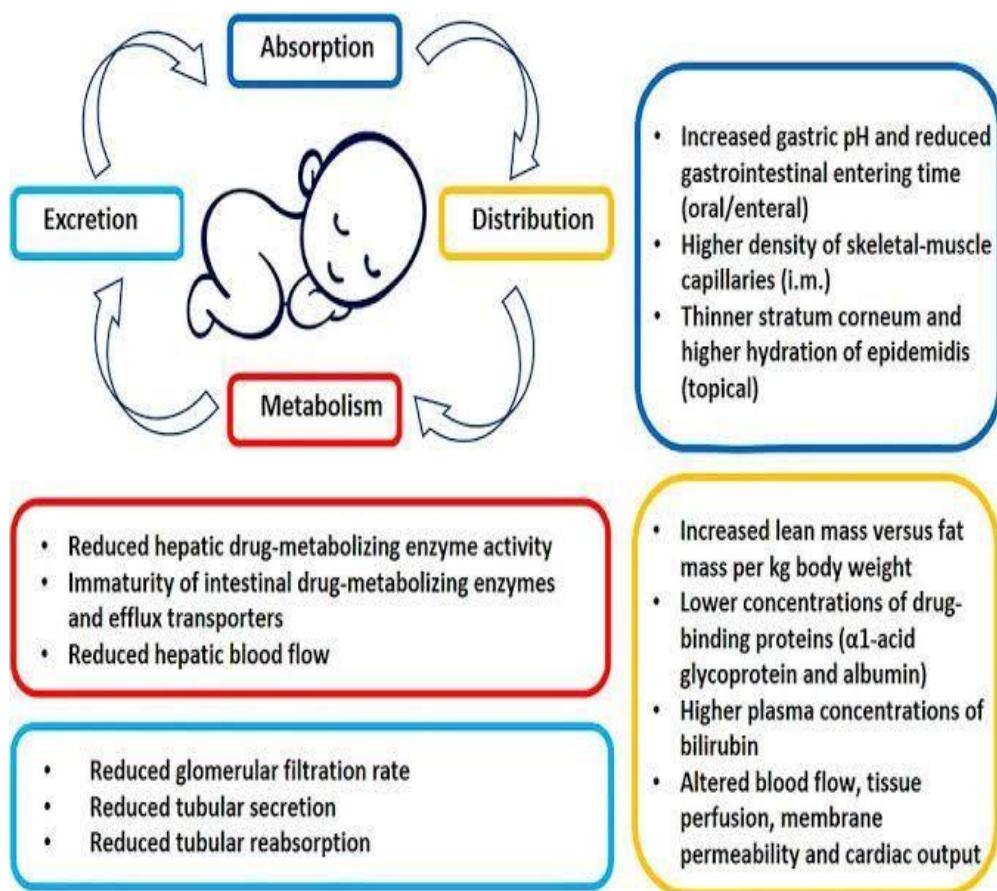


Figure 1: Drug disposition

Drug absorption

The absorption of drugs is different in children up to 2 years and is affected by the maturation process of the various organs.

At gastric level, there is a variation in pH, which is neutral at birth. In the first 24 hours after birth, it is 1–3 and returns to neutral around the eighth day, and subsequently it decreases to match the values of an adult around the second to third year of life. If the pH of the stomach is high, drugs that are weak acids are absorbed slower than drugs that are weak bases. For this reason, in newborns there is good gastric absorption of acid-labile drugs, such as benzylpenicillin, ampicillin, amoxicillin and erythromycin.

The rate of gastric emptying appears to be directly influenced by gestational and postnatal age as well as the type of feeding. Infants have a delayed gastric emptying (6–8 hours) that determines a delay in the absorption of drugs and in reaching the concentration peak, with a reduced concentration peak. Gastric emptying is also conditioned by the composition of the meal and is faster in neonates after they are fed an extensively hydrolysed formula than an intact protein or partially hydrolysed formula. In contrast, slower gastric emptying times have been reported with increasing caloric density and medium-chain triglycerides in premature infants. Gastric emptying time appears to approach adult values within the first 6–8 months of life.

The intramuscular absorption of drugs in newborns is negatively influenced by the reduced mass, perfusion and muscle contractility. However, it is also influenced by the physicochemical characteristics of the drugs such as the pH, molecular weight, solubility and dissolution rate. In fact, water-soluble drugs show an increased intramuscular absorption.

Absorption: Describes how the drug moves from the site of administration to the site of action. Distribution: Describes the journey of the drug through the bloodstream to various tissues of the body. Metabolism: Describes the process that breaks down the drug. Excretion: Describes the removal of the drug from the body.

The rectal absorption of drugs is generally increased in newborns; however, the variability in the depth of administration or in the retention of drugs can vary their absorption, similarly to adults. In fact, drugs administered at deep rectal level reach the hepatic level directly through the superior rectal veins, whereas drugs administered at distal rectal level reach the systemic circulation via the medium and inferior rectal veins.

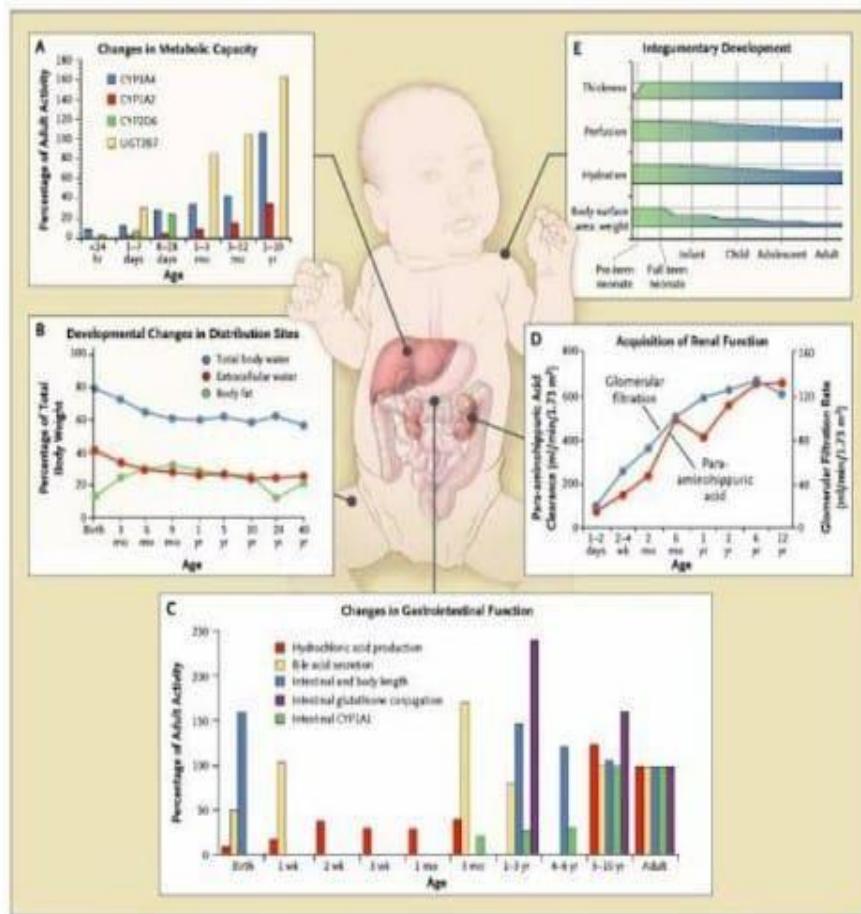


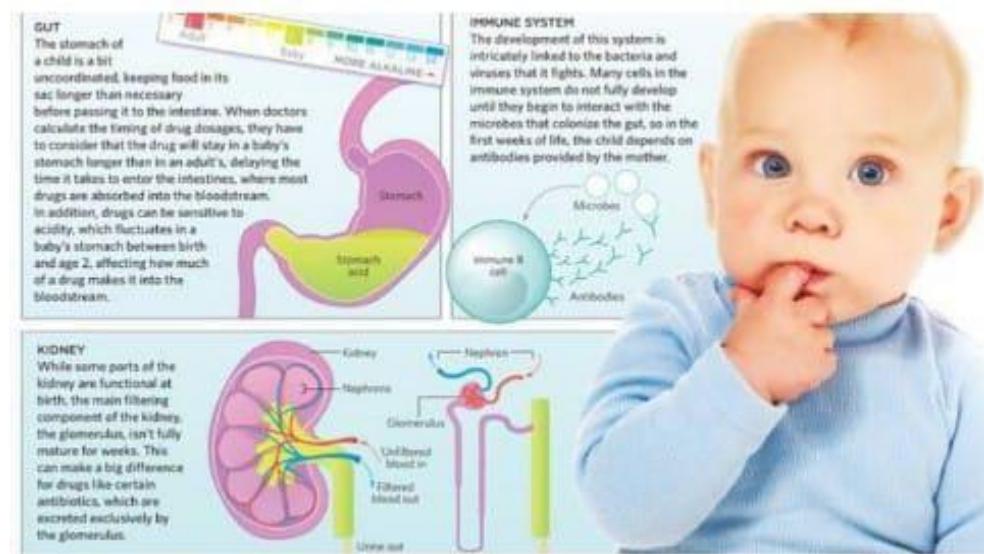
Figure 2: drug absorption

Drug distribution

After absorption, drugs are distributed in the various body compartments depending on their physicochemical properties such as molecular weight, ionization constant and solubility in water and in lipids. The distribution of the drug varies depending on the age of the individual and also on the permeability of the cell membrane, on the extent of its binding to proteins, the binding capacity of individual tissues and on the change in the volume of the extracellular fluids in proportion to the total quantity of water in the body.

Phase I and II reactions

Paediatric patients, especially neonates, exhibit distinct hepatic drug metabolism activity from adults due to differences in P450 expression during development. Mechanisms regulating paediatric gene expression and induction may also differ from those of adults. Drug metabolism occurs through phase **Drug metabolism**



Liver metabolism

In general, the rate of drug elimination by biotransformation in neonates and infants is slower than that in adults. During the first week of life, there are rapid physiological postnatal changes in the liver blood flow, including increasing portal vein blood flow, and gradual closure of the ductus venosus shunt. In addition, the loss of the umbilical blood supply causes changes in hepatic oxygenation. These relevant changes may affect the capacity

The CYP3A subfamily consists of CYP3A4, CYP3A5, CYP3A7 and CYP3A43. CYP3A43 is not known to play a significant role in hepatic metabolism. It has been established that CYP3A4 is the predominant CYP3A enzyme in adults, whereas CYP3A7 is the predominant CYP3A enzyme in foetus and infants. Moreover, CYP3A5 is expressed more in children and adolescents than in adults. Furthermore, there is a great deal of overlap of specificity of ability for CYP3A4 and CYP3A7 to metabolize therapeutic agents. Methods: Physiological data for neonates, 0.5-, 1, 2-, 5-, 10- and 15-

year-old children, and adults, of both sexes were compiled from the literature. The data comprised body weight and surface area, organ weights, vascular and interstitial spaces, extracellular body water, organ blood flows, cardiac output and glomerular filtration rate. Tissue: plasma partition coefficients were calculated from rat data and unbound fraction (f_u) of the drug in human plasma, and age-related changes in unbound intrinsic hepatic clearance were estimated from CYP1A2 and CYP2E1 (theophylline) and CYP3A4 (midazolam) activities in vitro. Volume of distribution (V_{dss}), total and renal clearance (CL and CL R) and elimination half-life ($t(1/2)$) were estimated by PBPK modelling, as functions of age, and compared with literature data.

In 2003, Stevens and colleagues published the results of examining the largest collection of foetal and paediatric 212 liver samples and demonstrated that CYP3A7 has a

Kidney excretion

Neonatal renal function is lower than expected on the basis of the body weight or body surface area, due to the reduced renal blood flow, lower capacity of the renal tubules to concentrate or acidify the urine, slower GFR and reduced transport system of organic ions for the active tubular secretion. The decreased GFR prolongs half half-lives of drugs, delaying clearance. The GFR is directly dependent on gestational age and this effect is more pronounced in preterm neonates. Consequently, in newborns and small children, drugs that require a renal excretion are eliminated much slower, with an increase in the plasma concentration and their potential toxicity (Many commonly used drugs, such as Principles of Drug Therapy (Robert M. Kliegman MD, in Nelson Textbook of Pediatrics, 2020).

Impact of Ontogeny on Drug Disposition

Development represents a continuum of biologic events that enable adaptation, somatic growth, neurobehavioral maturation, and eventually reproduction. The impact of development on the pharmacokinetics of a given drug is determined to a great degree by age-related changes in body composition and the acquisition of function in organs and organ systems important in determining drug metabolism and excretion. Although it is often convenient to classify pediatric patients on the basis of postnatal age in providing drug therapy, with neonates 1 mo of age, infants 1-24 mo, children 2-12 yr, and adolescents 12-18 yr, it is important to recognize that the changes in physiology are not linearly related to age and may not correspond to these age-defined breakpoints. In fact, the most dramatic changes in drug disposition occur during the 1st

18 mo of life, when the acquisition of organ function is most dynamic. It is important to note that the pharmacokinetics of a given drug may be altered in pediatric patients because of intrinsic (e.g., gender, genotype, ethnicity, inherited diseases) or extrinsic (e.g., acquired diseases, xenobiotic exposure, diet) factors that may occur during the 1st 2 decades of life.

Selection of an appropriate drug dose for a neonate, infant, child, or adolescent requires an understanding of the basic pharmacokinetic properties of a given compound and how the process of development impacts each facet of drug disposition. Accordingly, it is most useful to conceptualize pediatric pharmacokinetics by examining the impact of development on the physiologic variables that govern drug absorption, distribution, metabolism, and elimination (ADME).

Pediatrics encompasses a broad range of ages at which certain stages of life profoundly influence drug response and disposition. Dramatic pharmacokinetic, pharmacodynamic, and psychosocial changes occur as preterm infants mature toward term, as infants mature through the 1st few years of life, and as children reach puberty and adolescence aminoglycosides, have clearances strongly correlated with glomerular function.

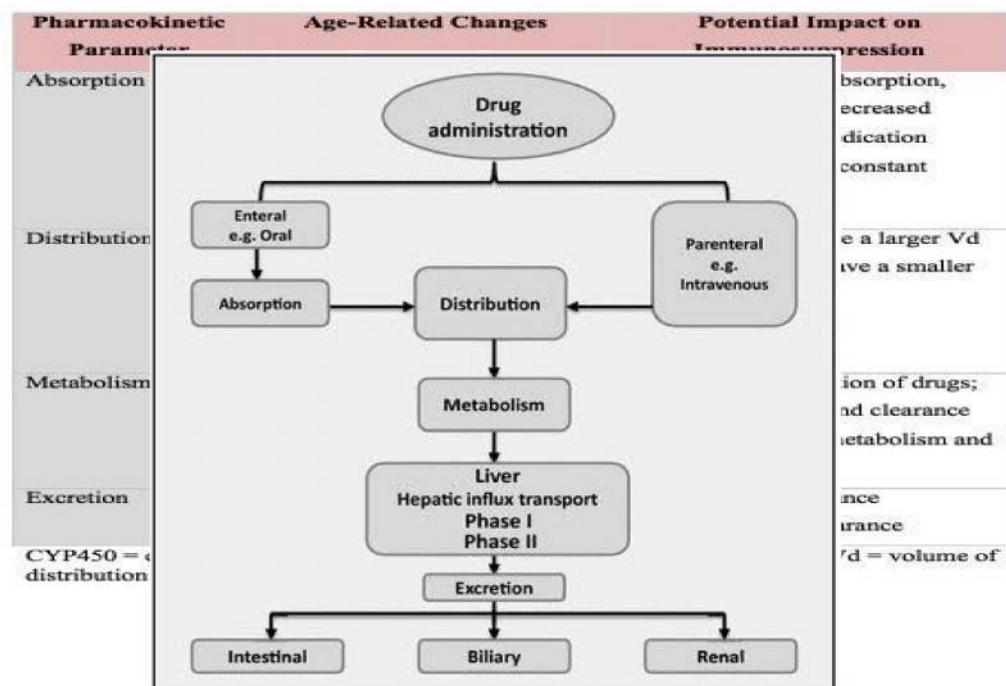


Figure 3: Flow chart of drug disposition

Conclusions

Paediatric and adult populations show significant differences with regard to the absorption, distribution, metabolism and excretion of various drugs. Newborns are neither small adults nor small children. In various paediatric age bands, it is possible to encounter different pharmacokinetic behaviours that are strongly influenced not only by age but also by body weight. Neonatal drug therapy should be based on a critical interpretation of available data and an understanding of foetal development and maturation processes and how diseases can affect the bio-arrangement of the drug in this specific population. Although there

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Pharmacy - Pain management

M.Noorul Jesya and M.Kannahi

PG and Research Department of Microbiology

Sengamala Thayaar Educational Trust Women's College (Autonomous)

Sundarakkottai, Mannargudi-614 016

Email id: kannahiamf@gmail.com, 2004034nooruljesya@gmail.com

Phone No: 8526334735, 6369512389

Introduction

Pain is a multidimensional experience accompanied mainly by actual or potential tissue damage affecting the quality of life. Pain is a subjective feeling, as no accurate or ideal tool exists to measure the level of pain experienced by a patient. To measure pain, a number of assessment scales are utilized such as the visual analog scale, numerical rating scale, verbal rating scale, brief pain inventory, and others. Pain is considered to be an indicator of many health issues.

A wide range of drugs are used to manage pain resulting from inflammation in response to tissue damage, chemical agents/pathogens (nociceptive pain) or nerve damage (neuropathic pain).

History

There are currently more than 100 million Americans suffering from chronic pain, including 65% to 80% of terminal cancer patients, 62% of nursing home residents, and many others who must combat pain on a daily basis.

Chronic pain afflicts more patients in the United States than diabetes, coronary heart disease, stroke, and cancer combined. It reduces a patient's independence and ability to perform many daily activities and puts a strain on social relationships, mood, and sleep patterns.

This decrease in overall functioning places a burden on the national economy, with total annual costs of health care and lost productivity attributable to pain ranging from \$560 billion to \$635 billion (2010 dollars) in the United States. With the number of patients suffering from pain increasing as the US population ages, the demand for pain management pharmacists will continue to rise.

Pharmacist role

Pharmacists can help minimize risk for patients using pain medications.

A comprehensive approach to care coordination that emphasizes medication reconciliation at multiple points of care (e.g., admission, unit transfer, discharge) is essential for optimizing patient outcomes. Pharmacists, who are trained as the medication experts on the health care team, can lead and champion this process.

Patients who have complex conditions and/or regimens that require services from multiple practitioners in different settings are at an increased risk for medication errors, drug-related adverse events, and poor clinical outcomes. Tam and colleagues conducted a systematic review of studies and found that up to 67% of patients had errors in prescription-medication history at hospital admission. One Canadian study conducted by Forster and colleagues found that 23% of patients discharged from an internal-medicine service experienced at least one adverse event; 72% of those were adverse drug events.⁹ The detection, management, and reporting of adverse drug events are among the many responsibilities of the pharmacist and play an important role in decreasing read missions.



Types of pain

The most widely accepted definition of pain is “an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage or described in such terms.” There are, however, as many different definitions of pain as there are ways that pain can manifest itself in complex patients. Each directly influences the course of therapy for each individual.

Pain is classified by its duration and etiology. Duration of pain is classified in 2 categories: acute pain and chronic pain. Acute pain is a response to injury or tissue damage that generally does not last longer than it takes for normal healing to occur. Typically, this pain lasts less than 6 months, but may become chronic if not adequately treated. Chronic pain, on the other hand, persists longer than the normal course of time associated with injury, typically more than 3 to 6 months. In many cases, the initial injury may not be readily identifiable.

Pharmaceutical medicines

Non-opioid medications: Step 1 - WHO Analgesic ladder Mild to Moderate pain

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen, diclofenac weaken and reduce the levels of chemical mediators (prostaglandins) produced during inflammation, relieving symptoms of pain, swelling and redness. They inhibit the enzyme cyclooxygenase (COX 2) which is integral in the synthesis of prostaglandins. During infection, the effect of prostaglandins on the hypothalamus results in a higher body temperature (pyrexia). NSAIDs weaken the production of prostaglandins enabling the temperature to reduce towards normal. NSAIDs do not just inhibit local prostaglandin production, but also throughout the body, producing side effects in other body tissues/systems such as the gastrointestinal tract (GIT). GIT side effects are explained by NSAIDs interference with the normal homeostasis role of prostaglandins (mediated by COX 1 enzyme) in maintaining gastric mucosa and regulating stomach. Side effects affecting GIT may include indigestion, nausea and vomiting, and diarrhoea and can result in ulceration and bleeding. Other side effects include rashes, photosensitivity, bronchospasm, dizziness and haematuria. NSAIDs must be used with caution in the elderly, and people with diabetes, asthma and impaired renal or cardiac function. NSAIDs are contraindicated for people with a previous history of adverse reaction, a history of peptic ulcer or clotting disorders and those taking anticoagulants or another NSAID medication.

2. Paracetamol as acetaminophen. Although it is the most widely used pain relieving medication the exact mechanism of action of paracetamol is relatively poorly understood. It is thought to act on the COX 3, a recently discovered type of COX present in the brain and spinal cord. Paracetamol has mainly anti-pyretic (reducing the levels of prostaglandins in the hypothalamus) and analgesic properties; it does not interfere with COX 2 and does not affect the other components of inflammation (swelling and redness). As paracetamol has no action on COX 1 at a therapeutic dose it has few side effects.¹ The maximum recommended daily therapeutic dose of paracetamol for adults is 4g (8 x500mg tablets). It is hepatotoxic at only 2-3 times the therapeutic dose causing necrosis of the liver and resulting in 226 deaths in 2013 in England and Wales

Aspirin also known as acetylsalicylic acid (ASA). Thromboxanes are inflammatory mediators derived from platelets that cause vasoconstriction and aggregation of platelets leading to clotting. Aspirin inhibits the production of COX 2 enzymes, which are also essential to the production of thromboxanes, inhibiting platelet aggregation and clots leading to its use in the treatment and prophylaxis of cardiovascular disease or myocardial infarction.

Compound analgesics: Step 2 on the WHO analgesic ladder – mild to moderate pain

Compound analgesics are a combination of drugs in a single tablet usually including codeine (a weak opiate) and aspirin or paracetamol. Examples include co-codamol and co-dydramol which contain codeine and paracetamol in various formulas (8/500, 10/500, 15/500, 30/500) where the first number refers to the amount of codeine and the second to paracetamol. Co-codaprin is a combination of codeine phosphate (8mg) with aspirin (400mg).

Tramaset contains a low dose (37.5mg) of the strong opioid tramadol combined with a reduced dose of paracetamol (325mg).

1. Compound analgesics may be used on their own or in combination with NSAIDs (such as ibuprofen). NSAIDs, paracetamol and opioids decrease pain via different mechanisms so used together can improve pain relief.
 2. Some low dose compound analgesics may be purchased over-the-counter (OTC) but most require a prescription.
-

Opioid medications: Step 3 on the WHO analgesic ladder – severe pain

Medications derived from morphine (or synthetic analogs) mimic the body's own analgesic system and are strongest and most effective painkillers currently available. They have a similar molecular structure as endogenous opioids (-endorphine, dynorphin and enkephalins) and produce the same effect. They work in the central nervous system by binding to opioid receptors in the pre- and post-synaptic membrane stopping the passage of neurotransmitters across the nerve synapse which blocks or attenuates the experience of pain.

Opioid medications include morphine, oxycodone, codeine, tramadol, buprenorphine, fentanyl and diamorphine (heroin). In people with chronic pain opioid medications may be given orally (as a capsule, tablet or liquid) or via a patch (transdermal). With either route slow or modified release preparations are often used to minimise fluctuations in pain relief and reduce the number of tablets that need to be administered. Modified / slow release medication also avoids people 'clockwatching' for the next dose. Examples of slow or modified release medicines which work over 12 or 24hrs include tramadol preparations such as Zydol or Zamadol. Fentanyl and Buprenorphine may be administered via transdermal patches which are applied every few days.

Opioid receptors are present in tissues throughout the body and the interaction of the drugs with these receptors is responsible for the side effects associated with opioid medications. In the GIT these include nausea and vomiting and, as a result of decreased gut motility, constipation. Opioids also reduce the sensitivity of the respiratory centres in the brain stem to CO₂ leading to respiratory depression. Other effects include drowsiness and dizziness and prolonged use can lead to hormonal changes which can lead to reduced libido, infertility and depression^{[6][7][1]}. Accidental overdose is a significant risk; the drug naloxone is used to reverse the effects of opioids and is used to treat a narcotic overdose. To avoid withdrawal symptoms opioid medications should be reduced slowly under medical guidance and not stopped abruptly.

The use of opioids for chronic non-cancer pain is controversial. Pain is rarely abolished and the use of analgesia is to enable the individual to participate in rehabilitation to restore function and maximise quality of life. Prolonged use of opioids can result in tolerance (where an increased dose of a drug is required to produce the same analgesic effect), psychological dependence and sometimes addiction and abuse. There is

evidence that people with chronic pain may not benefit from opioid use. People who use opioids for a prolonged time may develop hyperalgesia which is distinct from their original pain problem and may present as a more diffuse less defined pain. A Danish study reported significant associations between opioid use and an increase in moderate to severe pain as well as a reduction in quality of life scores and poorer self-rated health in people with chronic pain taking opioid medication versus those who were not. Using opioids was also linked to low levels of exercise, unemployment and higher health care usage. The Cochrane review found that there was no statistically significant difference in pain relief and functional improvement between strong opioids and NSAIDs for people with chronic low back pain.

Adjuvants

The WHO analgesic ladder recommends that patients are prescribed additional medication to manage the symptoms of neuropathic pain resulting from post hepatic neuralgia, phantom limb pain, peripheral neuropathy and pain caused by nerve compression e.g. severe sciatic pain, when these symptoms are not responding. These drugs include tricyclic antidepressants and antiepileptic drugs target proteins (neurotransmitters) within the cell membrane of the CNS. Because of their dual role, it is important that patients understand that they are prescribed these medications to control of troublesome pain symptoms rather than because of epilepsy or a mental health condition. NICE Guidelines CG173 recommends offering a choice of Amitriptyline, Gabapentin, Pregabalin or Duloxetine as initial treatment for neuropathic pain changing to another drug if the first is ineffective or poorly tolerated. More than one of these drugs should not be prescribed concurrently. Evidence cited by NICE suggests that compared to placebo these drugs had a significant effect on the symptoms of neuropathic pain. Although these medications are helpful and well tolerated by many people they can produce significant side effects which may result in people needing an alternative medication or declining this group of drugs

Topical analgesics

Topical analgesics can provide localised pain relief and are used to treat acute and chronic pain, such as musculoskeletal and neuropathic pain, as well as muscle pain related to trauma. They have low levels of systemic absorption which reduces the risk of side effects and limits interactions with other medications.

Local anaesthetics

Systemic local anaesthetics such as intravenous lignocaine may be used to treat chronic neuropathic pain conditions including fibromyalgia. These drugs act as sodium channel blockers. Sodium channels are thought to be only present in peripheral nerves. When nerves are damaged or irritated excitability and spontaneous firing increases mediated by the increased flow of sodium ions across the cell membrane. Sodium channel blockers slow or stop the flow of ions reducing the excitability of the cell and producing a decrease in the sensation of pain. Sodium channel blockers are also thought to have an effect on glutamate production in the dorsal horn reducing nerve cell activity.



Conclusion

The transition of care is a time when a patient is vulnerable to medication errors. As medication experts on the healthcare team, pharmacists play an integral role in ensuring the appropriate and safe use of medications. They also collaborate with other professionals between the inpatient and outpatient settings to help patients optimize their medication regimens and improve their care. Through their service as patient-care advocates, pharmacists play an important role in facilitating seamless care to promote better pain management and overall health outcomes.

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Medical 3D printing—"An Revolutionary technology in the field of medicine"

K. Saranya

Cardiopulmonary Technology-Intern, Department of Cardiology,
Faculty of Allied Health Sciences, Meenakshi Academy of Higher
Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.
Email ID: Saranya1112001@gmail.com

Abstract

Background: Advances in 3D printing also refer to as additive manufacturing are capturing attention in the health care profession due to their ability to improve treatment for several medical conditions. A radiologist as an example; would possibly create an actual replica of a patient's spine to help plan a surgical procedure or else a dentist ought to experiment a damaged tooth to make a crown that fits exactly into the affected person's mouth. In these both instances the doctors can use 3D printing technology to create products that specifically match the anatomy of the patient. This 3D printing technology is not limited only to planning surgeries or producing various customized dental restorations such as crowns; 3D printing has enabled the manufacturing of custom-designed prosthetic limbs, cranial implants or orthopedic implants consisting of hips and knees. At the same time its ability to change the manufacturing of medical and surgical products particularly high-hazard devices such as implants could affect the safety of the patient, creating new challenges for Food and Drug Administration (FDA) oversight. **Conclusion:** In this review we focus upon the how medical 3D printing is used in health care, FDA regulation for medical products that are made and what regulatory questions the organization faces. Based on this we'll discuss major ongoing development in 3D printing and other fascinating medical devices that are going to develop in this technology to enlighten medical field in the upcoming days.

Keywords: Medical 3-D Printing, Food and Drug Administration (FDA), Additive Manufacturing, Customized Medical Devices.

Introduction

Additive manufacturing commonly known as 3Dprinting was first developed in the year of 1980's and it involves taking a blue print or digital model of the subject that is then printed by using 3D technology in successive layers of an appropriate material to create a new kind of version of that subject^[1].

Unlike traditional methods in which the products are created by means of shaping a raw material into final from through carving, molding or grinding. This method of printing is an additive manufacturing technique that create an 3D object by making successive layers of raw materials such as metals, ceramics, plastics etc....^[2].

These objects are produced from a virtual document or digital file which are rendered by using a Magnetic Resonance Imaging (MRI) Technology or Computer Aided Design (CAD) drawing, which allows the manufacturers to easily adapt the product or to make changes as desired. 3D printing approaches can differ in the ways of how the layers are deposited and in the sort of different materials and substance used^[3].

A wide variety of 3D printers are available on the marketplace which are ranging from inexpensive and cheaper models, aimed at consumers and are capable of printing a small, simple parts, to commercial grade printers which produces drastically a significant larger and more complex Product. So far, most FDA reviewed products have been developedby using this 3D printing technology including medical devices such as orthopedic implants, dentistry implants and so on^[4]. Such a manufacturing approach offers a several clinical advantages, for an illustration; Medical research manufactures have used 3D printing technologies to create devices with complex geometrical structures such as knee replacement with a porous structure which can able to facilitate a tissue growth and integration^[5].

3D printing technology has a major advantage of creating a whole product or device components at once, while other manufacturing techniques may require several parts need to be separately fabricated and is screwed or welded together because of those ability and type of manufacturing technique & It does not rely on molds or several multiple pieces of specialized equipment. This can also be used for creating patients matched products based on patients' anatomy. For example-joint replacement, cranial implants and dental restoration are printed at the point of care that help guide, doctors and surgeons duringvarious medical operative procedures^[6].

The 3D printing technology-how it is regulated?

The food and drug administration (FDA) does not regulate 3D printers themselves; instead of that it regulates the medical and surgical products made via 3D printing. The type of regulatory review required relies upon the sort of product being made, the intended use of product and the potential risk which will be involved to the patients.

Medical Devices- The most common type of product which is being made using the technology of 3D printing at this time, or regulated by FDA's centre for devices and radiological health.

- ❖ These are classified into one of the three regulatory categories,
 - 1) **Class 1**-Devices which are low risk and include products such as bandages and hand-held surgical instruments.
 - 2) **Class 2**-Devices which are considered as moderate risk and it includes medical items such as infusion pump.
 - 3) **Class 3**-Devices which are considered as high risk, that comprises products that are life sustaining or life supporting, substantially significant in preventing the impairment of human health risk of illness and injury.
- ❖ A pacemaker is a best example for the class 3 devices.

FDA classifies these above said devices based on their level of risk involved and the regulatory controls necessary to give a reasonable assurance of safety and much greater effectiveness^{[7][8]}.

Medical 3D printing—“An savior in the time of crisis”

During the Covid 19 pandemic, the whole world faced a critical shortage of essential medical goods and products due to soaring demand and disruption to manufacturing and transportation. The most needed products were those in the shortest supply, were especially personal protective equipment and ventilators.

The flexibility and speed of 3D printing Technology come to the fore during this time, with many organisations putting printers to work to produce and much needed medical and surgical items. An UK-Based Organisation photosyntric was one such example. This company used its patented 3D medical printing Technology to widely and rapidly deliver millions of facial masks to protect a lot of Frontline workers.

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Like this, 3D printing Technology plays a vital role in this kind of crisis and if it has improved more means, they will surely pave the path for personalized care and high impact medical applications^[9].

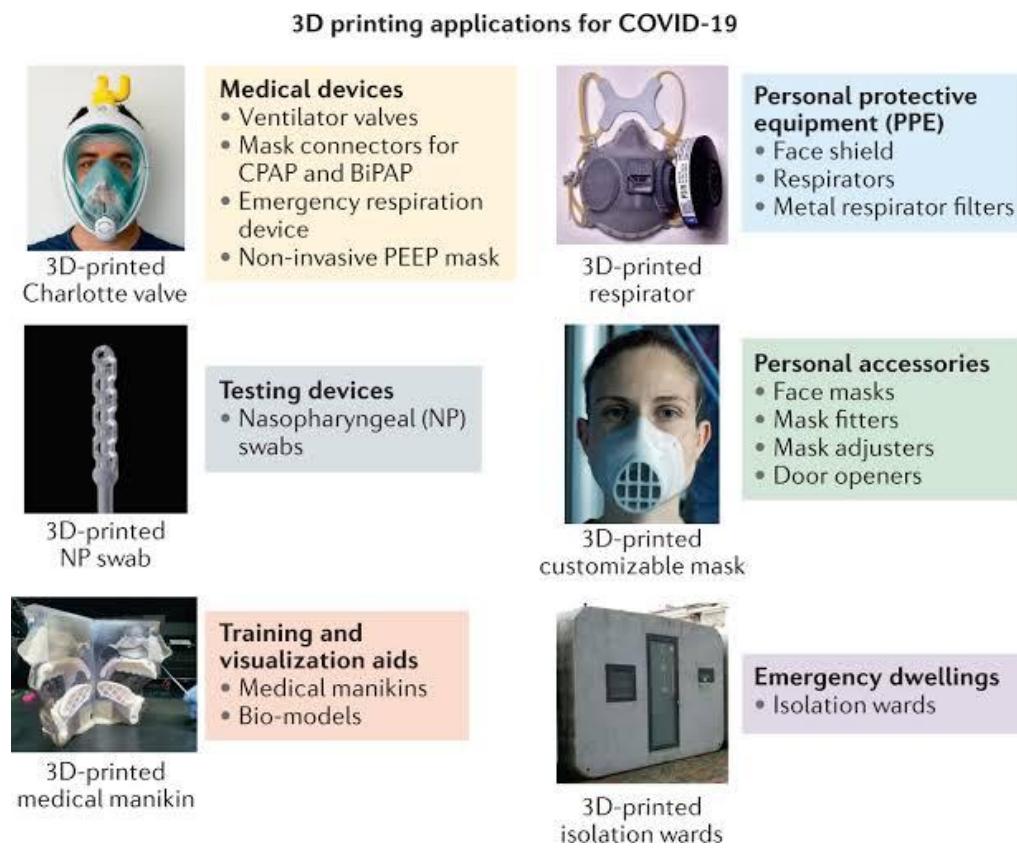


Figure 1

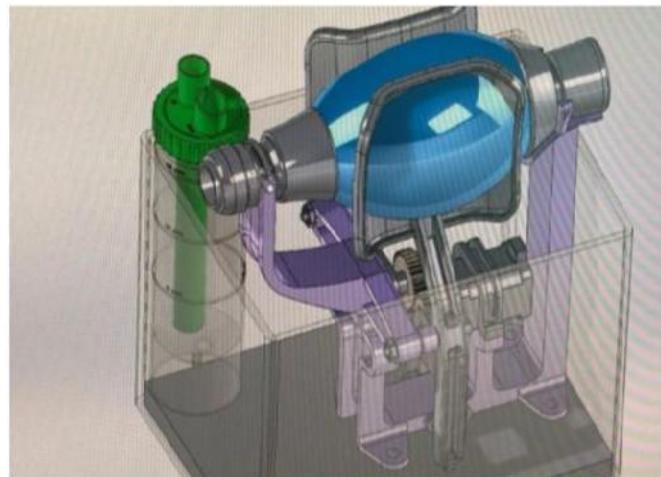
Medical 3D printing and its fascinating upcoming discoveries: -

3D printing has an enormous potential application in other product areas also, including researches underway and ongoing to use medical 3D printing technology to manufacture pharmaceutical with the potential for unique dosage form or formulations which includes those that might enable slower or faster absorption and metabolism^[9].

This could also try to make a personalized treatments that combines multiple drugs into "One Pill" or a "polypill" [9]. In addition to these researchers are using Bioprinters to create multiple cellular and tissue constructs such as skin grafts, organs and other humanoid anatomical structures.

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But this application are still in experimental phases only^{[10][11][12]}. Apart from this, the amazing evolution in medical 3D printing technology is, hospitals are already using this technology, especially most of an FDA approved machines which are used to create precise replicas of skulls, jaws, hearts, valves, fibulas,knee & hip implants and hearing aids^[13].



3-D Printed
Ventilators

Figure 2



Figure 3

Conclusion

This 3D printing technology has a significant promise in the healthcare field, particularly because of its ability to produce a highly customized product at the point of care. This technology in medical field and design need to think outside the norm for changing the field of healthcare. The three main pillars of this technology are the ability to treat more people whom were previously not feasible, to obtain outcome for patients and minimal time required under the direct case of medical specialist. Therefore, like any kind of new technology, 3D printing has introduced enormous advantages and chances of possibility in our medical field. However, it must be accompanied by an updated and current legislation in order to guarantee its correct use. But if we oversight this technology, it must be adapted in order to keep pace and ensure that the tons of benefits of this technology may outweigh the potential risks and complications^{[14][15]}.

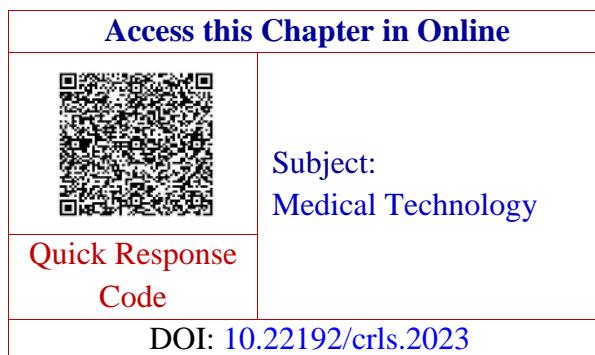
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A New breakthrough in cancer treatment – Dostarlimab ‘A miracle drug for Immunotherapy’

^{1*}**R. Tamilvanan**

Perfusionist, Department of Cardio-Thoracic and Vascular Surgery,
Faculty of Allied Health Sciences, Meenakshi Academy of Higher
Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

Corresponding Author,

^{1*}**R. Tamilvanan**, Perfusionist,

Department of Cardio-Thoracic and Vascular Surgery,
Faculty of Allied Health Sciences,
Meenakshi Academy of Higher Education and Research,
Chennai, Tamil Nadu, India.

Email ID: tamilrohit49@gmail.com

Abstract

Background: Immunotherapy is one of the main pillars of cancer treatment that has recently emerged as a beacon of hope for cancer patients. Certain immunotherapies, such as, Immune checkpoint inhibitor therapy, Monoclonal antibody have Provided extensive interest in response to their exceptional properties that activate the immune system to respond and fight against cancer cells, inhibiting their progression. In the era of rapid development in medical world, Dostarlimab, an anti-programmed cell death protein (PD-1) monoclonal antibody has amazed the medical profession by showing 100% complete cure of patients with colorectal cancer. Not only this, as a results obtained from successful clinical trials revealed that, no major side effects happenedto any of the participants in the study. Dostarlimab has also shown promising results in endometrial cancer, melanoma, ovarian cancer, breast cancer, head and neck cancertherapy. **Conclusion:** In this review, we focus upon the action of immunotherapy, especially about dorstarlimab and extensively emphasizing the miraculous therapy to activate T-cells for cancer treatment. Based on this, we discuss major ongoing clinical trials and other fascinating combination immunotherapies to enlighten future doctors and researchers about the response of dostarlimab against various cancers.

Keywords: Immunotherapy; Clinical trial; Dostarlimab; Colon cancer; PD-1 inhibitor, Drug delivery.

Introduction

Cancer remains one of the deadliest and scariest disease that humankind has ever encountered, and despite of years of research in this medical field it is still a leading health problem responsible for over 10 million deaths per year [1].

Various forms of treatment strategies have been brought to use, including treatment with drugs in chemotherapy, radiation in radiotherapy, immunotherapy and surgery. Immuno-oncology is the latest field of research in this area and its scope and full potential are yet to be explored. As a part of immunotherapy, specific parts of the patient's immune system are used to treat several range of diseases, including cancer and mostly solid tumors [2,3,4,5,6,7,8].

Cancer immunotherapy aims to re-activate the immune system, which has been suppressed by tumor cells in numerous ways. Several novel strategies involving immunotherapy are being developed to treat cancer or to minimize the associated cytotoxic effects caused as a result of different cancer therapies. Immunotherapies are very specific and once it is stimulated; they target the cancerous stem cell and even metastatic cancer, which in turn highlights their potential of reaching the smallest of tumors where surgeons might not. This also paved a way for development of cancer vaccines, which have shown potential results in minimizing tumor growth, but still fall short in complete eradication of tumour cells. Additionally, there are several antibody-based drugs are employed for cancer treatment which directly or indirectly relate to immunotherapy [9,10,11,12]

In 1986, the first immunotherapy agent, an antitumor cytokine designated interferon-alpha 2 was approved by the Food and Drug Administration (FDA) - US. N-a2 was first approved for the treatment of hairy cell leukemia (HCL) after studies showed that it had a high response rate in patients with advanced HCL. Then, in the year of 1995, FDA approved IFN-a2 for use as adjuvant therapy for stage IIB/III melanoma. When it was licensed for the treatment of metastatic melanoma and renal cell carcinoma in 1998, interleukin-2 (IL-2), a T-cell growth factor that aids in immunological modulation and T-cell proliferation, became the second anticancer cytokine approved by the FDA. Since the development of immunotherapies, a promise of revolutionizing the standard care in cancer treatment has existed and, in recent years, an effective class of immunotherapeutics known as checkpoint inhibitors has emerged as a cornerstone in the treatment for various cancers [13].

Upto now, there are several different types of immunotherapies are used to treat the cancer: immune checkpoint inhibitors, monoclonal antibodies, T-cell transfer therapy,vaccines, and immune system modulators or regulators. In addition to note that, a record number of antibody therapeutics have been granted with approval in either of the European Union (EU) or in the United States (US). In diseases like cancer, immunotherapies have drastically changed thegame for patients, since immunotherapies get the immune system properly engaged to eradicate cancer cells. For an illustration, the use of programmed cell death protein-1 (PD-1) and the cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) have shown that increased median overall survival rate and durable responses in patients across multiple tumors. Tons of people have benefited from immune checkpoint inhibitors (ICPIs); however, despite long-lasting and effective responses in a variety of tumor types, most patients either do not respond to this drug or develop resistance to the ICPIs. Additionally, the use of ICPI as a cancer treatment has the potential to cause major side effects, and therefore identification of patient populations that will benefit from ICPI as single medicines and in combination is immediately needed [14].

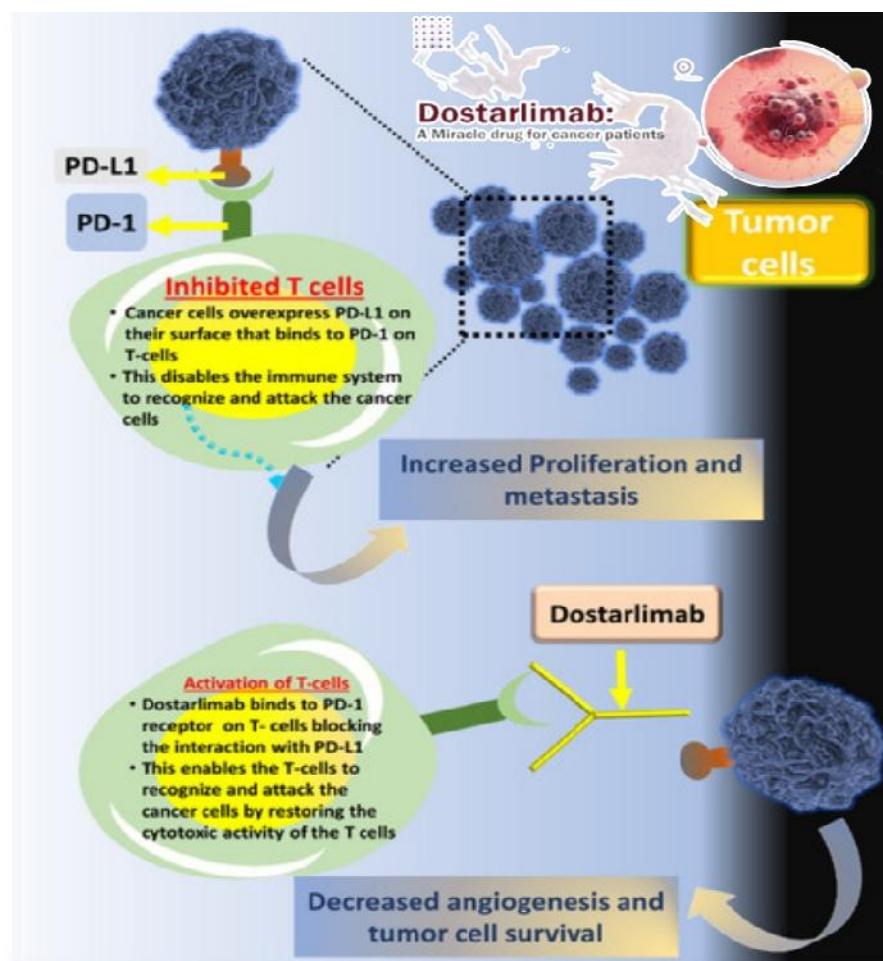
Regarding activated T-cells, PD-1 is an inhibitory immunological checkpoint receptor. It reduces the capabilities of activated effector T-cell such as Proliferation, Generation of Cytokines, and Cytotoxic activity by interacting with its ligands, Programmed cell death ligands 1 and 2 (PD-L1 and PD-L2). Then, one of the strategies through which tumor cells elude the immune system and interfere with cancer-specific immune responses is the upregulation of PD-L1. Preclinical and clinical investigations have shown that treatments that either bind to PD-1 receptor or ligand and effectively disrupt the receptor–ligand interaction can boostup the antitumor immunity and improve patient survival in a range of malignancies [15].

So far, the FDA has approved six PD-1 and PD-L1 inhibitors for clinical usage, collectively known as PD-L1. Patients suffering from cancer can choose from a variety of dose regimens, disease-specific treatments, tolerance profiles, and pricing alternatives due to the competitive environment of anti-PD-1 antibodies. On 17 August 2021, the Food and Drug Administration (FDA) has granted an accelerated approval to Dostarlimab, an monoclonal antibody, for adults with dMMR recurrent or advanced endometrial cancer that has progressed eventhough ongoing or prior treatment with the platinum-containing chemotherapy regimen, Tumors that exhibit the MSI-H or Dmmr biomarker mostly have an abnormal function of DNA repair mechanisms. Genes that should repair these problems to maintain the cell health are absent in these types of cancer. Dostarlimab, an PD-1 inhibitor, demonstrated a long-

lasting effect on dMMR tumors, and in 2022, reported a 100% remission rate for rectal cancer [16]. All patients had dMMR, a mutation present in 5 and 10% of rectal cancer cases (this mutation is also present in endometrial, prostate, and bladder tumors). This clinical trial gives a lot of hope and promises that we can match a tumor and the genetics of what is driving it, with therapy [15,16].

Mechanism of action: -

Dostarlimab (Jemperli™) or dostarlimab-gxly is a humanized monoclonal antibody (mAB) which acts as an antagonist for programmed death-1 (PD-1) receptors.



It is being developed by GlaxoSmithKline (GSK) under a license from AnaptysBio Inc for the treatment of several forms of cancer including endometrial cancer, ovarian cancer, colorectal cancer, cancer of the head and neck, small cell lung cancer (SCLC), squamous cell cancer (SCC), non-small cell lung cancer (NSCLC), fallopian tube cancer, pancreatic cancer, and many more.

According to preliminary finding results, obtained from the GARNET trial, dostarlimab has recently been approved in the year of 2021 for adults with advanced or recurrent advanced mismatch repair-deficient endometrial cancer (dMMR) in the USA and EU. The Recommended allowance dosage of dostarlimab is generally 500 mg every 3 weeks (For the first four doses) and 1000 mg every 6 weeks (After the fourth dose) is administered until the progression of disease or any unexpected toxicity has been noticed [17,18,19]





Major Milestones Of Dostarlimab against Cancer

Top 10 interesting facts about dostarlimab: -

Here are the Ten things to know about Dostarlimab, the drug that cured cancer during trial:

- 1) This clinical trial comprised a group of 18 patients. All eighteen were struggling with rectal cancer at the Memorial Sloan Kettering Cancer Center in Manhattan, United States.
- 2) In all these patients, rectal cancer was locally advanced. This means that tumours had spread within the rectum and in some cases, also to the lymph nodes, but not to other organs.
- 3) The drug named ‘Dostarlimab’ was administered to the patients for a period of six months. The medicine was given in prescribed dosages every three weeks for the allocated period of time.

4) At the end of this clinical trial, cancer was checked for and remained undetected through physical exam, endoscopy, positron emission tomography or PET scans or MRI scans.

5) This medicine costs nearly \$11,000 or Rs 8.55 lakh per dose (As per reports).

6) Dostarlimab works by the mechanism of unmasking of cancer cells, which in turn helps the immune system to identify and destroy them.

7) The rectal cancer study was inspired from Dr. Luis A. Diaz Jr. of Memorial Sloan Kettering Cancer Center led in 2017, after his clinical trial findings.

8) In this clinical trial the patients involved are, had also undergone to previous treatments to manage their cancer, including chemotherapy, radiation, and invasive surgery. Post the clinical trial, they were able to be taken off painful chemotherapy and radiation sessions.

9) After this trial, patients showed a complete absence of post-treatment complications and also any signs of recurrence of cancer in the patients until 25 months from the end of the trial.

10) This study was sponsored by the drug company named GlaxoSmithKline. [20]

Risk factors:

Before Using Dorstarlimab, we need to sort of some of the risks involved in taking this drug. This is a decision you and your physician will make. For this medicine, the following conditions should be considered:

- ❖ Allergic reactions
- ❖ Pediatric & Geriatric
- ❖ Drug Interactions or Multiple Drug reactions

Other Medical Problems: The presence of other medical problems may affect the use of Dostarlimab. So, make sure that consult your doctor if you have any other medical problems, especially:

- Colitis (inflammation of the bowels)
- Diabetic ketoacidosis
- Hepatitis (inflammation of the liver)
- Hyperthyroidism (high levels of thyroid hormone)
- Hypophysitis (inflammation of the pituitary gland)
- Hypothyroidism (low levels of thyroid hormone)

- Immune system problems
- Nephritis (inflammation of the kidneys)
- Pneumonitis (inflammation of the lungs)
- Type 1 diabetes—Use with caution. May make these conditions worse.
- Organ transplant (eg, kidney or liver transplant), recent—Use with caution. May increase risk for organ transplant rejection. [21]

Conclusion and Future prospects:

Dostarlimab mesmerized the medical field, by driving the patient's own immune system to act against the deadly disease of cancer and could potentiate the fast remission of neoplastic cells.

Generally, any invasion of pathogens in the human body turns the T-cell to “ON”, causing the immune-defense mechanism of the body to respond against them. These T-cells have proteins on their surface called immune checkpoint proteins. Most cancer cells alter these certain proteins that inactivate T-cells in response to their growth and proliferation. Thus, cancer cells switch “OFF” the immune-response button of T-cells. As a result of it, they can no longer detect, suppress and destroy the cancer cells. But, this problem can be overcome by immunotherapy, as it acts on tumors, disabling their function to act on T-cells. This in turn, enhance the ability of T-cells to immediately act against them and kill them.

Dostarlimab is one the potent immune checkpoint inhibitors that blocks the binding of PD-1 protein on T-cells to the PD-L1/2 ligands. Also it is under the trial for different cancer therapies and recently it has shown positive results and complete remission for the very first time in history of field of medicine.

The clinical trial was performed on a subset of twelve patients, whom were battling with colorectal cancer with mismatch repair deficiency (MMRd). Such kind of tumors are, however, non-responsive towards radiation or chemotherapy. Nonetheless in the above trial, the amazed result we got is, all of the 12 patients were completely cured and its suggesting that immunotherapy could turn out to be a major milestone in the history of cancer therapy.

It is essential to note that all the patients involved in this trial were at the same stage of cancer and were given no previous chemotherapy or surgical treatment. The treatment appeared to be effective within this group; however, it

is still difficult to suggest that the same response will be reported in large groups of individuals.

Furthermore, studies should be carried out at various locations with different circumstances for different types of cancers as well for better understanding of this drug. As of now, immunotherapy has not reached an established in our wider clinical market. But, in this context, the concept of nanotechnology could provide a new way of beginning in oncotherapy.

Overall, in this review we overlooked that the immunotherapeutic agent dostarlimab is a star compound against colorectal cancer and other cancers too & also how it works against cancer cells and its marvelous immunotherapeutic effects. Hence, dostarlimab seen as a miracle drug for rising hope against cancer treatment in the field of medicine.

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Biofilm formation -An overview and its control Measures

D.Sangeetha¹, V. Sumathi² and R.Ishwarya³

^{1&2} Assistant professor, Department of Microbiology, Faculty of Science,

¹E-mail: sangeethadau@gmail.com

²E-mail: sumathikarthidd@gmail.com

³ Research Scholar, Department of Microbiology, Faculty of Science
Annamalai University

³E-mail: saigayathriparis@gmail.com

Introduction

Biofilms are film-like structures generated on biotic and abiotic surfaces by the aggregation of bacterial cells. These naturally occurring biofilms pose a significant risk to humans (Wang *et al.*, 2007), accounting for 80% of bacterial infections (Stewart *et al.*, 1999). Biofilms are known to be responsible for the survival of pathogenic bacteria. Because of their role in certain infectious diseases and their development in a wide range of ecosystems in the food industry, medical equipment, and natural environments, they are extremely important for human health (Francolini and Donelli (2010); Lequette *et al.*, 2010).

Biofilms are structured aggregates of bacteria that can survive harsh environmental conditions and are resistant to host immunity and various chemotherapeutic agents (De la Fuente-Núñez *et al.*, 2013). Biofilms are communities of bacteria that are encapsulated in an extracellular polymeric substance (EPS) that is mainly composed of polysaccharides, nucleic acids, and proteins (Mayer *et al.*, 1999; Hayat *et al.*, 2019). Since infections caused by biofilm-forming bacteria are difficult to treat, there is an urgent need to find novel biofilm inhibitors. Antibiotics have a difficult time penetrating such biofilms and killing the hidden bacteria. Biofouling is a major issue in the chemical, medical, and pharmaceutical industries. Biofouling reduces the efficiency of chemical processes and is a persistent source of chronic diseases. Biofouling in drinking water treatment and distribution systems can be harmful. Pathogenic biofilms are a chronic source of disease in the human body. Biofilms are easily contaminated in medical devices and artificial organs. Since this review looks into biofilm formation, biofilm resistance mechanisms, biofilm control strategies, and the use of some natural products in biofilm control.

Biofilm-associated infections are increased in various fields and cause life-threatening infectious diseases. These biofilm producers are not responding the antibiotic treatment. At present an urgent requirement of alternative therapy or treatment to combat the biofilm producers. So, this chapter emphasizes the mechanism of biofilm formation and its control measures by the various natural compounds.

Characteristics of Biofilm Formation

The formation of biofilm is a progressive process. Primarily, bacterial cells move onto a surface and adhere reversibly to the surface. In the second step, irreversible adherence occurs with the microcolonies expansion that produces an EPS matrix. Subsequently, the progress of the mature 3-D biofilm architecture emerges. Matured biofilms are more resistant to the host immune defense and the action of antibacterial agents. During the dispersal of biofilm, the cells endure lysis and discharge from the biofilm community. Inside the host, bacteria produce biofilm on a biotic or an abiotic layer. The abiotic surface is typically coated with proteins or other biological molecules, forming a habituation film that changes cells adhesion. In biofilm formation, host cells can develop a fundamental part, and their components can be assimilated into the biofilm matrix (Lynch and Robertson, 2008; Romling and Balsalobre, 2012).

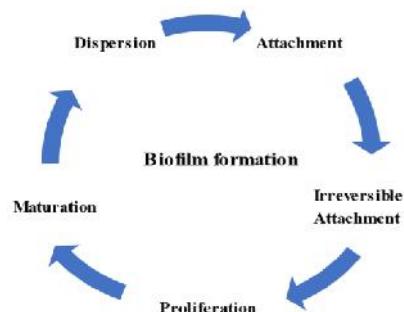


Fig:1 Basic Steps Involved in theformation of biofilm

The first two stages are highly critical in the development of biofilms and targeting one or both of these stages seems to be the ideal strategy for inhibition of biofilm formation. The attachment stage involves cytoskeletal elements (predominantly flagella, fimbriae) and lipopolysaccharides as key players. Surface signaling /communication of a group of bacteria, also termed as Quorum Sensing is a key player in the formation of biofilm.

Biofilm Mediated antibiotic resistance

Antibiotic resistance can be accomplished by the mutation of gene expression due to scarcity of the oxygen and nutrients level maintained inside the biofilm. Various phenotypic and genotypic changes are happened by the biofilm producers such as, different modulations of its actions, lipid biosynthesis, iron sequestration, DNA repair, and host immune modulation etc. (Li *et al.*, 2016). High concentrations of antibiotics are used bacteria quickly develop antibiotic resistance. There are four basic biochemical mechanisms by which bacteria resist the bactericidal and bacteriostatic effects of antibiotics:

Alteration of the antibiotic's target receptor molecule in the bacteria. Decreasing the accessibility of the antibiotics into the cell or increasing the removal of antibiotics from the cell. Destruction or deactivation of antibiotics. Generation of a new metabolic pathway by the bacteria, which is not inhibited by the bacteria (Dessen *et al.*, 2001; Hotta *et al.*, 1981). Delayed or failed penetration of antibiotics and altered bacterial growth within the biofilm, inducing resistance (Joo 2012; Fux *et al.*, 2003; Drago., 2017). Some bacteria like *P. aeruginosa* can acquire resistance via horizontal genetransfer (Gupta *et al.*, 2016). In some cases, bacteria can use multidrug efflux pumps to pump antibiotic agents out of the maturing biofilms and into the extracellular matrix, contributing to resistance (Hall 2017; Bjarnsholt *et al.*, 2013; Zhang *et al.*, 2008). Interactions between bacteria and fungi have also been found to be relevant in polymicrobial biofilms. For example, Adam *et al.* showed that a dual species biofilm of *S. epidermidis* and *C. albicans* had increased resistance to vancomycin due to a fungal matrix component that acted as a barrier to the antibiotic (Adam *et al.*, 2002).

Biofilm forming bacteria and its infections

Bacteria form biofilms as part of their survival mechanisms, and biofilms are thus ubiquitous in nature. Antoni van Leeuwenhoek observed and described biofilms using his primitive microscope on matter from his own teeth in 1683. However, medical microbiologists were uninterested in the biofilm lifestyle of microorganisms until the early 1970s, when Nils Høiby discovered a link between the aetiology of a persistent infection and bacteria aggregates in cystic fibrosis patients (Høiby 2017). Biofilms have since been identified as being involved in many clinical infections (Costerton *et al.*, 1999; Hall Stoodley&Stoodley 2009), and evidence is mounting that biofilms contribute to pathogenesis, particularly in chronic infections (Bjarnsholt 2013). Early childhood colonization of the nasopharynx by otopathogenic bacteria such as *Streptococcus pneumoniae* and non-typeable *Haemophilus*

influenzae (NT-Hi) significantly increases the risk conditions can lead to temporary or permanent hearing loss (Schilder,*et al.*,2016; Monasta*et al.*,2012; Chonmaitree*et al*.,2016).

The primary infection in endocarditis is a biofilm composed of both bacterial and host components located on the cardiac valve. *Staphylococci*, *streptococci*, and *enterococci* are the most commonly isolated microorganisms from IE cases. More than 80% of IE cases are caused by these species. Electron microscopy is used to identify biofilm in the context of endocarditis (Elgharably *et al.*, 2016). In 1987 using electron microscopy to show bacterial colonies embedded in a matrix material on valves of six IE cases before Costerton*et al.* recognised endocarditis as a biofilm infection (Marrie *et al.*, 1987).

There are various sites in the human body where biofilm infections may occur due to either a

Pre existing condition or a hospital acquired infection. Further, tissue related bacterial biofilm

Infections have been noted to occur more often in immunocompromised patients, and patients with underlying chronic illness such as cardiovascular disease, diabetes, skin barrier breakage

Cancer or especially if the infection is severe or starts early in the course of the illness. (Sivarajani *et al.*, 2018)

Wounds are damaging to living tissue caused by e.g., a trauma like cuts, abrasions, burns, and surgery, or as a consequence of underlying illnesses such as diabetes. Most Chronic wounds can be colonized with several different bacterial species whereas *Staphylococcus aureus* is most commonly isolated (Brackman *et al.*,2013). Aerobic bacteria, like *S. aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa*, are often found on the surface of chronic wounds while anaerobic species are predominant in deeper tissue (James *et al.*, 2008). The anaerobic bacteria that predominate chronic wounds of both humans and animals are *Bacteroides spp.*, *Fusobacterium spp.*, *Peptostreptococcus spp.*and *Clostridium spp.* (Percival *et al.*,2012).

Bacterial prostatitis (BP) generally presents with urinary tract infection (UTI), pain in the pelvic and genital region, and the occurrence of bacteria in expressed prostatic secretions. Similar to UTI, the species most commonly associated with BP are *E. coli*, *Proteus mirabilis*, *P. Aeruginosa*, *Klebsiella spp.* and other Enterobacteriaceae as well as *E. FAEALIS* (Yoon *et al.*,2012; Weidner *et al.*,1999; Wagenlehner *et al.*,2008). More research is

needed to understand the pathogenesis of chronic bacterial prostatitis and if bacterial persistence involves biofilm formation.

Table:1 Biofilm forming bacteria and their disease (Musk *et al.*,2006)

Biofilm forming bacteria	Biofilm Mediated Disease
<i>Burkholderiacepacian</i>	Cystic fibrosis lung infection
<i>Haemophilus influenzae</i>	Ear infection
<i>Neisseria gonorrhoeae</i>	Urethral infection
<i>Pseudomonas aeruginosa</i>	Bum wound infection, cystic fibrosis lung Infection
<i>Staphylococcus aureus</i>	Bum wounds, bacterial endocarditis, catheter Infection
<i>Staphylococcus epidermidis</i>	Nosocomial sepsis, catheter infection
<i>Steptococcusmutans</i>	Tooth decay

Biofilm inhibiting Compounds

Substantial financial and human efforts have been done in search for a new way that could facilitate the eradication of bacteria. Natural sources emerged as good candidates (Kohet *et al.*, 2013). They have been proved to have excellent antimicrobial activity (Sokovićet *et al.*, 2007), but recent studies also indicate they have very good anti-QS activity. Quorum Sensing is a key player in the formation of biofilm, the natural anti-biofilm agents either act solely or synergistically by diverse mechanisms. One of the ways by which biofilm can be controlled is through the use of antibiotics. However, due to poor and irrational antibiotics use which has made biofilm-related infections difficult to control, new biofilm control strategies are required (Banerjee ET AL., 2019).

Phytochemicals

There are broadly five classes of natural compounds that have high anti-biofilm properties. Those are phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes(Yong *et al.*, 2019). These entire compounds act on biofilm by six main mechanisms like substrate deprivation, membrane disruption, and binding to adhesin complex and cell wall; bind to proteins; interact with eukaryotic DNA; and block viral fusion (Cowan, 1999; Lu *et al.*, 2019). Researchers worked on bioactive compounds from medicinal plants for the discovery of novel natural anti-biofilm compounds.

This is normally done by evaluating the capacity of the test organism to form biofilm when the agent being evaluated is present. The concentration is chosen such that it does not interfere with other antimicrobial activities such as growth inhibition and killing. As such, sub-inhibitory concentration is normally considered for antiadhesion evaluation. A dose-dependent biofilm formation inhibition activity of methanolic extracts of Sophorasecundiflora, sphaeralcea ambigua, prosopislaevigata, opuntiaficus-indica, marrubiumvulgare, scutellariadrummondii, nothoscordum bivalve and Gutierrezia microcephala at subinhibitory concentrations against *E. coli* and *S. aureus* has been reported (Sánchez ET AL., 2016). Many plants and plant products have tested positive for interfering with quorum sensing thereby used in biofilm control.

Biosurfactants

Biosurfactants are surface active molecules that have used in research and industrial studies. They are identified as amphiphilic biomolecules produced by a wide range of microorganisms as secondary metabolites, Owning the ability of surface tension reduction like the industrial surfactants but with the advantage of being eco-friendly molecules that can be produced from renewable resources. Moreover, features associated with biosurfactants include low toxicity, biodegradability, cost-effectiveness and biocompatibility. (Varjani *et al.*, 2017). Biosurfactants (BS) hinder biofilm formation by varying the cell adhesion ability through less cell surface hydrophobicity, membrane disruption, and inhibited electron transport chain, thus restricting cellular energy demand (Satpute *et al.*, 2016). Biosurfactants are appropriate coating agents for medical implants such as urinal catheters, bone implants, etc. to inhibit biofilms originated from pathogenic organisms without using synthetic drugs. Rhamnolipids and sorphorolipids are reported to be potential agents for the inhibition of biofilms formed by Gram-negative and Gram-positive microbes (Sharahi *et al.*, 2019). This surfactant is a heteropolysaccharide, with

two functional carbonyls and hydroxyl groups, and has oil-emulsifying capacity.

Antimicrobial Peptides

AMPs are broad-acting antimicrobial agents widely used in the treatment of both fungal and bacterial biofilms (Pletzer *et al.*, 2016). These peptides disrupt biofilms developed on medical devices such as catheters, artificial valves, stents, dentures, etc. occupied in hospital-acquired infections by *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Enterococcus faecium*, *Acinetobacter*, and *Enterobacter spp.* (ESKAPE), and non ESKAPE pathogens (Rajput and Kumar, 2018). AMPs are substitute to traditional antibiotics that are less vulnerable to bacterial resistance by attacking the bacterial cell membrane (Hirtet *et al.*, 2018). AMPs occur naturally in humans, animals, plants, and microbes and act on bacterial cell membranes by interacting with membrane phospholipids electrostatically, followed by insertion into membrane, thus killing bacteria. Essential to exploit the structure of different naturally occurring AMPs to develop novel therapeutic peptides with improved stability and activity in comparison with their natural counterparts.

The biofilm is disturbed through the different AMP by the transmembrane pore mechanism, which will lead to the final condition of cell death. The study of Pulido *et al.*, 2016 reveals that the total permeabilization effect was visualized by confocal lazer scanning microscopy and Sytox green permeabilization assay. Indeed the AMP action over the membrane depolarization and permeabilization facilitates the antimicrobial and biofilm inhibitory activities.

Conclusion

Bacteria Biofilm formation takes place in a series of well-regulated events and is the most common bacterial lifestyle in most natural and man-made environments. The ability of bacteria to colonise surfaces and form biofilms is regarded as a serious issue that has been linked to negative consequences in many fields such as food, water, pharmacy, and healthcare. Various techniques and approaches for removing harmful biofilms have been developed, with the majority of them focusing on bacterial attachment interference. Furthermore, surface modification and QS signals can be used to promote beneficial biofilm formation in a variety of industrial and environmental settings. Thus, novel strategies, such as the use of antiadhesion agents or the use of natural, bacterially produced signals, are being developed to block a specific biofilm step without killing the bacteria.

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Pollution of Soil and their Effect on Soil Microorganisms

Dr. Indu Singh

P.G. Department of Zoology, K.N.I.P.S.S. Sultanpur (U.P.), India

E-mail: desiredindu@gmail.com

Introduction

As the world's population grows, so does the demand for food, which necessitates increased and sustainable food production through intensive agriculture, public health considerations, and proper use of natural resources. To satisfy this demand, agriculture must be improved with advanced agricultural technologies, and soil quality must be maintained (Jones et al., 2013). Soil quality plays a major role in today's production. Concerns about soil pollution have grown in significance in the current sense of fertilizer, waste, metals, and chemical usage, among other things. The existence of xenobiotics (human-made) chemicals or other changes in the natural soil environment cause soil contamination or pollution as part of land degradation (Gianfreda, and Rao, 2008). Industrial activity, agricultural chemicals, and excessive waste disposal are the most common causes. It's important to realize that all soils contain substances that are harmful or toxic to humans and other living things. The concentration of such compounds in unpolluted soil, on the other hand, is low enough that they do not pose a threat to the environment. The soil is said to be polluted when the concentration of one or more toxic substances is high enough to damage living organisms. Soil pollution is described as the contamination of soil with toxic chemicals or other contaminants in such a way that the soil quality is lowered and it becomes uninhabitable for living organisms (e.g. microbes, plants, insects etc.) (Varjani et al., 2019). Anthropogenic practices are primarily responsible for this increase. Heavy metals, plastics, polycyclic aromatic hydrocarbons, and other non-biodegradable wastes are considered to be major soil pollutants that have a direct impact on soil microbes and earthworms (Keshavarzi et al., 2018).

One of the main factors causing soil pollution is the ever-increasing use of chemicals such as pesticides, herbicides, insecticides, and fertilizers, which increase salinity in the soil, making it unsuitable for crop production and negatively affecting the microorganisms present in the soil, causing the soil to lose its fertility and resulting in the loss of minerals present in the soil, thus

causing soil pollution (Tsion and Steven, 2019). Other types of soil contamination typically arise from the rupture of underground storage tanks, acid rain falling onto the soil, radioactive fallout, percolation of contaminated surface water to subsurface strata, fuel leakages from automobiles, unfavorable and harmful irrigation practices, leakages from sanitary sewage, leaching of wastes from landfills or direct discharge of industrial wastes to the soil, improper septic system and management and maintenance, that get washed away due to rain and seep into the nearby soil and unhealthy waste management techniques, which are characterized by release of sewage into the large dumping grounds and nearby streams or rivers (Havugimana *et al.*, 2015).

1. Effect of heavy metals on soil microbes

Heavy metal contamination is a serious global environmental issue because it harms the growth of plants, soil indigenous species such as bacteria, earthworms, and other soil-dwelling organisms in terms of population size, diversity, and activities. Cd, Pb, As, Cu, Ni, and other heavy metals are the most harmful (Xie *et al.*, 2016). It was discovered that the impact of heavy metals on soil microflora and their usable concentration have a strong negative relationship. As a result, factors that influence heavy metal bioavailability in the soil may have an effect on the metals' toxicity to the soil microbial community. Metal pollution in the soil causes a significant decrease in microbial biomass. In certain cases, this decline occurs at low metal concentrations (Khan and Scullion, 2002). Metal concentrations near the EC in soil (Exciding concentrations-determined limits) are likely to trigger a significant microbial biomass inhibition with long-term consequences for soil productivity.

Heavy metals are refractory contaminants that are widespread and valuable. Heavy metal toxicity is mainly determined by their bioavailability, or the number of species that are ultimately absorbed into the body by absorption, migration, and transformation. High concentrations of heavy metals on the toxicity of microorganisms may have two reasons, heavy metals and microorganisms have a strong affinity, and it is easy with some biological macromolecules such as enzyme activity center (Dutta *et al.*, 2019), and electron-donating groups such as nucleic acid base, mercapto protein, and phosphate combination, resulting in the inactivation of these biological macromolecules, more than the ability of organisms to bear, resulting in biological disease and death, from a short-term perspective, heavy metal pollution will lead to the degradation of microbiological diversity of those who lack the pressure on the outside world, and at the same time lead to those who

can adapt to those pressures increased; secondly, due to heavy metals cannot be microbial degradation of the majority of ligand metallothionein. With the food chain of enrichment and transmission, a large number of metallothionein and small molecules like glycine and taurine are simple to accumulate, endangering all biological, particularly human health and life protection.

Heavy metals in the soil can affect the growth of soil microbes as well. Soil microbes are involved in almost all soil biochemical reactions, and they play an important role in the production of soil organic matter and its decomposition of harmful compounds, biochemical cycles, and the formation of soil structure. Soil microbial properties, such as the underlying soil respiration rate and enzyme activity, which are influenced by soil pH, organic matter, and other chemical properties, are negatively affected by heavy soil contamination (Stefanowicz et al., 2020). Low concentrations of heavy metal polluted soil are, in most cases, conducive to CO₂ release; high concentrations of heavy metal contamination, major inhibition of soil respiration; extreme heavy metal pollution may impair soil microbial activity, posing a serious threat to soil ecosystem function, according to studies (Shahid et al., 2017).

2. Effect of polycyclic aromatic hydrocarbon on soil microflora

PAHs (polycyclic aromatic hydrocarbons) are chemical contaminants that are volatile. PAHs have carcinogenic and mutagenic properties. It has also shown genotoxic effects (IARC, 2010). Even at low levels, PAHs have the ability to damage the environment and/or human health. High levels of PAHs in soil, in particular, may have a negative impact on total bacterial and fungal species, microbial metabolic processes, and enzyme activities. PAHs can influence microbial community composition by exerting pressure on sensitive soil microorganisms.

Polycyclic aromatic hydrocarbons (often abbreviated to PAHs) are organic compounds that:

- Contain only carbon and hydrogen atoms.
- Contain more than one aromatic ring in their chemical structures.

Naphthalene, anthracene, and phenalene are all forms of PAHs. Polycyclic aromatic hydrocarbons (PAHs) have been related to a variety of cancers. In humans, these organic compounds can cause cardiovascular diseases. Coke (coal) refining, car emissions, cigarette smoke, and shale oil production are all sources of PAHs in the soil (Aydin et al., 2014).

3. Effect of industrial wastes on soil microflora

The growing industrialization has led to soil contamination by industrial waste disposal units. There is a unique character of each type of soil. This individuality is distinguished by the profile of the soil which consists of a series of layers that vary from the percolation of waste water released into the earth to wash the contaminants down to the following horizons (Parajuli, 2019). The effluents emitted from industries into the earth are made up of several harmful substances, mineral acids, bases, etc. which, due to their preservation and adsorption, are deposited in the earth for a period of time (Vahidi et al., 2016). The mineral ingredients in the discharged effluents present in trace amounts favor the growth of certain algal, fungal and bacterial colonies which, in their turn, modify the soil texture. Plants growing in polluted soils may also pick up some of the chemicals deposited. The saprophytic soil and air microflora are attracted by organic effluents with high concentrations of biodegradable organic matter released to the soil, which can proliferate in certain instances, leading to bad outcomes or fungal conditions (Kumar and Dwivedi, 2018). Soil contamination can occur when industrial waste is discharged into the environment. The following are some common soil contaminants that can be traced back to industrial waste. Inadequate disposal of highly toxic chemical/industrial wastes will seriously pollute the soil. The accumulation of radioactive waste in deposits, for example, will result in waste being drained into the soil. This waste can also contaminate groundwater. There are a number of dangerous substances in chemical pesticides (Arias-Estévez et al., 2008).

- Chlorinated industrial solvents
- Dioxins produced from the manufacture of pesticides and the incineration of waste.
- Plasticizers/dispersants
- Polychlorinated biphenyls (PCBs)

Many petroleum hydrocarbon waste products are produced by the petroleum industry. Some of these wastes are found to be carcinogenic in nature, such as benzene and methylbenzene.

4. Effect of pesticides on soil microflora

Pesticides are pest-controlling, pest-killing, or pest-repelling agents commonly used in agriculture. They protect the plant from pests such as insects, rats, weeds, nematodes, and microbes. As we all know, the world's population is rapidly increasing, which necessitates an increase in food production. Pesticides play an important role here because they are necessary to prevent pests from destroying crop yields (Popp et al., 2013). To keep pests away from the plant, various strategies have been used over a period of time. Previously, both venomous and nutritious plants were grown in the same region. Insects were held away from the nutritious vegetation by the poisonous plants. The Ebers papyrus contains information on the preparation methods used to remove insects during ancient times, also used sulfides. Then, in the 15th century, arsenic and mercury were introduced, and they were used until 1950. Paul Muller discovered the first modern synthetic insecticide, DDT (dichloro-diphenyl-trichloroethane), an organochlorine pesticide, in the 1940s. Its widespread use is due to its efficacy against insect-borne human diseases as well as the defense of crops and livestock from insects. The adverse effects of DDT were first published in Rachel Carson's book "Silent Spring" in 1962. As a result, the US Environmental Protection Agency (EPA) issued a cancellation order in 1972, and the use of DDT was outlawed in several nations. Synthetic insecticides such as organophosphates, carbamates, and pyrethroids have started replacing DDT (Coats, 1994). Pesticides help to reduce pest-related crop losses. They also help to extend the life of the crops. This results in a higher yield percentage while still ensuring the availability of high-quality food at a fair price. Good nutrition is provided by high-quality food, which enhances one's quality of life. They also reduce the amount of fuel used to remove weeds and are effective enough to combat invasive species that pose a significant threat to the area's native species. Pesticides improve not only the farmer's financial situation, but also the agricultural economy of the region. Insects or the bacteria borne by insects cause a variety of deadly diseases in humans and livestock. Insecticides are used to kill insects save lives, minimize pain, enhance quality of life, lower veterinary and medication prices, and boost livestock yield quality and quantity. All of this contributes to a rise in person and national production and economic growth (Mahmood et al., 2006; Table 1).

Table:1 Some pesticides and their functions

Pesticide	Target & Function	Examples
Algicide	algae	Copper Sulphate, oxyfluorfen
Antifoulants	Organisms attached to underground surfaces.	Tributyltin oxide (TBTO)
Bactericides	Bacteria.	Tetracycline, streptomycin
Disinfectants and sanitizers	Disease causing microbes.	Sodium chloride
Fungicide	Fungi.	Bordeaux mixture
<u>Nematicides</u>	<u>Nematodes.</u>	<u>Methylbromide,</u> <u>chlorpyrifos</u>

Pesticides are divided into five groups by their acute oral and dermal toxicity, according to the World Health Organization. Aside from that, many pesticides (such as Aldoxycarb, Butacarb, Cycluron, Erbon, and others) have been phased out (Hun et al., 2021; Table 2).

Table-2. Classification of pesticides and some examples

Class	Oral: LD ₅₀ in body	Dermal: LD ₅₀ in body	Examples	mg/kg body	mg/kg
		weight of rat	weight of rat		
Ia-	Extremely hazardous	<5	<50	Disulfoton, Captafol	
Ib-	Highly hazardous	5-50	50-200	Dichlorvos, Triazophos	
II-	Moderately hazardous	50-2000	200-2000	Benfuracarb, Carbosulfan	
III-	Slightly hazardous	Over 2000	Over 2000	Butylate, Malathion	
U-	Unlikely to present acute hazard	5000 or more	5000 or more	Bioresmethrin, Carpropamid	

a. Effect of pesticides on soil fertility

Bacteria, fungi, actinobacteria and algae together form the soil microflora. They play a major role in the maintenance of soil fertility by actively participating in the processes of nutrient cycling as well as decomposition. Soil fertility has a direct effect on the crop productivity. Beside

soil fertility, for increasing the crop yield, crops need to be protected from pest attack and pesticides are used for this purpose. But these pesticides apart from acting upon the pests, exhibit negative effects on the soil microbes. The extent to which they affect the soil microbiota depends on the concentration applied, its bioavailability, absorption, bioactivity, toxicity and degradability along with soil's texture, organic matter, tillage system and vegetation (Meena et al., 2020) The application of most of the pesticides at field recommended dose may temporarily alter the microbial population thereby altering the soil enzymatic activities. Herbicides like acetochlor, atrazine, 2,4-D Ethyl Ester when applied initially decreases the microflora population but after certain period of time, the microbes get adapted and resistant to the herbicides(Tyagi et al., 2018). They may degrade the pesticides and use its degraded components as a source of nutrition thereby resulting in the increase in their number. Similar effect on soil bacterial, fungal and actinomycetes population is also exhibited by pendimethalin, oxyfluorfen, propaquizafop, glyphosate, paraquat, glufosinate ammonium and metsulfuron-methyl⁵⁸ (Zain et al., 2013).

High dose of pesticide when applied mostly disturbs the microbial population, for example high dose of glyphosate when applied frequently over a long period of time, affects the soil fungal communities (Vazquez et al., 2020). Algal communities are affected by pesticides like diuron, diquat, S-metolachlor and bensulfuron-methyl which act as inhibitors of various metabolic processes (Deng et al., 2012). Similarly, many insecticides when applied may also have positive or negative effect on the microbial communities present in the soil. The rate of their effect depends upon the type and the quantity of insecticides applied. Lower concentration of insecticides has a positive effect on microbial population while with increased concentration, they exert a negative effect on the microbial population. Baythroid, an insecticide, when applied exhibits a positive effect on both bacterial and fungal population (Lodhi et al., 2000). A positive effect on fungal population is also exhibited by cypermethrin. Cypermethrin when applied in combination with a fungicide called mancozeb, enhances the bacterial population. The same insecticide i.e., cypermethrin in combination with thiamethoxam affects microbes involved in ammonification, nitrification and denitrification. Fungicides like carbendzim, Copper Oxy Chloride and mancozeb have been reported to affect the fungal population (Kumar et al., 2018). Insecticides like miraj and malathion and fungicide like mancozeb affects actinomycetes population. Most of the pesticides affect microbial biomass carbon (MBC). Herbicides at half recommended rate don't have any effect on MBC but at recommended rate and double recommended rate reduces the MBC. Some

pesticides like quizalofop-pethyl also exhibit inconsistent effects on MBC (Shah et al., 2016).

b. Effect on mycorrhizal fungi

A symbiotic association between fungi and the plant roots facilitate P, NO₃ and NH₄ uptake by the plant. In this association, the mycorrhizal species enters into root cell wall and colonize in the cortex where they form arbuscules and vesicles. They also form extraradical mycelium which expands in the soil and form spores and hyphae. This association enhances the plant growth by facilitating absorption of water and minerals by the hyphae whereas the absorbed nutrients are transferred to the plant at the site of arbuscules. Pesticides when applied affects the mycorrhizal fungus in many ways. Glyphosate affects the colonization of mycorrhiza as well as the arbuscules (Helander et al., 2018). Besides having an adverse effect on root mycorrhization, it also affects hyphae, reduces spore biomass, vesicles and propagules. Alachlor reduces the mycorrhizal spores and the infection intensity. Apart from concentration, the effect of a pesticide on this symbiotic association may also vary with variation in plant species. For example, glyphosate, a herbicide exhibits a positive effect on the mycorrhizal vesicles in *Festu capratensis* but at the same time doesn't have any effect in case of *Elymus repens* (Helander et al., 2018).

Triclopyr, imazapyr and sulfometuron methyl has been reported to have no significant effect on ectomycorrhiza irrespective of the physio-chemical properties of the soil (Busse et al., 2004). The effect of pesticides may also vary with soil type. Sulfentrazone, isoxaflutole and oxyfluorfen affects the colonization of arbuscularmycorrhizal fungi in both clayey soil as well as sandy loam soil. They do not affect the viability of spores in case of sandy loam soil whereas in clayey soil, sulfentrazone increases the number of non-viable spores. Insecticides also show varying effect on arbuscularmycorrhizal fungi. Some insecticides like spinosad, pyrethrum and terpenes don't exhibit any significant effect on the diversity or colonization of mycorrhiza whereas azadirachtin affects the both. Similarly, fungicides like benomyl, bavistin and mancozeb decreases the mycorrhizal colonization as well as spore number whereas captan increases both mycorrhizal colonization as well as spore number (Channabasava et al., 2015).

c. Effect on biological cycle

Soil microbes are involved in processes of nitrogen cycle like ammonification which is an enzymatic conversion of organic nitrogen into ammonium, nitrification in which there is conversion of ammonium into nitrite

by *Nitrosomonas* and then nitrate by *Nitrobacter*, denitrification in which nitrite or nitrate are converted into gaseous form of nitrogen *i.e.*, nitrous oxide and nitrogen. The gaseous nitrogen conversion into nitrates or nitrites by diazotrophs is called nitrogen fixation. A large amount of atmospheric nitrogen is fixed by symbiotic nitrogen fixation. *Rhizobium* as well as the leguminous plant involved in this symbiotic association are generally affected by high concentration of pesticides. Glyphosate at its lower concentration enhances the nodule formation but at high concentration exhibit negative effect on it. Nodulation is also affected by higher concentration of fluchloralin and 2,4-D, methabenzthiazuron, terbutrynand linuron (Khan et al., 2006). But more detrimental effect is exhibited by metibuzin which affects nodulation even at its lower concentration.

Tetramethylthiuram disulfide (TMTD) is a fungicide which when applied at higher concentration results in nodule senescence along with reduction in the number of nodules (Gorshkov et al., 2020). The activity of nitrogenase enzyme which catalyzes the conversion of nitrogen to ammonia is also affected by the use of pesticides. Pivot SL 100 (herbicide) and Funaben T (fungicide) are among those pesticides which reduces the nitrogenase activity of *Rhizobium* (Niewiadomska et al., 2004). The process of ammonification is affected by herbicide like Mocarz 75 WG whereas the rate of nitrification and denitrification is affected by atrazine and glyphosate (Zhang et al., 2018). Pesticides also affect *Azotobacter* which is a freeliving anaerobic nitrogen fixing bacteria. They are positively regulated by pesticides like glyphosate at field dose or dose slightly higher than that of the recommended field dose but a very high dose has a negative effect on them (Adero et al., 2020). Imazetapir at field dose also doesn't have any negative impact on *Azotobacter*. But some pesticides, for example dimethoate and bayleton 50 even at their recommended dosproves to be toxic to the nitrogen fixing bacterial population (Khudhur et al., 2013).

d.Effect on enzymatic activities

Soil harbors a large community of microflora as well as many enzymes which catalyzes various reactions. Pesticides alter the soil enzymatic activities like dehydrogenase, phosphatase (acid and alkaline), β -glucosidase, urease, cellulase, aryl-sulfatase, amylase, invertase etc. which plays an important role in nutrient transformation thereby affecting the soil fertility, quality and productivity. The effect on the soil enzymatic activities depends upon the type of pesticide applied and their concentration. Dehydrogenase is an enzyme involved in respiration. When a pesticide is applied at a dose that is toxic to the

microbes, the microbial population decreases and this in turn decreases the dehydrogenase. So, we can say that the dehydrogenase activity act as an indicator of any changes in the microbial population. Imazethapyr when applied at recommended and half recommended rate, temporarily reduces the dehydrogenase activity while quizalofop-*p*-ethyl do not exhibit any effect on the enzyme. When pesticides are applied at their double recommended rate, the dehydrogenase activity is generally affected.

Phosphatase is an enzymes that removes phosphoryl group. On the basis of soil pH, phosphatase present may be acid phosphatase or alkaline phosphatase. Some pesticides like imazethapyr and quizalofop-*p*-ethyl exhibit temporarily effect on acid and alkaline phosphatase (Saha et al., 2016). The urease activity is initially increased by atrazine, pendimethalin, tembotriione and topramezone but then decreases whereas a combination of topramezone and atrazine initially decreases the activity followed by increase in activity and finally decreases the activity. Amylase and invertase activity are affected by an insecticide known as baythroid (Lodhi et al., 2000). Pesticides like propiconazole and chlorothalonil at high concentration exhibit negative effect on cellulase and invertase activities (Ramudu et al., 2011).

e. Effect on others

Soil macrofauna includes earthworms, ants, termites etc. which are involved in the decomposition of organic matter, adding and mixing of humus in the soil. This results in the increase of soil fertility. Pesticides affect these soil dwelling faunas and the effect depends on the concentration of the pesticide applied and the treatment period. The growth and survival of earthworm is affected by pesticides like propyzamide, metribuzin and benfluralin (Travlos et al., 2017). The reproductive rate is affected by herbicides like glyphosate, insecticides like imidacloprid, fipronil, thiametoxam and fungicides like captan. The cocoon production of earthworm is affected by herbicide butachlor. Mortality and mobility of termites is also affected by application of herbicides like 2,4 D and atrazine (Ejomah et al., 2020). The number of larvae and the worker ants reduces with the application of some pesticide like thiamethoxam (Schlappi et al., 2020). The brood tending ability of queen of fire ant and incipient colonies development is affected by pesticide like imidacloprid. The fecundity and the survival of queen ants is also affected by benzimidazole fungicides (Heneberg et al., 2018).

Pesticides, the crop protection shield should be used efficiently. The injudicious use of these useful substances at a concentration above the recommended dose, possesses a great threat not only to the soil dwelling micro

and macro-organisms but also to human health. The one and only way to eradicate the toxic effect of these pesticides on environment and human health is to make farmers aware of the field recommended dose of pesticides and the long-lasting consequences of their high dose.

5. Effects of soil pollution on environment

Soil contamination has a variety of negative consequences for habitats, as well as human, plant, and animal health. The adverse effects of soil contamination can be caused by direct contact with contaminated soil or by contact with other resources such as water or food grown on or in direct contact with polluted soil (Lu et al., 2015). However, unintended pesticide diffusion into the atmosphere (commonly referred to as "pesticide drift") raises a number of environmental issues, including water and soil contamination (Warren et al., 2003). Figure 2 shows some of the most significant soil pollutants.

According to pollution issues, soil pollution contributes to air pollution by releasing volatile compounds into the atmosphere, because the more toxic compounds soil contains, the more air pollution it produces. Toxic chemicals may also lead to water pollution if they leach into groundwater or if contaminated runoff or sewage, which may contain dangerous heavy metals, reaches streams, lakes, or rivers (Anju et al., 2010). These heavy metals can build up in soils to the point that they are no longer able to sustain plant life if they are applied often or in large quantities. Furthermore, soil contamination allows large amounts of nitrogen to escape through ammonia volatilization and denitrification, and the decomposition of organic materials in soil can release sulfur dioxide and other sulfur compounds, resulting in acid rain. Furthermore, acidic soils produced by the deposition of acidic compounds such as sulfur dioxide caused by the burning of fossil fuels develop an acidic atmosphere that harms microorganisms that improve soil structure by breaking down organic material and assisting water flow. Soil contamination can disrupt plant metabolism, lowering crop yields and causing pollutants to move up the food chain through trees and plants that absorb those (Peralta-Videa et al., 2009).

Acid rain pollutes soils, which disrupts soil chemistry and reduces the plant's ability to absorb nutrients and perform photosynthesis (Shu et al., 2019). Soil contamination also results in the loss of soil and natural nutrients, making it difficult for plants to survive in such soil, leading to soil erosion and disrupting the balance of flora and fauna in the region (Sylvain et al., 2011). Although aluminum is found naturally in the atmosphere, soil contamination can cause inorganic forms to be mobilized, which are highly toxic to plants and

can potentially leach into ground water, compounding their effects (Naumburg et al., 2005). Soil contamination raises salinity, rendering the soil unfit for vegetation and rendering it useless and barren. Any crops would be toxic enough to cause severe health issues in people who ate them if they were able to thrive in these conditions (Shrivastava and Kumar, 2015). Another possible consequence of soil contamination is the production of toxic dust. Furthermore, polluted soils containing high levels of nitrogen and phosphorus will leach into rivers, causing algal blooms and aquatic plant death due to dissolved oxygen depletion (Snedaker and Getter, 1985). Finally, acidic deposition in the soil will impair the soil's ability to buffer pH changes, causing plants to die off due to inhospitable conditions.

6. Conclusion: Soil microorganisms play an important role in soil health and quality by performing a variety of processes. They are essential for organic matter turnover, nutrient release, and soil structure stabilization, as well as ensuring soil fertility. Organic and inorganic pollutants such as pesticides, heavy metals, toxic hydrocarbons, antibiotics, and others may disrupt soil homeostasis. The antimicrobial activity of these chemicals can inhibit the development of soil microorganisms differentially by affecting the microbial composition of the soil, which can change the ecological role of the soil. Microbial parameters are thought to be useful measures for assessing soil fertility and quality status because of their fast response and sensitivity to pollutants. Hence, we have to develop a wholistic viable green approach to minimize the entry of pollutants into soil to preserve a valuable microbial resource in soil.

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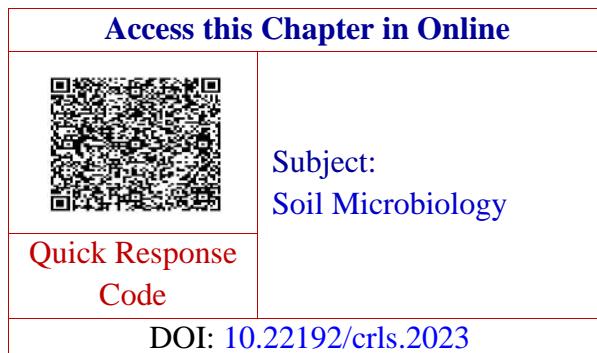
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Effect of various carbon and nitrogen sources for production of biosurfactants from fungi isolated from Victoria park reserved forest soil.

Hitakshi Maniya¹, Amrita Bhayani², Vrutti Gohel³,

Trusha Gajaria*, Kena P. Anshuman*

(phitu901@gmail.com,bhayani.amrita9116@gmail.com,

vruttigohel122@gmail.com)

corresponding author –

dr.kenaanush@gmail.com*,tgajaria10@gmail.com*

(Gyanmanjari Science College, Bhavnagar; Sir P.P. Insti. Of Sci, MK
Bhavnagar Uni, Bhavnagar, Guj, India)

Abstract

Biosurfactants are surface active compounds produced by microorganisms. Now a days increase interest in biosurfactants in recent years because of their environmental advantages over conventional surfactants. They are amphiphilic, produced in living spaces or excreted extracellular hydrophobic and hydrophilic moieties that confer on the organism the ability to accumulate between fluid phases thus reducing surface tension. The aim of this study is to investigate the production of biosurfactants by fungi (viz. *Penicillium sp.* and *Aspergillus sp.*) isolated from Victoria park reserved forest soil, Bhavnagar. Some advantages of biosurfactants are biodegradability, low toxicity, better surface and interfacial activity, anticancer activity. Several factors are influencing biosurfactants production, e.g. the nature of the carbon source, nitrogen source, the C: N ratio, temperature, aeration, pH etc. Biosurfactants have several applications in agriculture, industry, medicine, petroleum sectors, food industries and cosmetic industries. Biosurfactant production potential of test fungi was examined based on oil spreading assay. Result suggested that *Aspergillus sp.* is the potential biosurfactant producer followed by *Penicillium sp.* The Various carbon and nitrogen sources also affect the production of biosurfactants.

Key Words: Biosurfactants, Fungi, *Aspergillus sp.*, *Penicillium sp.*, oil spreading assay, carbon and nitrogen sources

Introduction

Biologically synthesized surface active agents are known as ‘Biosurfactants’. Biosurfactants produced by diverse microorganisms. Surfactants are among the most versatile material in chemical and process industry. All biosurfactants are amphiphilic in nature they consists of two parts – a polar (hydrophilic) moiety and non polar (hydrophobic) group(Shah *et al.*, 2016). It is produced in living surfaces mostly on microbial cell surfaces or excreted extracellular. Due to their amphiphilic structure biosurfactants increase the surface area of hydrophobic water insoluble substances, increases the water bioavailability of such substances and change the properties of the bacterial cell surface. Surface activity makes surfactant excellent emulsifier, foaming and dispersing agents.

Biosurfactants reducing surface and interfacial tension they reduce the repulsive forces between two dissimilar phases and allow these two phases to mix and interact more easily. The physiological role of biosurfactant production in microorganism the ability to makes substrate readily available for uptake by the cells in adverse environment condition. Some factors influencing biosurfactant production are the nature of the carbon source, nitrogen source, the C:N ratio, temperature, aeration and pH(Sena *et al.*, 2018).

Biosurfactants are mainly produced by bacteria and yeast but in recent year studies have highlighted on production by filamentous fungi as well (Gogoi *et al.*, 2016; Sena *et al.*, 2018).In this study investigated the production of biosurfactant by fungi isolated from Victoria Park Reserved Forest, Bhavnagar.

Biosurfactants were used in several industry including organic chemical, petroleum,petrochemical, mining,Agrochemical, fertilizer, food, beverages, cosmetics, pharmaceutical and many more (Desai and Banat, 1997).

Classification and properties:

Biosurfactants are categorized by their chemical composition,molecular weight, physicochemical property and mode of action and microbial origin. They are also classified according to their dissociation pattern in water.They are organic compounds belonging to various classes i.e. glycolipids, lipopeptides, fatty acids, phospholipids, neutral lipids and lipopolysaccharides(Rosenberg,1986; Desai and Banat, 1997).

Various carbon and nitrogen sources play very important role in biosurfactant production. The quality and quantity of biosurfactants production are affected and influenced by the nature of carbon substrate.Nitrogen is

important in the biosurfactant production medium because it is essential for microbial growth as protein and enzyme synthesis depends on it. In our study different carbon and nitrogen compounds have been used for the production of biosurfactant such as soybean, sucrose, starch, peptone, yeast extract and ammonium nitrate.

Screening methods for biosurfactants efficiency(Amalesh *et al.*,2012):

There are many methods for the screening of biofurfactants efficiency, these are as follows:

Hemolytic activity:

The isolated strain was streaked on the blood agar plate and plate was incubated at 37°C for 48 to 72 hours. After incubation halo zone around the colonies indicate positive result. Zone is classified as alpha, beta and gamma. -hemolysis was observed when colony showed greenish zone around its inoculation, -hemolysis was observed when a clear white zone was observed around the inoculated colony and -hemolysis was recorded when there was no change around the colony.

Drop collapsing test:

Drop collapsing test is the qualitative process useful for the screening of biosurfactants. The isolates were placed on the surface of hydrocarbon. The collaps of hydrocarbon indicate positive result. A drop of water used as a control.

Oil spreading assay:

On empty petri plate two different layers were formed. The first layer is of water and second layer is of hydrocarbon. The 24 hrs old cell free extract broth (Filtrate) of isolate is added on petri dish. The clear zone formation around the culture indicates positive result. Measure the diameter of clear zone. A drop of water used as a control.

Emulsification index test:

Emulsification index is the quantitative process. Add 2ml of hydrocarbon in test tube along with 2ml of 48 hrs grown culture broth. Then it was vortex for 2 min and allowed to stand by 24 hrs. After 24 hrs of incubation emulsification index was calculated according to standard methodologies.

Applications of biosurfactants (Desai and Banat,1997;Nguyen *et al.*, 2008; Fakruddin M 2012, Banat *et al.*, 2014):

-) Biosurfactant used in agriculture, enhance the solubility of hazardous chemical compound such as PAH.
-) Biosurfactant used in commercial laundry detergents shows good emulsion formation capability with vegetable oils.
-) Biosurfactant used as biopesticide: Biosurfactant produced by several bacteria exhibit insecticidal activity against fruit fly drosophila.
-) Biosurfactant also used in medicine: Antifungal, Antiviral, Antimicrobial activity, Biosurfactant exerts its toxicity on the cell membrane permeability.
-) Anticancer activity
-) Anti adhesive agents
-) Immunological adjuvants
-) Gene delivery
-) Biosurfactant in food processing industry
-) Biosurfactant in cosmetic industry.
-) Biosurfactant in petroleum.
-) Biosurfactant in microbial enhanced oil recovery.
-) It is low toxic and non hazardous compound
-) It has high Biodegradability
-) Biocompatibility and digestibility which allow their application in cosmetic, pharmaceutical and as functional food additive.

Materials and methods:

Isolation of biosurfactantproducing fungi:

The soil samples were collected from the different places of Victoria park reserved forest, Bhavnagar. The soil samples were plated on potato dextrose agar (PDA). After five days of incubation at room temperature colonies were examined. The fungal growth were identify by Morphological and microscopically.

Media and Growth condition (With various carbon and nitrogen sources):

The selection of biosurfactant producers were performed in Erlenmeyer flask containing 100 ml of culture medium with various carbon and nitrogen source such as soybean, sucrose, starch, peptone, yeast extract, sodium nitrate (1.5%).The medium was inoculated with spores of isolates at a concentration of $\sim 2 \times 10^6$ spores/ml (Sena *et al.*, 2018).

Then the flasks were incubated in static condition (without shaking) at room temperature for 5 days. Fungal biomass removed by filtration with

whatman filter paper. The filtrate was separated for using detection of biosurfactant by Oil spreading assay.

Oil spreading assay:

For this assay 50 ml of distilled water was taken in sterile petridish and 20 μ L crude oil was added to the surface of distilled water to form thin oil layer. Then 10 μ Lof culture filtrate are gently placed on the centre of the oil layer. If biosurfactant is present in the filtrate the oil is displaced and clear zone is formed (Morikawa *et al.*, 2000; Youssef *et al.*, 2004).

Results and Discussions:

The fungi were isolated on PDA from the soil collected from Victoria park reserved forest. Among this two isolates of *Aspergillus sp.* and *Penicillium sp.* were used for further screening of biosurfactant producers.

Different Carbon and Nitrogen sources were taken for biosurfactant production, such as Soybean, sucrose, starch, peptone, yeast extract and sodium nitrate (Table-1). In optimize condition the fungal isolates showed different growth(Fig.1).



Fig. 1-Fungal growth with different Carbon and Nitrogen sources

Table – 1– Various carbon and nitrogen sources with fungal isolates:

Source	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>
Soybean, peptone	P1	A1
Soybean, yeast extract	P2	A2
Soybean, sodium nitrate	P3	A3
sucrose, peptone	P4	A4
sucrose, yeast extract	P5	A5
sucrose, sodium nitrate	P6	A6
starch, peptone	P7	A7
starch, yeast extract	P8	A8
starch, sodium nitrat	P9	A9

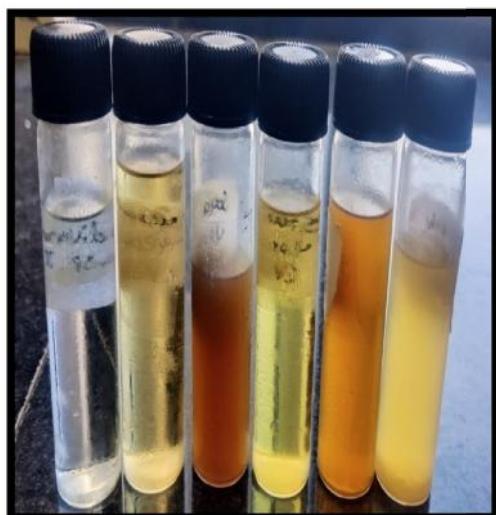


Fig.2 Culture filtrate

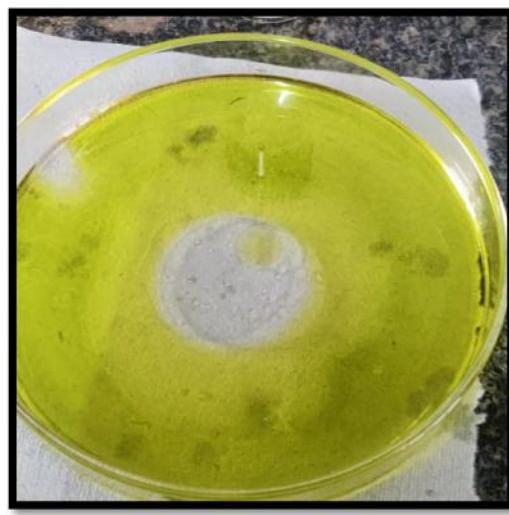


Fig.3 - oil Spreading Assay

The dry biomass showed the growth efficiency in different medium. The filtrate was used for oil spreading assay (Fig.2). The different values were obtained of oil spreading assay which was shown in Table-2.

Table – 2– Oil spreading assay of fungal isolates.

<i>Penicillium sp.</i>	Oil spreading assay
P1	-ve
P2	+ve
P3	-ve
P4	-ve
P5	-ve
P6	-ve
P7	-ve
P8	+ve
P9	-ve
<i>Aspergillus sp.</i>	Oil spreading assay
A1	+ve
A2	+ve
A3	+ve
A4	-ve
A5	+ve
A6	+ve
A7	-ve
A8	+ve
A9	-ve

From the above results we can conclude that among the two test fungi *Aspergillus sp.* and *Penicillium sp.* can produced biosurfactant which was revealed by the respective assay showed clear zone (Fig.3).

The oil spreading activity was observed highest in the *Aspergillus sp.* in isolates media A1, A2, A3, A5, A6 and A8. And it is also observed in *Penicillium sp.* in isolates P2 and P8. Maximum positive results were evaluated in *Aspergillus sp.* by performing oil spreading assay. Based on these findings *Aspergillus sp.* is the potential biosurfactant producer followed by *Penicillium sp.*

The Varying carbon and nitrogen source was also affect the growth of fungi and production of biosurfactants. For *Aspergillus sp.* the Soybean, yeast extract, Sodium nitrate, peptone and Starch were the substrate of choice for biosurfactant production. And for *Penicillium sp.* soybean, yeast extract and starch were the substrate of choice for biosurfactant production. Thus, yeast

extract, soybean and starch are chief ingredients to enhance efficiency of biosurfactant production.

Several fungi like *Aspergillus sp.*, *Penicillium sp.*, *Fusarium sp.* etc were produced biosurfactants (Ferreira *et al.*, 2016; Sena *et al.*, 2018).

Sena *et al.*, 2018 isolated fungi from Amazon forest and checked for biosurfactant production with various carbon and nitrogen sources. Result showed soybean oil is best carbon source for biosurfactant production and yeast as nitrogen source. Our results support to their findings.

Mineral nitrogen source sodium nitrate was not support biosurfactant production. While, in our study sodium nitrate in combination with soybean produced biosurfactant by *Aspergillus sp.* but not with *Penicillium sp.*. More over yeast extract also one of the important nutrients for both fungi for production of biosurfactant.

Our result also support Batista *et al.*, 2006 were glucose was better carbon source for screening of biosurfactant. Accorsini *et al.*, 2012 also used soybean oil and glycerol as low cost substrate for biosurfactant production from yeast.

Thus, various carbon and nitrogen sources enhance biosurfactants production. Other factors like temperature, pH, aeration or agitation, salt etc could be used to enhance production.

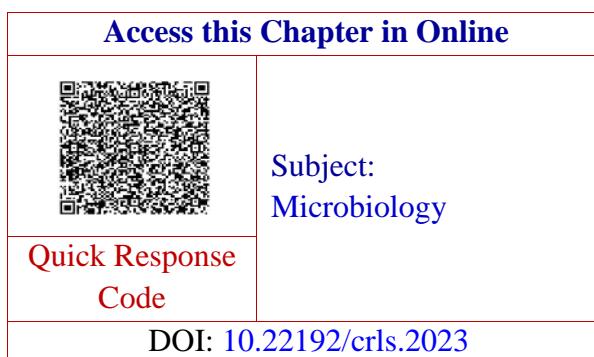
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Effect of Methylene Blue dye on Pathogenic Microorganisms

Hirva Shah¹, Kajal Swain¹, Rohan Bambhaniya¹ and
Kena P. Anshuman*

Sir P. P. Institute Of Sci., M.K. Bhavnagar University, Bhavnagar,
Gujarat, India

(hirva2104@gmail.com,kalpeshswain8310@gmail.com,rohanbambhaniy07@gmail.com,dr.kenaanshu@gmail.com *)

Abstract

This qualitative research study was conducted to illustrate the effect of methylene blue, a dye on pathogenic microorganisms. In 2019, methylene blue was used in the treatment of Covid-19 as it has potent antiviral activity against SARS-COV 2. In this study, the antibacterial assay of pathogenic organisms were carried out against gram negative bacteria (*E.coli*, *Proteus vulgaris*, *Enterobacter aerogenes*) and gram positive bacteria (*Staphylococcus aureus*) through disc diffusion technique using different concentration of methylene blue (0.1%, 0.3%, 0.5% and 1%). The zone of inhibition shows the susceptibility of the bacteria towards the methylene blue. Based on antibacterial assay result, methylene blue showed antibacterial activity. Result suggested that *E.coli* has least zone of inhibition at 0.3% methylene blue concentration, while maximum with *Enterobacter aerogenes* and *Proteus vulgaris* at 1% concentrations. But methylene blue has beneficial as well as side effects too. Thus study showed a significant killing effect on bacteria and significantly increased its antibacterial activity.

Key words: Methyleneblue, dye, antimicrobial effect, pathogenic microorganisms, COVID-19

Introduction

Methylene blue is an oxidation reduction agent. It has chemical formula C₁₆ H₁₈CIN₃S. It was synthesized in 1876 as an aniline-based dye for the textile industry (Berneth, 2008), but Robert Koch and Paul Ehrlich were realize its potential for use in microscopy stains (Ehrlich, 1881; Oz *et al.*, 2011).

Methylene blue was the first compound to be administered to humans and was shown to be effective in the treatment of malaria in Africa (Guttmann

& Ehrlich, 1891; Oz *et al.*, 2011). It was also the first synthetic compound used in clinical therapy as an antiseptic and the first antiseptic dye to be used therapeutically.

Applications of Methylene blue:

1. One of the most common clinical applications of methylene blue is for the treatment of methemoglobinemia, a condition in which blood loses its oxygen carrying capacity. In this disease methylene blue acts by reacting with RBC and form leucomethylene blue, which is a reducing agent of oxidized haemoglobin converting the ferric ion (Fe^{+++}) back to its oxygen carrying ferrous state (Fe^{+}) (Sills and Zinkham, 1994).
2. It is used in human and veterinary medicine for some therapeutic and diagnostic procedures, including as a stain in bacteriology, as a targeting agent for melanoma, as an antihaemoglobinaemic, as an antidote etc.
3. It is used as antiseptic and antimicrobial agent in *Staphylococcus aureus* and HIV-1 infection (Floyd *et al.*, 2004) and as disinfectant too (O'Neil *et al.*, 2006; Oz *et al.*, 2011).
4. It is also used in hypotension, an antiseptic in Urinary tract infection (UTI) infections, treatment of hypoxia and hyperdynamic circulation in liver cirrhosis and severe hepatopulmonary syndrome.
5. It is also used as redox indicator, peroxide generator, in water testing and in agriculture etc

Side effects of Methylene blue:

1. In humans, large intravenous doses of methylene blue (~500 mg) have been reported to cause nausea, abdominal and chest pain, cyanosis, methemoglobinemia, sweating, dizziness, headache, and confusion (Clifton and Leikin, 2003; Oz *et al.*, 2011).
2. Toxic effect in infants exposed to methylene blue during prenatal or perinatal diagnostic or therapeutic procedures is well documented: hyperbilirubinaemia, haemolytic anaemia, formation of Heinz bodies, erythrocytic blister cells, skin discolouration and photosensitization are the most common adverse effects (Sills & Zinkham, 1994; Cragan, 1999).

Here we have tested different concentration of Methylene blue with four pathogenic test organisms like, *E. coli*, *Enterobacter aerogenes*, *Proteus vulgaris* and *Staphylococcus aureus*.

General characteristics of microorganisms(Upasani, 2010):

1. *Escherichia coli*

Taxonomy:

Family I: *Enterobacteriaceae*

Genus: *Escherichia*

Species: *coli*

Habitat:

E. coli is common inhabitant of human intestinal tract (colon). It is also found in water, food, soil and sewage contaminated with fecal matter. Hence *E.coli* is used as an indicator organism for fecal contamination.

General Characteristics:

E. coli is Gram-negative, short rods, facultative anaerobic, motile (peritrichous flagella), non sporulating, maybe non-pathogenic or pathogenic. Many strains are fimbriated.

Pathogenicity:

E. coli cause Urinary tract infection (UTI) like cystitis, pyelonephritis, glomerulonephritis, gastroenteritis, traveller's diarrhea, infantile diarrhea, septicaemia.

2. *Enterobacter aerogenes*:

Tanonomy:

Family I:*Enterobacteriaceae*

Genus: *Enterobacter*

Species: *aerogenes*

Habitat:

They are found in humans and animal, as well as in soil, water, dairy products and sewage.

General Characteristics:

Enterobacter sp. is gram negative, rod-shaped, facultative anaerobic, non-sporulating, non-motile and encapsulated bacteria.

Pathogenicity:

Enterobacter sp. acts sometimes as a secondary pathogen. *Enterobacter aerogenes* and *Enterobacter cloacae* can cause wound infections, nosocomial infections and urinary tract infections (UTI).

3. *Proteus vulgaris*

Taxonomy:

Family: *Enterobacteriaceae*

Genus: *Proteus*

Species: *vulgaris*

Habitat:

Proteus vulgaris is commonly found in the intestine of humans and some animals. It may also be found in the soil and sewage.

General Characteristics:

P. vulgaris is Gram negative, short rods, facultative anaerobic, non-sporulating, non-capsulated, which may be pleomorphic, motile (peritrichous flagella).

Pathogenicity:

P. vulgaris is the primary causative agent in case of urinary tract infections in the human beings. The pathogen promotes stone formation by development of alkalinity due to its urease activity. The stone encourage the infection by retention of urine in the tract and causes cystitis, pyelitis, pyelonephritis.

Some strains also causes secondary infections like wound infections, hospital acquired infections and summer diarrhea in infants.

4. *Staphylococcus aureus*

Taxonomy:

Family: *Staphylococcaceae*

Genus: *Staphylococcus*

Species: *aureus*

Habitat:

Staphylococcus aureus are common inhabitants of nasal secretions and on the skin. This organism is most frequently associated to various superficial and/ or deep seated infections.

General Characteristics:

S. aureus is Gram positive cocci, aerobic or facultatively anaerobic, occurring in grape like clusters. They are non-motile, non-sporulating and non capsulated (in fresh cultures the cells may show presence of capsule). They can survive over a wide temperature range 10-42 °C and tolerate comparatively high concentrations of NaCl (up to 7%) and low moisture.

Pathogenicity:

S. aureus produces many toxins that are responsible for its ability to invade the body or damage tissue. The organism is an opportunistic pathogen. The organism is associated with respiratory tract infections, cutaneous infections like boils, abscesses, furuncles, carbuncles etc. They also cause deep infections like osteomyelitis, otitis, endocarditis, mastitis. Most infections are pyogenic. It also causes food poisoning by releasing the enterotoxin in the food causing vomiting and nausea when ingested. *S. aureus* produces a toxin that causes toxic shock syndrome. It can cause surgical wounds infection. The virulence is mainly due to its ability to produce , and haemolysins, leucocidins, enterotoxin and certain enzymes.

Materials and Method:

Young culture: Prepare 24hrs young culture of *E. coli*, *Enterobacter aerogenes*, *proteus vulgaris* and *Staphylococcus aureus*

Antimicrobial activity by disc method:

1. Inoculate 0.2ml of young test cultures in melted nutrient agar previously cooled to 50°C.
2. Mix well and pour it in to the sterile empty petridish.
3. Allowed it to solidify.
4. Deep disc in different concentrations of Methylene blue dye.
5. Put discs in the centre of the petri dish and press gently.
6. The plates kept in the refrigerator at 4°C for 20 mins.
7. Incubate all the plates in upright position at 37 °C for 24 hour.
8. Next day observe the zone of inhibition and measure the zone size.

Table.1 - Zone of inhibition of Methylene blue with different Microorganisms

Sr No.	Microorganism	Zone of inhibition at different Methyleneblue concentratin			
		0.1%	0.3%	0.5%	1%
1	<i>E. coli</i>	—	10mm	12mm	14mm
2.	<i>Enterobacter aerogenes</i>	—	12mm	14mm	15mm
3.	<i>Proteus vulgaris</i>	—	11mm	13mm	15mm
4	<i>Staphylococcus aureus</i>	—	11mm	12mm	14mm

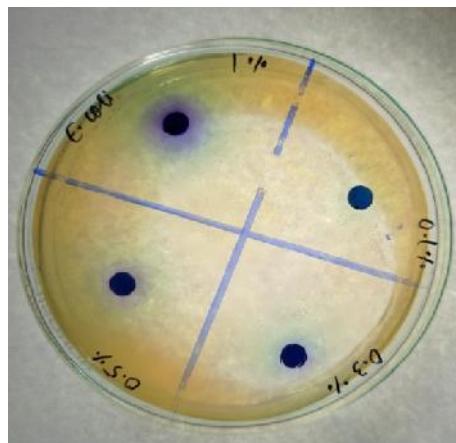


Fig.1 – Zone of inhibition with *E.coli*

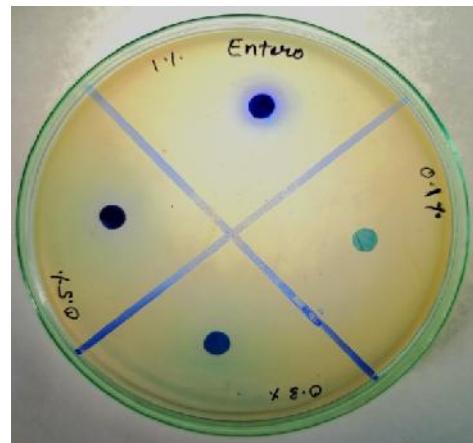
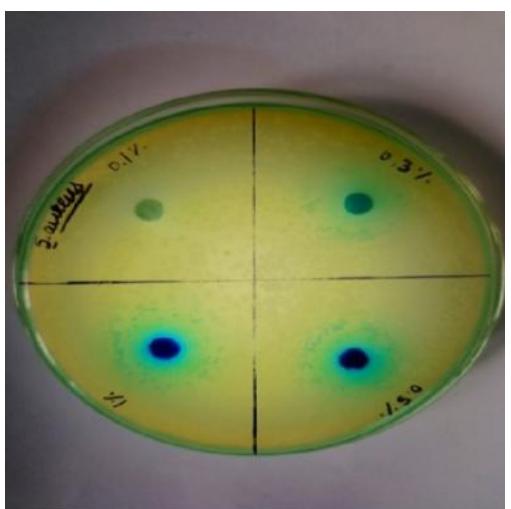
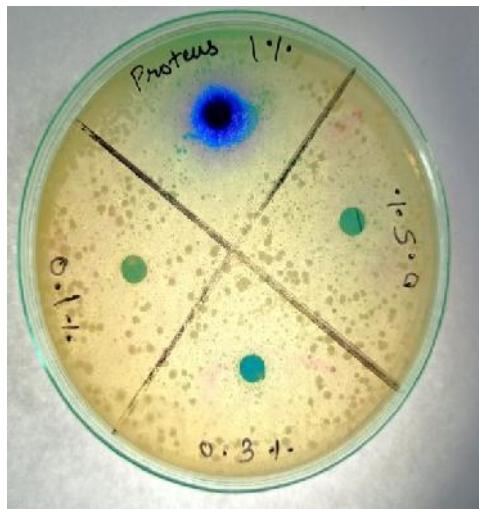


Fig.2 – Zone of inhibition with *Enterobacter aerogenes*

**Fig.3 – Zone of inhibition with *S. aureus*****Fig.4 – Zone of inhibition with *proteus sp.***

Results and discussions:

Result suggested that *Escherichia coli* has shown zone of inhibition with different concentrations of methylene blue 0.3%, 0.5% and 1% i.e. 10mm, 12mm and 14mm respectively. While *Enterobacter aerogenes* has shown zone of inhibition at 0.3%, 0.5% and in 1% methylene blue concentrations, i.e. 12mm, 14mm and 15mm respectively.

Zone of inhibition of *Proteus vulgaris* with different methylene blue concentrations were 11mm, 13mm and 15mm and *Staphylococcus aureus* has shown zone of inhibitions were 11mm ,12mm and 14mm at 0.3%, 0.5% and 1% concentrations respectively.

As shown in Table-1, Fig. 1,2,3,4 result suggested that *E.coil* has least zone of inhibition at 0.3% methylene blue concentration, while maximum with *Enterobacter aerogenes* and *Proteus vulgaris* at 1% concentrations i.e 15mm. So methylene blue has shown antimicrobial effect. No zone of inhibition observed with any isolates at 0.1% methylene blue concentration. But still Minimum Inhibition Concentration (MIC) is required to determine its concentration. The range and the degree of drug/antibiotic sensitivity of an organism varies (sensitive, moderately sensitive and resistant). It has to be checked especially for pathogenic strain, so that proper drug and dosage can be chosen by the doctor for treatment of infection. The drug sensitivity profile is also useful for taxonomic and epidemiological studies.

Methylene blue is safe when used in dose of <2 mg/kg. Methylene blue may be a treatment option for Corona virus disease of 2019 especially when combined with Non steroid anti-inflammatory medicines. It inhibits nitric oxide formation by inhibiting nitric oxide synthase and promote oxygen saturation and it also inhibits cytokine production because cytokine activate nitric oxide synthase (Dabholkar *et al.*, 2021; Ginimuge and Jyothi, 2010; Ghahestani *et al.*, 2020). It may also slow down the progression of the Alzheimer's disease (Oz *et al.*, 2009).

Methylene blue is cheap and easily available. But simultaneously it has many side effects too. So it's better to study individual persons history and then to use as antimicrobial agent.

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Immunohematology

N.Uma Maheswari and M. Agathiya

PG and Research Department of Microbiology.
STET Women's (Autonomous) College, Sundarakkottai,
Mannargudi, Thiruvarur, TamilNadu, India.
Email id: umasamyamf@gmail.com

Introduction

Immuno-hematology is the study of immune system of the blood. It encompasses the study of antigens expressed on surface of erythrocytes (RBC) that controls blood grouping in humans. The knowledge from immunohematological studies help us to understand principles for blood transfusion required due to accident, disease or surgery, graft acceptance or rejection, disorders resulting from blood group incompatibilities. The International society of blood transfusion now recognizes 285 specificities, 245 of which belong to one of 29 blood group systems.

Table 1: Human blood group systems

No	Blood group	Representa-tion	No.of antigens	Gene	Located in chrome
1	ABO	ABO	4	ABO	9
2	MNS	MNS	43	GYPA, GYPB TYPE	4
3	P	P1	1	R1	22
4	Rh	RH	49	RHD RHCE	1
5	Lutheran	LU	20	LU	19
6	Kell	KEL	25	KEL	7
7	Lewis	LE	6	FUT3	19
8	Durry	FY	6	FY	1
9	Kidd	JK	3	SLC14AI	18
10	Diego	DI	21	SLC4AEI	17
11	Yt	YT	2	ACHE	7
12	Xg	XG	2	XG, MIC2	X/Y
13	Scianna	SC	5	ERMAP	1
14	Dombrock	DO	5	DO	12

15	Colton	CO	3	AQPI	7
16	LandsteinerWiener	LW	3	LW	19
17	Chido/Rodgers	CH/RG	9	C4A,C4B	6
18	H	H	1	FUT2	19
19	Kx	XK	1	XK	X
20	Gerbich	GE	8	GYPC	2
21	Cromer	CROM	12	DAF	1
22	Knops	KN	8	CR1	1
23	Indian	IN	2	CD44	11
24	Ok	OK	1	CD147	19
25	Raph	RAPH	1	CD151	11
26	John Milton Hagen	JMH	1	SEMA7A	15
27	I	I	1	GCNT2	6
28	Globoside	GLOB	1	B3GALT3	3
29	Gill	GIL	1	AQP3	9

ABO blood groups

Blood groups were discovered in 1900 by Landsteiner. Initially individuals with A,B,O Blood groups were reported. Later in 1902, AB blood group was discovered by Sturli and decastelo.

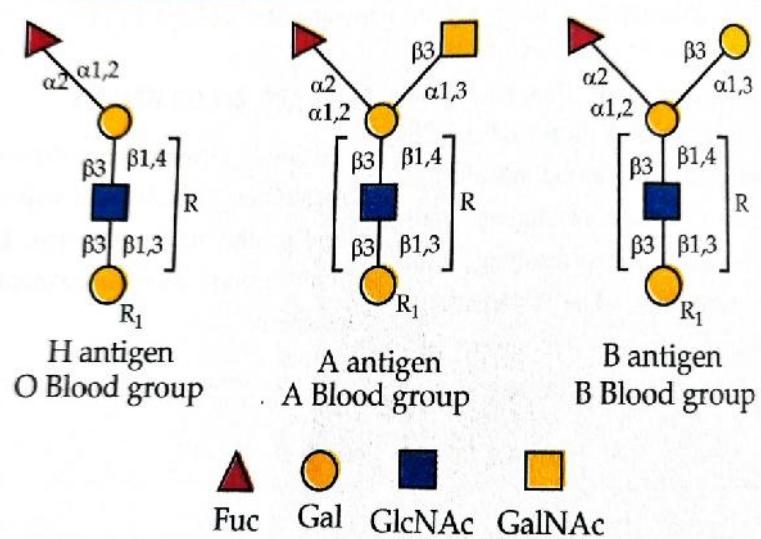


Fig.1: Structure of A,B and O oligosaccharide antigens

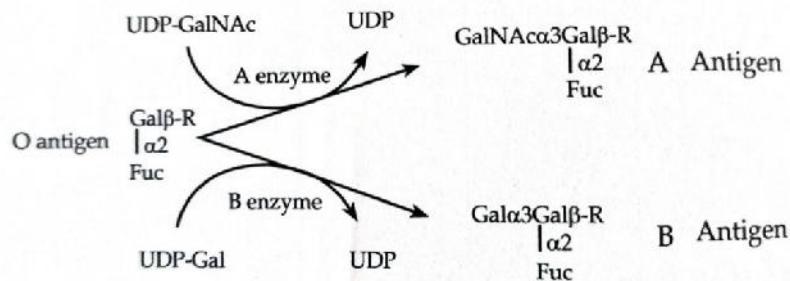


Fig.2:Biosynthetic pathways for conversion of H determinants to A or B determinants, R=represents the core structure.

Antigens

ABO blood groups in human are attributed to the expression of glycoproteins on the cell surface of red blood cells. An individual expressing the A blood group has the A antigen expressed on its RBC. Similarly an individual with B blood group has the B antigen expressed on its RBC. Individual with AB blood groups has both A and B antigens expressed on its RBC. Individuals with O blood groups, do not express either A or B antigens .

Nature of antigens

ABO antigens are proteins molecules with terminal sugar residues. The antigens differ from each other in the arrangement of sugar residues .An additional N acetylglucosamine linked to the terminal galactose constitute the A antigen.Presence of an additional galactose on the terminal residues constitutes the B antigen.

Antibodies

Individuals have antibodies in the serum to the complementary antigens.Antibodies to the ABO blood group are usually of IgM type however IgG is also found.A blood group individual has antibodies against 'B' antigen in their Sera.'B' blood group individuals have antibodies against 'A' antigen in their Sera,.An individual with 'AB' blood group has no antibodies in their Sera against 'A' or 'B' antigen.Individuals with O blood group have antibodies against both A and B antigens in their Sera.

	A	B	AB	O
Red blood cell type				
Antibodies present				
Antigens present				None

Fig.3: ABO blood group

Genetics of blood groups

Multiple alleles are involved in control of blood groups. The genes controlling the A and B blood group are dominant over O blood groups. However both A and B genes express co-dominance in AB blood groups. There are three alleles A,B and O at the ABO locus on chromosome 9. Thus the genes for A blood group is either I^aI^a or I^aI^o and B blood group is either I^bI^b or I^bI^o . However in AB blood group I^aI^b is expressed. ABO locus is on the long arm of chromosome 9. It contains 7 exons that span more than 18kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most O alleles and results in a loss of enzymatic activity.

Blood transfusions and ABO blood groups

Blood grouping: Human blood contains antibody in the serum that reacts with corresponding blood group antigen. An antibody that reacts with antigen A is termed as anti-A antibody and an antibody that reacts with antigen B is called anti-B antibody. A individuals do not have anti-A antibody and B individuals do not have any anti-B antibody. AB individuals do not have either anti-A or anti-B antibody and O individuals have both anti-A and anti-B antibodies. RhD is an important antigen in determining blood. The term “positive” or “negative”

refers to either the presence or absence of the RhD antigen in the individual. Anti-RhD antibody does not occur naturally as compared to anti-A and anti-B antibodies.

The ABO blood grouping method is based on the principle of agglutination. A drop of blood of an individual is reacted with known monoclonal antibodies and the agglutination reaction is observed for. If the antigen is present in the RBC of the individual, then adding corresponding antibody to it will cause agglutination/clumping of blood. Thus if the blood agglutinates with anti-A antibody, the blood group is A and if it agglutinates with anti-B, then it is B blood group. On the other hand, if agglutination is seen with both anti-A and anti-B, it is Group AB and if there is no agglutination it is Group O.

While bloodgrouping if the blood agglutinates in presence of anti-RhD antibody, it is denoted as positive and if the blood does not agglutinate, it is denoted as negative.

ABO incompatibility during transfusion reaction is due to the presence of antibodies in the serum that are complimentary to the antigens. When a patient of blood group A is given blood from a patient of blood group B, the anti B antibodies in the Sera of the recipient reacts with the donors RBC and activate complement system thus leading to cell lysis called acute intramuscular hemolysis which inturn leads to release of hemoglobin and finally cause kidney failure and can even be fatal causing death. The erythrocyte fragments released in blood causes the blood clotting system to activate leading to disseminated intravascular coagulation. A table is given to show the compatibility of donor & recipient during blood transfusion reaction.

Table 2: Blood group matching

	O-	O+	B-	B+	A-	A+	AB-	AB+
AB+	✓	✓	✓	✓	✓	✓	✓	✓
AB-	✓				✓		✓	
A+	✓				✓	✓		
A-	✓				✓			
B+	✓	✓	✓	✓				
B-	✓		✓					
O+	✓	✓						
O-	✓							

Universal donor and recipient: People with blood group O can donate blood to anyone and are known as universal donors. People with blood group AB can accept blood from all donors and are called universal recipients. People with group A or B can receive matching blood or group O blood.

Bombay Phenotype

- This is an extremely rare ABO group named after its discovery in "Bombay".
- Although initially discovered in East Indians, it is now reported across the globe in Caucasians, Negroes, Japanese and other populations.
- Individuals with this blood type has RBC which lacks A,B and H antigens and their Sera contain anti-A and anti-B and anti-H. The anti-H cannot be detected in the ABO group but would be detectable in pretransfusion tests, e.g., their Sera would agglutinate group O donor cells, which have the H antigen.
- Bombay blood group results from the inheritance of two rare recessive hh genes which inhibits the formation of A,B and H antigens which occur at a locus other than the ABO gene locus. The h gene is very rare. Individuals with Bombay phenotype often result from consanguineous marriages in which parents are blood relatives (e.g., first cousins). Due to inbreeding occurs, the frequency of rare homozygotes increases.
- For such individual they show similarity as group O in routine ABO grouping as shown below

Antibody	anti-a,b	anti-a	anti-b
Reaction with Sera	-	-	-

- Individuals with this type of blood group show incompatibility when crossmatched with RBC of all normal ABO groups
- If they require blood transfusion. They need blood from another Bombay Individual during blood transfusion and donors must be their blood relatives.

Rh blood group

1. Rh blood group was discovered by Landsteiner and Weiner in 1940 and named after the organism it was discovered in i.e., Rhesus monkey.
2. By this system human who are Rh positive have proteins including RhAG and RhD or RhCcEe
3. Rh negative individuals do not express any of these antigens.
4. Rh locus is on chromosome 1p34-1p36 expressed in erythroid lineages in humans.
5. Rh polypeptide has been sequenced .It contains 417 amino acids.
6. The complex of the Rh protein family has an estimated density of 170 000 Daltons and consist of a tetrahedron with 2 RhAG molecules stabilized by both N- terminal and C- terminal domain associations
7. Rh proteins remain associated with membrane proteins and in RBC
8. Rh complex together with spectrin- based skeleton through protein 4.2 and ankyrin contributes to the mechanical dynamics of RBC membrane
9. It is one of the most immunogenic and polymorphic human blood group system.
10. Rh homologues have been identified in several mammalian species as well as in primitive organisms,suggesting their distribution to cells other than RBC,as in higher vertebrates ,thus conferring other biological role in non-erythroid cells.
11. Molecular evolutionary analyses of these Rh homologues exist as early as in fishes.

Hemolytic disease of the newborn (HDN)(Erythroblastosis fetalis)

The Rh blood group system is the major cause of hemolytic anemia in the newborn.

Symptoms

Anemia, splenomegalyhepatomegaly, and edema.

Cause

- ❖ It is caused by maternal anti erythrocyte antibodies across the placenta .
- ❖ It happens when the Rh-mother has a Rh +ve child.
- ❖ The mother become sensitized to the antigen upon birth of such child.
- ❖ During the process of birth the child's blood mixes with the mother and the mothers body produces antibodies against these Rh antigens.
- ❖ This does not affect the first child.

- ❖ However in case of the second pregnancy, of the mother bearing the second Rh positive child, the consequences are fatal for the child as the maternal antibodies mostly IgG against the Rh antigen crosses the placenta and binds to the fetal RBC.
- ❖ This antibody coated RBC bind to receptors of Fc portions of IgG on monocytes and macrophages causing immune adherence mostly in the spleen and liver.
- ❖ This leads to the lysis of RBC s causing extravascular hemolysis of the fetus.
- ❖ RBC lysis in fetus may also lead to fetal anemia, hyperbilirubinemia leading to brain damage
- ❖ Sometimes this may cause spontaneous abortion or child stillborn.

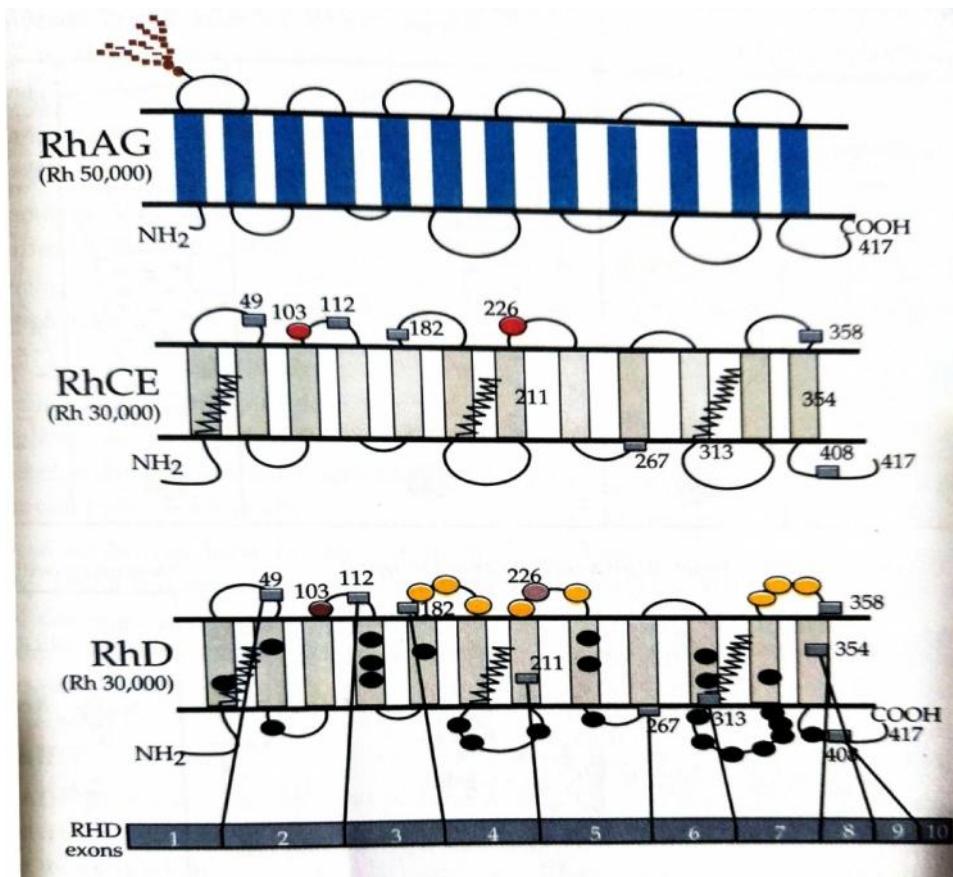


Fig.4: Schematic representation of structure of different Rh antigens

Prophylaxis

1. The recommended treatment involves intramuscular injection of 500IU of anti-D immunoglobulin called as RhoGAM to the mother within 72 hours of delivery of the first child who is Rh positive.
2. These antibodies bind to RBC from the baby which has entered the mothers body and destroys them preventing mother from being exposed to the Rh antigen &thereby preventing antibody production.

DAT Test

DAT or direct antiglobulin test is done to detect the presence of maternal antibodies on the baby's RBC.

This is done by a Coomb's test

Wherein isolated foetal RBC is incubated with goat antibody to human IgG antibody. If maternal RBC is bound to fetal RBC the cells agglutinate.

Development of the HDN caused by Rh incompatibility can be tested by taking maternal serum at regular intervals during pregnancy

Rh factor

The Rh factor is an antigen that some people may have on their red blood cells. Those who have this antigen are Rh-positive, while those without it are Rh-negative. Knowing a person's Rh status is key to understanding their blood compatibility.

This compatibility is particularly important during pregnancy and when receiving a blood transfusion. In the event of incompatible blood, the body will respond by producing antibodies against the Rh antigen. These antibodies will destroy red blood cells with the Rh antigen, which can cause health complications

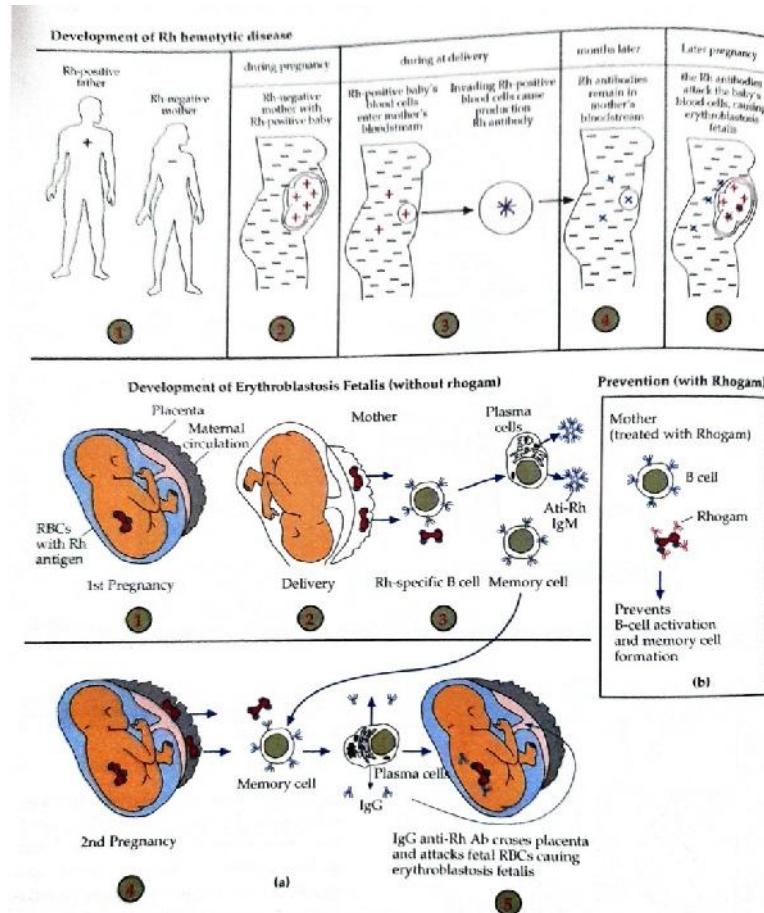


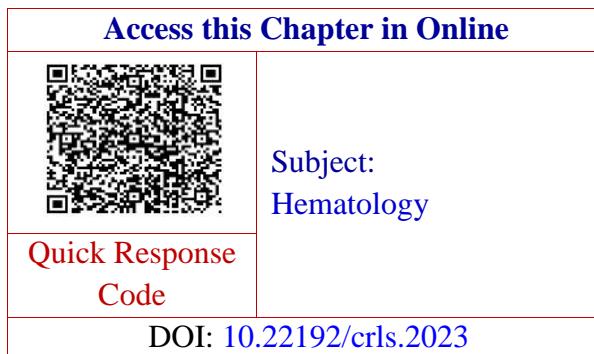
Fig.5: Hemocyte disease of the new born development of erythroblastosis fetalis (hemolytic fetus (a) and effect of treatment with anti-Rh antibody or Rhogam disease of the new born)caused when an Rh mother carriers and Rh(b)

Advances in blood group detection

1. Blood group detection kits with monoclonal antibodies against A,B Rh antigens are commercially available.
2. DNA analyses can be valuable for the prediction of Rh antigens when suitable panels of monoclonal antibodies are not readily available, or the antibodies are not available in the needed strength or volume.

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Hypersensitivity

M. Suhaina Parveen and M. Kannahi

PG and Research Department of Microbiology
Sengamala Thayaar Educational Trust Women's College (Autonomous),
Sundarakkottai, Mannargudi-614 016.
Ph.no:6369512389, 7540050540
Mail id: kannahiamf@gmail.com, 2004045suhaina@gmail.com

Introduction

The human immune system is an essential part of defense against infection; however the general defending immune system can occasionally produce harmful response to the host. Such responses are identified as hypersensitivity response, as well as the study of these is called immunopathology. This term came from the impression that the persons who have been formerly exposed to an antigen, marked noticeable responses to that antigen later and are consequently supposed to be sensitized. Hypersensitivity responses can be produced through endogenous self-antigens or exogenous antigens. Immune reactions for endogenous or self-antigens effects in autoimmune diseases. On the other hand, immune response may take variety of forms against exogenous antigens such as microbial and non-microbial (i.e. drugs, foods, pollens, chemicals and dust) components. Some of the most common reactions to exogenous antigens cause the group of disease known as allergy which has variety of symptoms ranging from itching, rash, fever, asthma and anaphylaxis. Almost any substance capable of inducing an immune response is a potential allergen. Development of hypersensitivity to any particular allergen is due to a complex interaction of genetic susceptibility and exposure. (Allergic rhinitis due to ragweed pollen is common, but it is not problem for those who live in areas where ragweeds do not grow.) The necessity for exposure is the basis of the most common treatment, removal of the offending antigen. A growing number of diseases/syndromes are being shown to have some symptoms due to immune reactions. The purpose of this chapter is to present a general idea to the main components and properties of hypersensitivity reaction.



Hypersensitivity reactions can involve immunological or non-immunological mechanisms, with the latter also being known as idiosyncratic reactions or pseudo-allergy. Johansson and colleagues Johansson *et al* (2001) states that “Hypersensitivity causes objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects.” The stimulus could include both exogenous and endogenous antigens and haptens. Hypersensitivity is the exaggerated immune response to protect the human from foreign bodies known as antigens. When the antigen is detected by the immune system, a hyperimmune response starts and the hypersensitivity reaction starts. This reaction is not always desirable as it may harm humans. Hypersensitivity reactions may lead to various consequences ranging from mild symptoms to severe shock causing death. Antigens or causative agents may be either small particles such as pollen grains or large particles such as drugs including antibiotics. Antigens are detected by T cells or antibodies by recognizing epitopes on the surface of the antigen. Some antigens may have the same epitope, so antibodies are able to detect more than one antigen with the same epitope and interact with them. This phenomenon is known as cross-reactivity.

Types of hypersensitivity

Type of Reactions	Clinical syndrome	Time required for manifestation	Mediators
Type 1: IgE type	1. Anaphylaxis 2. Atopy	Minutes	IgE, histamine and other agent
Type 2: cytolytic and cytotoxic	Antibody mediated damage, anemia, agranulocytosis etc.	Variable hours to days	IgG, IgM
Type 3: immune complex	1. arthus reaction 2. serum sickness	Variable : hours to days	IgM, IgG,C and leucocytes
Type 4: delayed hypersensitivity	1. Tuberculin 2. contact dermatitis	Hours to days	T- cells, lymphokines, macrophages.

Allergy (Atopy or Type – I Hypersensitivity)

Type 1 hypersensitivity occurs within seconds to minutes. Therefore, it is called immediate hypersensitivity. Generally, this type is not harmful, however, the antigen in this reaction may be either harmless as pollen grain, drugs, and food or harmful antigen as venoms. Type I reactions involve two types of white blood cells (mast cells and basophils), as well as immunoglobulin E (IgE) antibodies. Upon the initial exposure to an allergen, the immune system produces IgE antibodies which bind to the cell membranes of mast cells and basophils. The antibodies are specific to a particular allergen and serve to detect the allergen upon subsequent exposure.

Etiology

The main antibody exaggerated by the immune response during this reaction is called immunoglobulin E (IgE) which attacks soluble antigens. The interaction between IgE and the antigen releases histamine and inflammatory mediators.

There are two stages of this immune response after exposure

- First stage, called the sensitization stage, is where the host contacts the antigen for the first time. This contact is asymptomatic as the host recognizes the antigen for the first time.
- Second stage is the late phase reaction at which the sensitized host is exposed to the antigen again leading to the development of type I hypersensitivity reaction.

Types of antigen involved

There are various types of antigens that can exaggerate the immune response such as:

- Food: some foods may cause allergies like Nuts, Soy, and wheat
- Animal source: Bee bites, cats, and rat dander
- Environmental source: Dust, pollen, and molds
- Drug allergy: antibiotics are the main drugs that induce allergic reactions. However, other such as propofol and isoflurane anesthesia drugs may induce hypersensitivity as well.

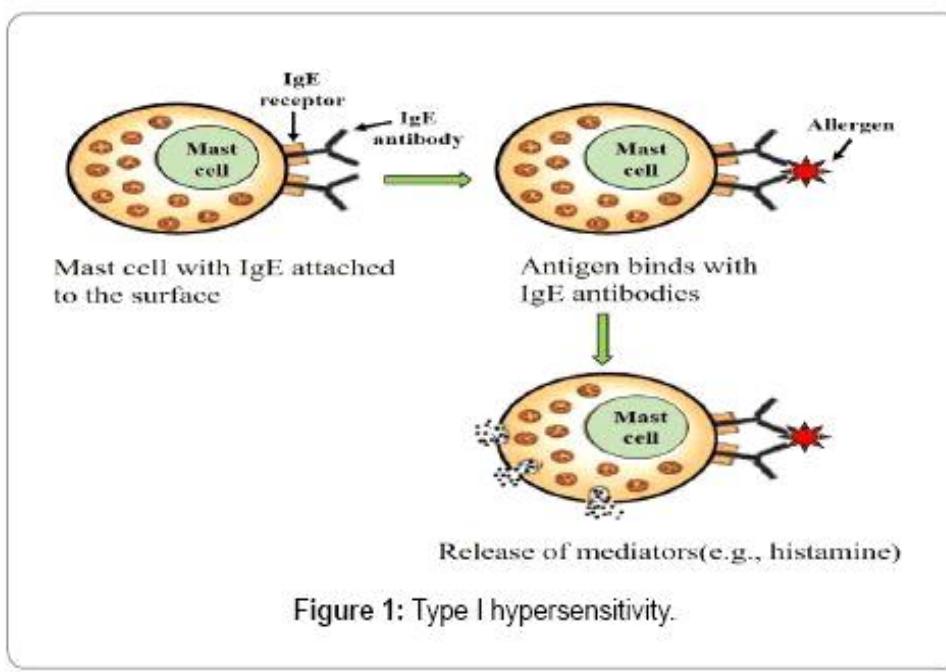


Figure 1: Type I hypersensitivity.

Epidemiological and Clinical features

Symptoms of the reaction may affect any part of the body such as the following hypersensitivity examples:

- Rhinitis: allergy in the nose.
- Conjunctivitis: it is an ocular allergy.
- Dermatological allergy: hypersensitive skin suffers from dermal hypersensitivity reactions like eczema.

Epidemiology of these reactions according to their causes are as follows:

- ✓ Asthma affects about 5% of the population. Of those, about half have attacks brought on by known allergens. Many of the others are thought to be triggered by allergens that have not yet been identified.
- ✓ Allergic rhinitis is the most common immune mediated clinical entity. It may be seasonal or more-or-less continuous depending on the allergen.

Diagnosis and Treatment

Symptomatic relief is provided by drug therapy. Antihistamines in either spray or oral form are the most commonly used drugs. Newer forms are available that produce much less drowsiness. Overuse of nasal sprays may lead to a rebound effect (rhinitis medicamentosa). Comolyn sodium in aerosols and less often in eye drops provide excellent relief when used prophylactically with few if any long-term side effects. It acts to stabilize the membranes of mast cells and basophils so that the vasoactive factors are never released. Nasal sprays containing a locally acting corticosteroid are used with some success in long-term prophylaxis. Corticosteroids are anti-inflammatory and cytotoxic to most leukocytes, but their exact mechanisms of action in relieving allergic rhinitis are unknown. It bypasses most of the side effects of systemic corticosteroid treatment. The slight burning and epistaxis associated with this spray does not appear to be dangerous. Long-term use should be monitored for localized effects of cortisol; e.g., mucosal atrophy. Long term relief is provided by desensitization via intradermal injections of allergen mixtures. The goal is to induce the formation of IgG antibodies against the allergens which would then remove them from circulation before the IgE on the mast cells had a chance to see the allergen.

Type II tissue injury (Type II Hypersensitivity)

Type II hypersensitivities, also called cytotoxic hypersensitivities, are the result of antibody (IgG and IgM) interactions with body cells and tissues that lead to cell destruction. Once bound to a cell, the antibody initiates a cascade of events, known as complement, that causes inflammation and cell lysis. Two common type II hypersensitivities are hemolytic transfusion reactions and hemolytic disease of newborns.

Damage can be accomplished via three different mechanisms

- Antibody binding to cell surface receptors and altering its activity.
- Activation of the complement pathway.
- Antibody-dependent cellular cytotoxicity.

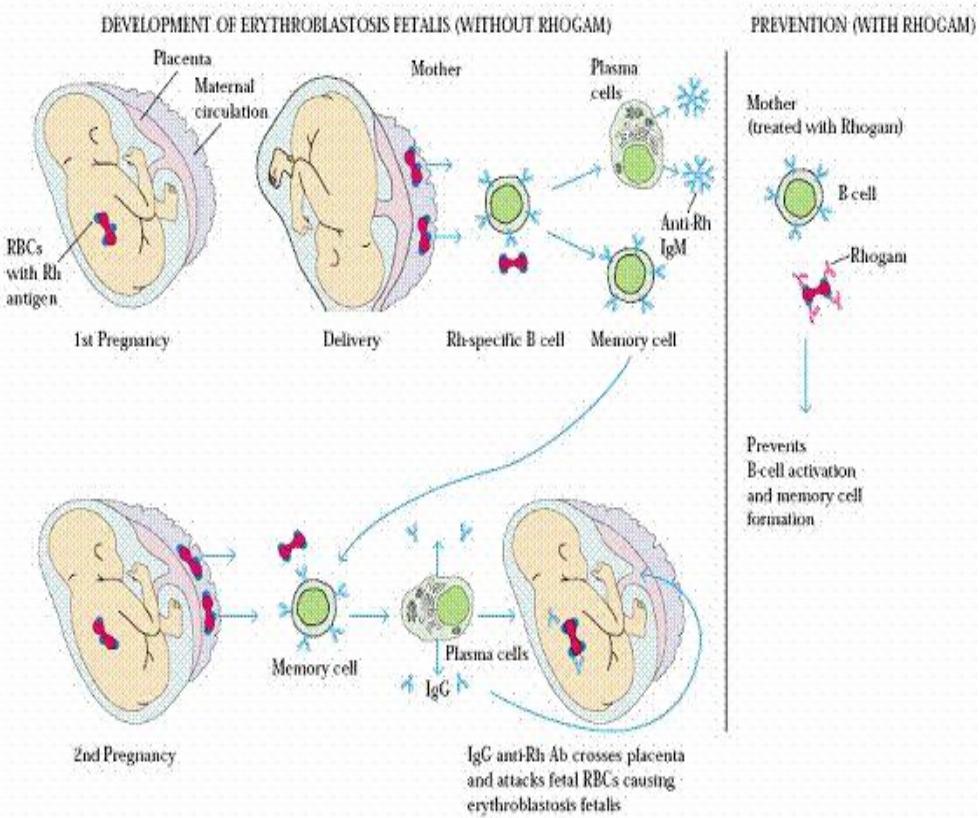
Disease	Autoantibody target
Autoimmune hemolytic anemia	Red blood cells
Goodpasture syndrome	Glomerular basement membrane
Graves disease	Thyroid stimulating hormone receptor
Immune thrombocytopenia	Platelets
Myasthenia gravis	Muscle acetylcholine receptor

Examples

Cytotoxic reactions are intermediated by IgM and IgG. One of the best examples of cytotoxic reactions is the Rh-incompatibility of a newborn. Other examples are blood transfusion reactions, Good pasture's syndrome and autoimmune

The pathophysiology of type II hypersensitivity reactions can be broadly classified into three types

- Cell depletion or destruction without inflammation
- Inflammation mediated by complement or Fc receptor
- Cellular dysfunction by antibodies



Immune Complex Disease (Type –III Hypersensitivity)

Type III hypersensitivities are caused by the formation of immune complexes in body tissues. Immune complexes are masses of antigens with antibodies bound to them. These antigen-antibody complexes contain greater antibody (IgG) concentrations than antigen concentrations. The small complexes can settle on tissue surfaces, where they trigger inflammatory responses. The location and size of these complexes make it difficult for phagocytic cells, like macrophages, to remove them by phagocytosis. Instead, the antigen-antibody complexes are exposed to enzymes that break down the complexes but also damage underlying tissue in the process.

Diseases caused by this reactions

The most common diseases involving a type III hypersensitivity reaction are:

- Serum sickness
- Post-streptococcal glomerulonephritis
- Systemic lupus erythematosus
- Farmers' lung (hypersensitivity pneumonitis)
- Rheumatoid arthritis.

This damage takes place in three stages which are:

- Immune complex formation: at this stage, the antigen is detected by the antibody and interacts with it forming an immune complex.
- Immune complex deposition: the antigen-antibody complex precipitates in joints and glomeruli of the kidney. This occurs when the ratio of antigens is higher than that of antibodies.
- Inflammatory reaction: the classical pathway begins when the complex is precipitated which leads to the release of C3a, C5a, macrophages, and neutrophils. The release of these mediators damages tissue. hypersensitivity symptoms differ according to the site of inflammation; it is presented as arthritis when the inflammation is at joints and glomerulonephritis when glomeruli are inflamed.
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Summary of Immune Complex Diseases		
<u>Disease</u>	<u>Symptoms</u>	<u>Treatment</u>
Serum sickness	Fever, joint pain, dermatitis, lymphadenopathy, proteinuria, lung failure	Removal of complexes, organ function support
Polyarteritis nodosa	Pain whose location varies with time, hypertension due to vascular injury	Immunosuppression
Systemic lupus	Polyarthralgia, erythema around eyes, lung and kidney failure	Immunosuppression
Allergic broncopulmonary aspergillosis	Asthma, episodes of fever, cough, chest pains & malaise	Corticosteroids to control inflammation
Some cancers	Similar to serum sickness	Remove tumor mass

Delayed Hypersensitivity (Type –IV Hypersensitivity)

Type IV hypersensitivities do not involve antibody actions but rather T cell lymphocyte activity. These cells are involved in cell mediated immunity, a response to body cells that have become infected or carry foreign antigens. Type IV reactions are delayed reactions, as it takes some time for a response to occur. Exposure to a particular antigen on the skin or an inhaled antigen induces T cell responses that result in the production of memory T cells.

The reaction happens as follows:

- The body is exposed to the antigen.
- Leukocytes are attracted to the antigen.
- Macrophages engulf the antigen.
- Monocytes are presented to T-cells.
- T-cells begin to be activated and sensitized.
- T-cells begin to secrete cytokines and chemokines.
- Tissue damage occurs.

Four subtypes of type 4 hypersensitivity reaction:

- IVa
- IVb
- IVc
- IVd.

Summary of Delayed Hypersensitivity Diseases

Disease	Pathology	Treatment
Contact dermatitis	Localized dermatitis	Removal of allergen, topical cortisone
Hypersensitivity pneumonitis	Acute = dyspna, cough, malaise, fever, chills (also subacute & chronic forms)	Systemic corticosteroids
Schistosomiasis	Granulomas that block portal veins in response to schistosome eggs trapped there	Treat underlying disease
Granuloma formation	Build-up of mononuclear cells and giant cells around a persistent antigen.	Treat underlying disease
Drug allergies	Response to drugs bound to tissue proteins.	Immunosuppression

Diagnosis:

Identification of the antigen is usually via patch-testing. Intradermal injection of small amounts of the suspected allergen is still used, particularly in testing for tuberculosis, but the patch test is easier and is less likely to produce necrosis. A good history will often provide needed clues. Definitive diagnosis may require histological examination of tissue samples. The particular symptoms developed depend on the tissue affected.

Type	Antigens	Immune reactant	Reaction
Type I hypersensitivity: Anaphylaxis, Drug allergy, Food allergy, asthma	Drug, food, dust, egg, Insect venoms, nuts, fish	IgE	Inflammation, oedema, Eczema, diarrhea.
Type II hypersensitivity: Hemolytic disease of the new born, Good pasture syndrome, drug sensitivity	Rh antigen, acetylcholine receptor	Antibody, IgG, IgM	Paralysis, anemia, nephritis
Type III hypersensitivity: Serum sickness, Arthus reaction, Systemic lupus erythematosus	Auto-antigen, DNA, mushroom spores	Immune complexes, Basophile complement	Joint inflammation, nephritis
Type IV hypersensitivity: Delayed hypersensitivity	Poison Ivy, proteins, food, dust, chemicals, bacteria	T cells, Macrophages	Skin inflammation
Type V hypersensitivity: Graves' disease	Thyroid stimulating hormone receptor	Antibody	Thyotoxicosis

The treatment of immediate hypersensitivity reactions includes

- Allergic bronchial asthma can be treated with any of the following: inhaled short- and long-acting bronchodilators (anticholinergics) along with inhaled corticosteroids, leukotriene antagonists, use of disodium cromoglycate, and environmental control. Experimentally, a low dose of methotrexate or cyclosporin and omalizumab (a monoclonal anti-IgE antibody) has been used.

- Treatment of autoimmune disorders (e.g., SLE) include one or a combination of NSAIDs and hydroxychloroquine, azathioprine, methotrexate, mycophenolate, cyclophosphamide, low dose IL-2, intravenous immunoglobulins, and belimumab.
- Omalizumab is a monoclonal antibody that interacts with the binding site of the high-affinity IgE receptor on mast cells. It is an engineered, humanized recombinant immunoglobulin. Moderate to severe allergic bronchial asthma can improve with omalizumab.

The treatment of delayed hypersensitivity reactions includes

- The most common drugs to treat tuberculosis include isoniazid, rifampin, ethambutol, and pyrazinamide. For drug-resistant TB, a combination of antibiotics such as amikacin, kanamycin, or capreomycin should be used.
- The most common drugs to treat leprosy include rifampicin and clofazimine in combination with dapsone for multibacillary leprosy. A single dose of antimicrobial combination to cure single lesion paucibacillary leprosy comprises ofloxacin, rifampicin, and minocycline.
- Praziquantel can be useful for treating infections caused by all Schistosoma species.
- Hydroxychloroquine and chloroquine can use in the therapy of sarcoidosis involving the skin, lungs, and the nervous system.
- The use of anti-TNF monoclonal antibodies such as adalimumab and certolizumab have been approved for Crohn disease.

Conclusion

Hypersensitivity reaction to food, insect venom, spore and drugs are not rare. Appreciatively, recent clinical research such as HLA tetramers and microarray techniques are likely to provide clinical application for hypersensitivity reaction. Hypersensitivity generally results when an inequity happens among the effector mechanisms and the regulator mechanisms that typically function to limit such reactions. The progress of hypersensitivity I responses is related to the inheritance of specific genes. the regulations for control of allergic reactions should be followed to prevent yourself from getting hypersensitivity reactions.

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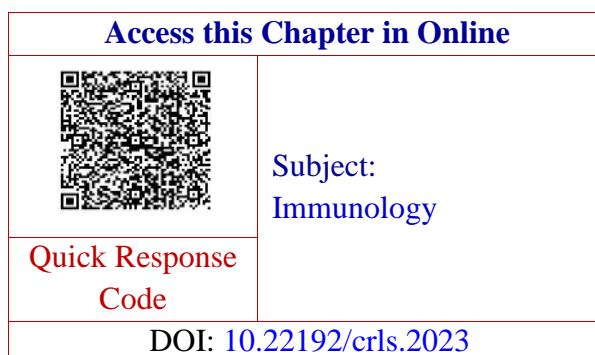
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Immunology - Bronchitis

K. Yoga & M. Kannahi

PG and Research Department of Microbiology, STET Women's College
(Autonomous), Sundarakottai, Mannargudi (Tk), Thiruvarur (Dt),
Tamilnadu, India.

Contact – 9363327928, 6369512389
Email Id: kannahiamf@gmail.com

Introduction

Bronchitis is characterized by bronchial inflammation that results in cough and sputum production. This inflammation can be acute in nature, usually resulting from a viral infection, or it may be a long-standing manifestation of chronic obstructive pulmonary disease. Acute infectious bronchitis differs from chronic bronchitis with respect to etiology, pathophysiology, and treatment.

History

Patients with acute bronchitis present with a productive cough, malaise, difficulty breathing, and wheezing. Usually, their cough is the predominant complaint and the sputum is clear or yellowish, although sometimes it can be purulent. Purulent sputum does not correlate with bacterial infection or antibiotic use.[9] Cough after acute bronchitis typically persists for 10 to 20 days but occasionally may last for 4 or more weeks. The median duration of cough after acute bronchitis is 18 days.[10] Paroxysms of cough accompanied by inspiratory whoop or post-tussive emesis should raise concerns for pertussis. A prodrome of upper respiratory infection (URI) symptoms like runny nose, sore throat, fever, and malaise are common. A low-grade fever may be present as well. High-grade fevers in the setting of acute bronchitis are unusual and further diagnostic.

On physical exam, lung auscultation may be significant for wheezing; pneumonia should be suspected when rales, rhonchi, or egophony are appreciated. Tachycardia can be present reflecting fever as well as dehydration secondary to the viral illness. The rest of the systems are typically within normal limits.

Bronchitis Types

Chronic bronchitis has similar symptoms to acute bronchitis, but it is an ongoing illness.

One definition states that a person has [chronic bronchitis](#) if they have a daily, productive cough for at least 3 months of the year, 2 or more years in a row.

The [National Library of Medicine](#) describe it as a type of chronic obstructive pulmonary disease (COPD) in which the bronchial tubes produce a lot of mucus. It either does not go away, or it goes away and keeps coming back.

The [Centers for Disease Control and Prevention](#)Trusted Source (CDC) note that a person who develops [emphysema](#) alongside chronic bronchitis will receive a diagnosis of COPD. This is a serious and potentially life threatening condition.

Learn more [here](#) about COPD.

Symptoms

For either acute bronchitis or chronic bronchitis, signs and symptoms may include:

- Cough
- Production of mucus (sputum), which can be clear, white, yellowish-gray or green in color — rarely, it may be streaked with blood
- Fatigue
- Shortness of breath
- Slight fever and chills
- Chest discomfort

If you have acute bronchitis, you might have cold symptoms, such as a mild headache or body aches. While these symptoms usually improve in about a week, you may have a nagging cough that lingers for several weeks.

Chronic bronchitis is defined as a productive cough that lasts at least three months, with recurring bouts occurring for at least two consecutive years.

If you have chronic bronchitis, you're likely to have periods when your cough or other symptoms worsen. At those times, you may have an acute infection on top of chronic bronchitis.

When to see a doctor

See your doctor if your cough:

- Lasts more than three weeks
- Prevents you from sleeping
- Is accompanied by fever higher than 100.4 F (38 C)
- Produces discolored mucus
- Produces blood
- Is associated with wheezing or shortness of breath.

Causes

Acute bronchitis is usually caused by viruses, typically the same viruses that cause colds and flu (influenza). Antibiotics don't kill viruses, so this type of medication isn't useful in most cases of bronchitis.

The most common cause of chronic bronchitis is cigarette smoking. Air pollution and dust or toxic gases in the environment or workplace also can contribute to the condition.

Risk factors

Factors that increase your risk of bronchitis include:

- **Cigarette smoke.** People who smoke or who live with a smoker are at higher risk of both acute bronchitis and chronic bronchitis.
- **Low resistance.** This may result from another acute illness, such as a cold, or from a chronic condition that compromises your immune system. Older adults, infants and young children have greater vulnerability to infection.
- **Exposure to irritants on the job.** Your risk of developing bronchitis is greater if you work around certain lung irritants, such as grains or textiles, or are exposed to chemical fumes.

- **Gastric reflux.** Repeated bouts of severe heartburn can irritate your throat and make you more prone to developing bronchitis.

Complications

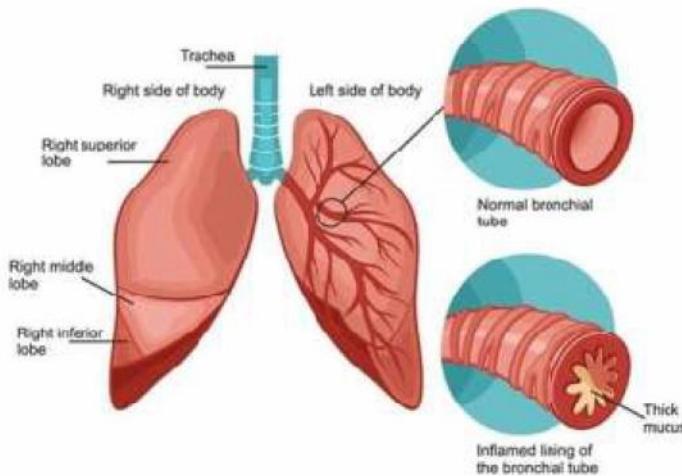
Although a single episode of bronchitis usually isn't cause for concern, it can lead to pneumonia in some people. Repeated bouts of bronchitis, however, may mean that you have chronic obstructive pulmonary disease (COPD).

Prevention

To reduce your risk of bronchitis, follow these tips:

- **Avoid cigarette smoke.** Cigarette smoke increases your risk of chronic bronchitis.
- **Get vaccinated.** Many cases of acute bronchitis result from influenza, a virus. Getting a yearly flu vaccine can help protect you from getting the flu. You may also want to consider vaccination that protects against some types of pneumonia.
- **Wash your hands.** To reduce your risk of catching a viral infection, wash your hands frequently and get in the habit of using alcohol-based hand sanitizers.
- **Wear a surgical mask.** If you have COPD, you might consider wearing a face mask at work if you're exposed to dust or fumes, and when you're going to be among crowds, such as while traveling.

BRONCHITIS



ACUTE COUGH / BRONCHITIS

Antibiotic Treatment Table

Antibiotics are NOT indicated for most cases of acute cough / bronchitis.

If antibiotic deemed necessary in high risk individual or systemically unwell, consider the following:

Drug	Dose	Duration	Notes
1st choice options			
Amoxicillin	500mg every 8 hours	5 days	Avoid in penicillin allergy
Doxycycline <i>(First choice in penicillin allergy)</i>	200mg every 24 hours*	5 days	Avoid in pregnancy. Advise to take with a glass of water and sit upright for 30 minutes after taking. Can take with food or milk if gastritis is an issue. Absorption of doxycycline significantly impaired by antacids, iron/calcium/magnesium/zinc-containing products.
2nd choice options			
Clarithromycin <i>(Second choice in penicillin allergy)</i>	500mg every 12 hours	5 days	Clarithromycin suitable only in 2 nd and 3 rd trimester in pregnancy. Alternative macrolide for all trimesters of pregnancy: Azithromycin 500mg stat then 250mg every 24 hours from Day 2 to Day 5.

ACUTE COUGH / BRONCHITIS

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Physical Assessment

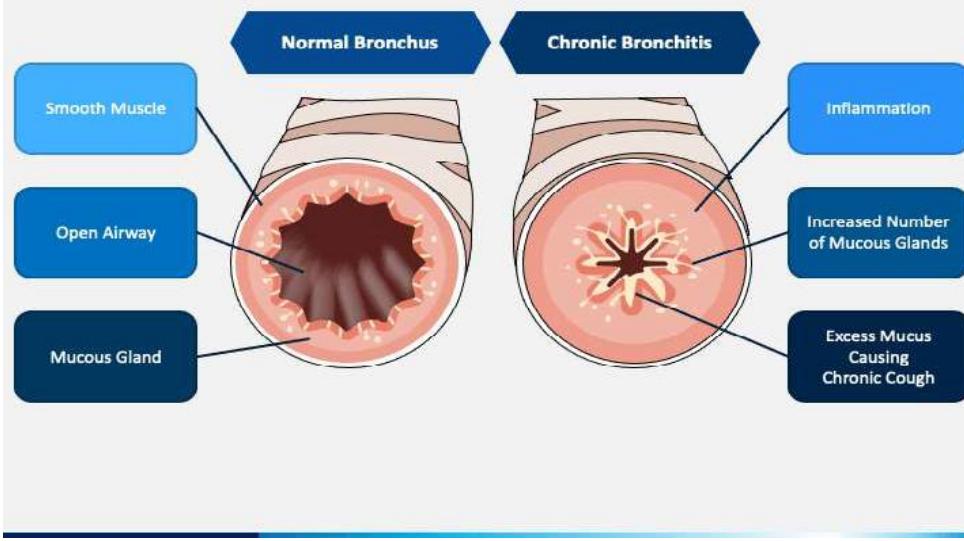
	Technique	Normal Findings	Abnormal Findings
Skin	Inspection	<ul style="list-style-type: none"> • Skin is brown and generally equal • No edema • Good skin turgor • No lesion • Temp. is warm & cool 	<ul style="list-style-type: none"> • None
	Palpation		
Nails	Inspection	<ul style="list-style-type: none"> • Clean, smooth • Pink to light brown nail beds 	<ul style="list-style-type: none"> • None
Hair	Inspection	<ul style="list-style-type: none"> • No lesion • No dandruff • Even in distribution 	<ul style="list-style-type: none"> • None
Head	Inspection	<ul style="list-style-type: none"> • Symmetrical in movement & position • Face is symmetrical • Normocephalic 	<ul style="list-style-type: none"> • None
Eyes	Inspection	<ul style="list-style-type: none"> • Symmetrical in position • Sclera is white & glossy • PERRLA • Brisk reaction to light 	<ul style="list-style-type: none"> • Pale conjunctiva
Ears	Inspection	<ul style="list-style-type: none"> • Equal in size • Symmetrical • No swelling or discharges 	

Physical Assessment

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CHRONIC BRONCHITIS

Pathophysiology of Chronic Bronchitis



Conclusion

- Bronchiolitis is mainly a clinical diagnosis.
- Diagnostic laboratory and radiographic tests play a limited role.
- Bronchodilators and steroids lack Significant Clinical effectiveness.
- Supplemental oxygen indicated if $\text{Pao}_2 < 90\%$ consistently.
- Assess patients for risk factors when making final disposition decisions
- Most patients recover suction, O_2 & fluids only.

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Intraoperative Bronchospasm

Ms. Yuvasree Harinath

Anesthesia Technologist, Institute of Anesthesiology and Critical Care,
Madras Medical College, Chennai, Tamil Nadu, India.

Email ID: yuvir20001@gmail.com

Abstract

Bronchospasm or Bronchial spasm is a sudden constriction of the muscles in the walls of the bronchioles. It is caused by the release of substances from mast cells or basophils under the influence of anaphylatoxins. It causes difficulty in breathing which ranges from mild to severe. Bronchospasm during general anesthesia can present in isolation or as a component of a more serious underlying pathology such as anaphylaxis. It is characterized by prolonged expiration, wheeze and increased peak airway pressures during Intermittent Positive Airway Pressure (IPPV). Untreated it can cause hypoxia, hypotension and increased morbidity and mortality. Suspected bronchospasm during anesthesia should be assessed and treated promptly. In this review I had focused on current updates of bronchospasm, its etiology, Pathophysiology, Clinical features, differential diagnosis, prevention and treatment.

Keywords: Bronchial Spasm, Bronchospasm, Lung Volumes, Anesthetic Drugs, Bronchi, Hypoxia, Spirometry Volumes, Pulmonary Function Test, Ventilation Modes.

Abbreviations

- **ETT** - Endotracheal tube
- **NSAID** - Nonsteroidal Anti-inflammatory Drugs
- **FEV1** - Forced Expiratory Volume in First second
- **FE** - Forced Concentration of Expiratory Gases
- **ERV** - Expiratory Reserve Volume
- **FEF** - Forced Expiratory Flow
- **HTN** - Hypertension
- **RV** - Residual Volume

- **FRC** - Function Residual Capacity
- **TLC** - Total Lung Capacity
- **LMA** - Laryngeal Mask Airway
- **ETCO₂** - End Tidal Carbon dioxide
- **ECG** – Electrocardiography
- **NIBP** - Non-Invasive Blood Pressure
- **ABG** - Arterial Blood Gas

Introduction

Bronchospasm is an abnormal contraction of the smooth muscle of bronchi, resulting in acute narrowing and obstruction of the respiratory airway, which is caused by the release of substances from mast cells or basophils under the influence of anaphylatoxins. It causes difficulty in breathing which ranges from mild to severe, so it is consider as nightmare for Anesthetist and other healthcare professionals. Bronchospasm during general anesthesia can present in isolation or as a component of a more serious underlying pathology such as anaphylaxis. It is characterized by prolonged expiration, wheeze and increased peak airway pressures during Intermittent Positive Airway Pressure (IPPV). Untreated it can cause hypoxia, hypotension and increased morbidity and mortality.

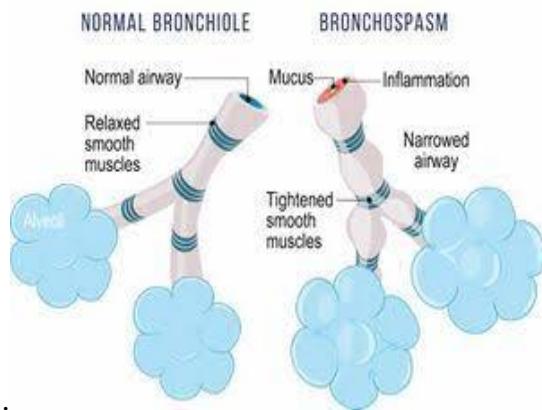


Figure 1

Etiology

Respiratory causes

❖ Upper Airway

- J Tumours of pharynx and larynx
- J Tracheomalacia
- J Laryngeal edema/infection
- J Foreign body

❖ Lower Airway

- J COPD
- J Bronchial Asthma
- J Bronchitis
- J Bronchiectasis
- J Cystic fibrosis

❖ Pulmonary

- J Infection
- J Pulmonary edema
- J Pneumonia

Mechanical irritation

- J Airway manipulation
- J Endotracheal intubation
- J Endobronchial intubation
- J Secretions in large airway
- J Aspiration of gastric contents

Drugs

❖ Anesthetics

- J Desflurane in smokers
- J Isoflurane inhalation induction

❖ **Others**

-) Morphine
-) Mivacurium, atracurium, rapacuronium, doxacurium
-) Beta blockers
-) Aspirin, NSAIDs
-) Cholinesterase inhibitors: neostigmine
-) Atropine > 1 mg
-) Glycopyrrolate > 0.5 mg

Allergy

- ❖ Allergens, latex allergy, anaphylaxis
- ❖ Drugs
 -) Antibiotics
 -) IV contrast
 -) Transfusion reactions

Others

-) Light anesthetic plane
-) Distended urinary bladder
-) Peritoneal retractions
-) Smoke inhalation
-) Carcinoid tumour

Etiology:

As per anesthetic stages

❖ **Causes during induction**

-) Airway irritation
-) Aspiration
-) Anaphylaxis
-) Pulmonary edema

- J Displaced ETT
- J Unknown

❖ Causes during maintenance (*most common*)

- J Anaphylaxis
- J Aspiration
- J Airway irritation
- J Pneumothorax
- J Endobronchial intubation
- J Pulmonary edema
- J Drug Induced: -Vancomycin ,Protamine

❖ Causes at extubation

- J Airway irritation
- J Extubation spasm
- J Pulmonary edema
- J Aspiration
- J Anaphylaxis/allergy
- J Accidental extubation

Pathophysiology

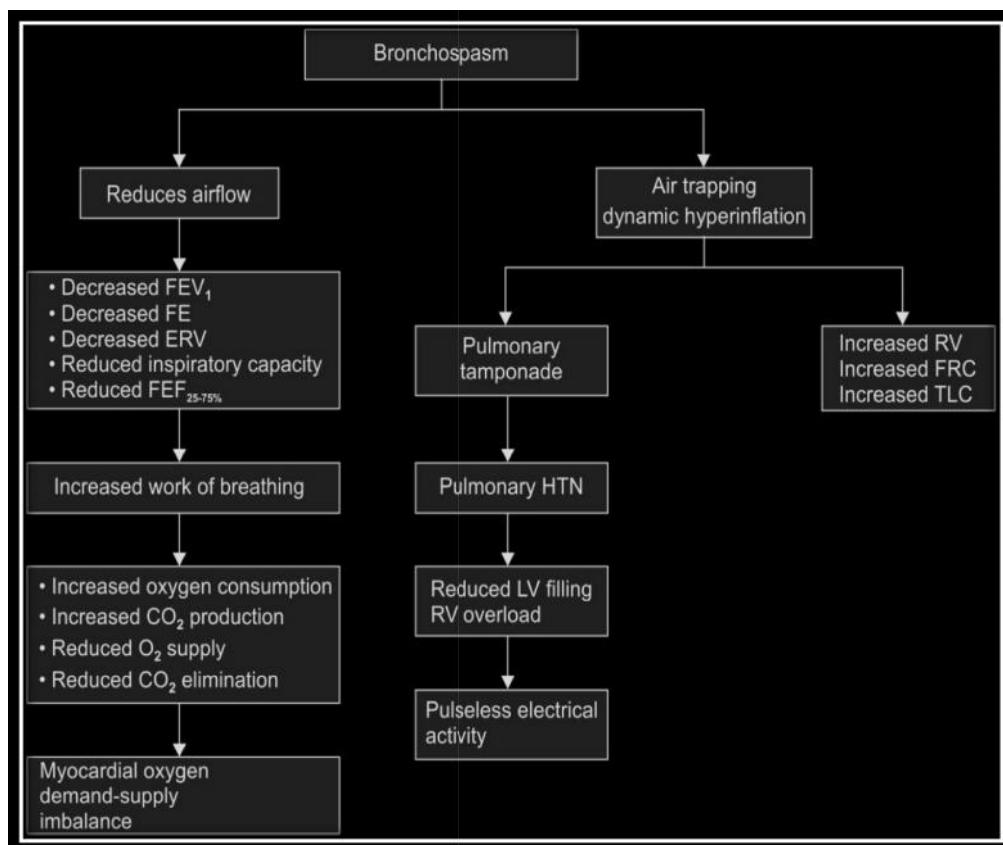


Figure 2

Clinical features

❖ Signs and symptoms

-) Reduced chest excursion
-) Coughing
-) Reduced breath sounds
-) Prolonged expiratory phase
-) Wheezing/rhonchi
-) *Silent chest:* Critically reduced air entry

❖ **Monitors**

-) Hypoxia, hypercarbia
-) Reduced phase II slope on capnograph
-) Reduced compliance of reservoir bag
-) Reduced tidal volume
-) Increased peak inspiratory pressure

Differential diagnosis

❖ **Causes of unilateral rhonchi**

-) Endobronchial intubation
-) Foreign body: Dislodged tooth
-) Tension pneumothorax
-) Kinked endotracheal tube
-) Obstructed endotracheal tube (ETT)
-) Interstitial pulmonary edema

❖ **Causes of raised peak airway pressure**

-) Increased inspiratory flow rate
-) Excessive tidal volume
-) Increased intrapleural pressure
-) Coughing/ bucking
-) Steep Trendelenburg position
-) Pleural effusion
-) Tension pneumothorax
-) Ascites
-) Abdominal gas insufflation/packs
-) Increased resistance in ETT: narrowing/kinking/ secretions

Prevention

- Bronchodilators and steroids before induction in susceptible patients
 - Prefer LMAs over ETT
 - Neostigmine for reversal of NMB may precipitate spasm
 - Careful suctioning at emergence in deep planes
 - Adequate analgesia at emergence
 - Deep extubation preferred
- Postoperative period:
 - Adequate analgesia
 - Bronchodilator therapy
 - Incentive spirometry
 - Deep breathing exercises
 - Early mobilization

Treatment

❖ Monitors

-]) Pulse oximetry
-]) ETCO₂, ECG
-]) NIBP, ABG
-]) Peak inspiratory pressure
-]) Frequent auscultation
-]) Auscultate chest and respiratory limb of anesthesia circuit to confirm wheeze

❖ Ventilation

-]) Turn to manual bag ventilation
-]) Switch to 100% FiO₂
-]) Heliox mixture with 21-30% O₂ (low viscosity of 0.52 kg/m³)

❖ **Deepen anesthetic plane**

-) Increase concentration of isoflurane/sevoflurane
-) Ketamine *agent of choice* for asthmatics
-) Propofol also can be used

❖ **Beta 2 agonists**

-) Through nebulization/MDI via airway adaptor
-) Salbutamol:
 - 2.5 mg via nebulisation
 - Repeated every 20 minutes for 3 times
 - Then given 2-4 hourly
-) Terbutaline 0.25 mg S/C every 20 minutes for 3 doses
-) Albuterol, levalbuterol and pirbuterol are others

❖ **High dose steroids**

- IV methyl-prednisolone 125 mg bolus followed by 40-60 mg IV Q6H
- IV hydrocortisone 200 mg Q4-6H
- IV dexamethasone

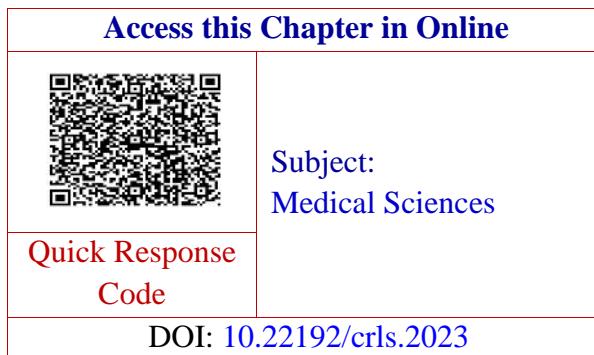
❖ **Others**

-) **Ipratropium bromide:** -
 - 0.5 mg through nebulization every 30 mins for 3 times
 - Followed by 2-4 hourly thereafter
-) Epinephrine 0.3 mL of 1:1000 solution bolus followed by 0.5-2 mg/min infusion
 -) MgSO₄ 1-2 gms iv.
 -) Aminophylline:
 - 5 mg/kg slow iv bolus, followed by 5 mg/kg/hr infusion
 -) Racemic epinephrine nebulization

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In vitro assessment of microbial growth inhibition properties and phytochemical analysis of *Ocimum sanctum* against UTI causing Gram positive cocci and *Candida* spp.

S.Geetha

Research Scholar, PG and Research Department of Microbiology,
Kamban College of Arts and Science For Women
Tiruvannamalai, 606603.

J. Ishwarya

Assistant Professor, PG and Research Department of Microbiology,
Kamban College of Arts and Science For Women
Tiruvannamalai 606603.

Dr. D. Sangeetha

Department of Microbiology, Faculty of Science, Annamalai University,
Annamalai Nagar – 608 002, Tamil Nadu, India.

Abstract

Background

A urinary tract infection (UTI) is the second most common disease after respiratory infection. The incidence of UTI is greater in women as compared to men due to female anatomy. Urinary Tract Infections are mainly caused by both gram-negative and gram-positive bacteria, although fungi and some viruses have also been reported. Currently several potent antibiotics are available for the treatment of UTI, but increasing drug resistance among bacteria and fungi has made therapy of UTI difficult. This situation leads to the development of new therapeutic agents that are less expensive and have fewer adverse effect. The synergistic effect of the mixture of phytochemicals present in plants play an important role to use plant extracts as antimicrobial agents.

Aim

The present study was designed to analyze invitro the antibacterial and antifungal activity of hexane and ethanolic extracts of *O.sanctum* leaf, stem and seed against UTI causing gram positive cocci and *candida* spp and the characterization of extracts by phytochemical and GC-MS analysis.

Materials and methods

Collected isolates were identified and confirmed by standard microbiological procedure. Drugsusceptibility pattern of isolates were screened by Kirby-Bauer Disc diffusion method as per CLSI guidelines. Antimicrobial activity of hexane andethanolicextract of *O.sanctum* leaves, stem and seeds were screened against UTI causing drug resistant gram positive cocci and *candida*spp by agar well diffusion method. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were evaluate to check the effectiveness of the extracts. Both qualitative and quantitative characterization of plant extracts were done by phytochemical analysis, Thin layer chromatography(TLC)and Gas Chromatography – Mass spectrometry (GC-MS).

Results

Total of 52 UTI strains were collected and identified in the following order of *Staphylococcus aureus* 21(40%), *Enterococcus* spp 15(29%) *candida* spp 11(21%) and *Staphylococcus saprophyticus* 5(10%). Drug resistant assay revealed that *Staphylococcus* spp were highly resistant to Oxacillin 21 (81%) and *Enterococcus* spp were resistant to piperacillin 11(73%). Out of 21 *Staphylococcus aureus* 15 were MRSA. *Candida* spp were highly resistant to Itraconazole and Fluconazole. Antimicrobial activity of hexane and ethanolic extracts of *O. sanctum* leaf, stem and seed were screened against drug resistant strains of gram positive cocci, *Candida* sp and correlate with ATCC strains such as *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC25955. Compared to ethanolic extract hexane extract shown activity to maximum number of gram positive cocci (N=26). Whereas *candida*spp were more sensitive to ethanolic extract of *O.sanctum* leaf (23mm). MIC and MBC value of hexane leaf was ranging from 125 μ g/ml -1000 μ g/ml, hexane stem and seed were 250 μ g/ml and 500 μ g/ml respectively. MIC and MBC of ethanolic leaf was noted as 125 μ g/ml -500 μ g/ml. MIC and MFC of hexane leaf was ranging from 62.5 μ g/ml - 500 μ g/ml, hexane stem was 250 μ g/ml, hexane

seed was 125-500 μ g/ml andethanolic extract of leaf was 62.5and 250 μ g/ml was noted against *candida* spp. The TLC chromatogram revealed the presence of a compoundwith Rf value 0.56 in *O. sanctum* hexane leaf extracts, Rf value 0.31for *O. sanctum* hexane stem and ethanolic extracts of leaf with Rf value 0.42. Phytochemical study explained that hexane leaf contained tannins, phlobatannins, steroids, saponins, flavonoids, alkaloids, cardiac glycosides, German kids andquinones. Hexane stem contained phlobatannins, alkaloids, steroids, cardiac glycosides, geraniums and quinones. GC-MS study stated that γ - sitosterol (30.5%), caryophyllene (11.83%) and cyclohexane(7.98%) were the major three component present in hexane leaf extract. squalene (34.95%)tetracosane(23.84%) and hexacontane (6.31%) were the predominant components in hexane stem extracts.

Conclusion

The present study confirmed the efficacy of solvent extracts of *O.sanctum* as natural antimicrobial agents and suggested the possibility of employing them for the treatment of Urinary tract infection.

Key words: UTI, Gram positive cocci, *candida*, antibacterial activity, MIC, MBC, MFC,TLC, GC-MS analysis.

Introduction

Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria, or fungi in the urine to severe infection of the organs of the urinary system with resultant sepsis [1]. According to the National Institute for Health and Clinical Excellence (NIHCE) guidelines, urinary tract infection is defined as a combination of clinical features and the presence of bacteria or fungi in urine. About 150 million people developed urinary tract infection in each year [2]. Compared to men, urinary infection are more common in women. Up to 10% of women have a urinary tract infection in a year and half of women having infection at least once at some point in their lives. Urinary tract infection has occur most frequently between the ages of 16 and 35 years.

The causative agents for Urinary Tract Infection are both Gram-negative and Gram-positive bacteria, as well as certain fungi. The predominant causative agent for both uncomplicated and complicated UTIs is Uropathogenic *Escherichia coli* (UPEC). Other causative agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella Pneumoniae*, *Staphylococcus Saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*,

Staphylococcus aureus and Candida spp. For complicated UTIs, the order of prevalence for causative agents, following Uropathogenic *E.coli* as most common, is Enterococcus spp., K. Pneumoniae, Candida spp., S.aureus, P. mirabilis, P. Aeruginosa and group B Streptococcus (GBS) [2]. Gram-positive bacteria are common cause of urinary tract infection (UTI), particularly among individuals who are elderly, pregnant, or who have other risk factors for UTI [3].

Candida albicans and non-C.Albicans candida (NACA) species are considered as one of the major microbial normal flora in the oral cavity, alimentary canal and vagina in healthy people. Moreover, they form colonize on the external side of the urethral opening. Immune deficiency conditions may lead to an imbalance between C.albicans, NACA yeasts and other host normal flora. In this situation, normal flora *Candida* may convert into opportunistic pathogenic microorganisms creating candidal UTIs in the host. The presence of C.albicans and NACA species in urine is known as candiduria, which may occur in both asymptomatic and symptomatic Urinary tract infection cases [4]. Candida albicans is the common cause of nosocomial fungal urinary tract infections; yet, a rapid change in the distribution of Candida species is undergoing.

Urinary tract infections also often result in chronic recurrence, resulting in the frequent use of antibiotics or long-term antimicrobial prophylaxis that exposes patients to the consequences of long-term changes in normal microbiota of the vagina or gastrointestinal tract and also the development of drug resistant pathogens. Although recurrent UTIs usually are not life-threatening, the high incidence significantly increases healthcare costs and can negatively impact patients' quality of life [5]. Based on the alarming growth of uropathogens that are resistant to existing drugs and the side effects of antibiotics, new therapeutic agents that are less expensive and have fewer adverse effects need to be developed. Medicinal plant treatment may be a viable solution for the effective treatment of infectious diseases [6].

The use of medicinal plants in traditional medicine has been described in literature dating back several 1000 years ago[7].In modern complementary and alternative medical practice, plants are the principal source of therapeutics and each part of the plant, including the seeds, root, stem, leaves, and fruit, potentially contains pharmacoactive components [8-10]. The main bioactive components in medicinal plants are considered to be combinations of secondary metabolites [11]. There are many advantages and benefits associated with the use of medicinal plants; the main one is their cost-effectiveness and

global availability. They are safe as compared to other medicinal products and the lack of major side-effects are other clear advantages.

Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds which are useful both in agriculture and medicine. Among these, *Ocimum tenuiflorum*, also known as *Ocimum Sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been described as the “Queen of plants” and the “mother medicine of nature” due to its perceived medicinal qualities. The stem and leaves of *O. sanctum* possess several bioactive compounds such as saponins, flavonoids, triterpenoids, tannins as well as phenolic compounds, which have great therapeutic importance in curing many diseases, and are also responsible for antimicrobial activity [12]. Possessing enormous number of bioactive components *O. sanctum* potentially inhibit the growth of several bacterial pathogens including *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella entericaserovartyphi* (*S. typhi*), *Staphylococcus aureus* (*Staph. aureus*) and *Bacillus subtilis* [13].

Based on this background this study was designed to evaluate the antibacterial and antifungal activity of solvent extracts of *Ocimum sanctum* leaf, stem and seed against UTI causing gram positive cocci and *Candida* spp.

Materials and Methodology

The present investigation was made to prove that the validity of our traditional medicine by invitro assessment of microbial growth inhibition properties and phytochemical analysis of *Ocimum sanctum* against UTI causing Gram Positive Cocci and *Candida* spp.. The assessment work carried out in Madras University, Taramani, Chennai. In the period of November 2019 to February 2020.

Collection of sample:

The urine samples were collected from hospitalized patients who were suspected of UTI, in chennai. The urine samples were transferred to a University of Madras, Taramani, Chennai (Sterile Containers).

Identification of isolates:

Urine analysis was performed by checking pH, colour and turbidity of urine. A smear was prepared from a drop of urine and done Gram's staining to determine the presence of pus cells, epithelial cells, crystals along with Gram negative, Gram positive bacteria and yeast cells. All the urine samples were processed for the identification of isolates by standard microbiologic procedure.

Fifty two isolates were collected from the patients and the collected isolates were inoculated in macconkey agar, blood agar (HI media INDIA), CLED (Cystine lactose electrolyte deficient) medium, SDA medium and Chrom agar medium. Then incubation at 37°C for 24 hours and identification was done using standard conventional, morphology, culture and biochemical tests (Bergey's manual).

Identification of bacteria:

The colony morphology was observed and the cell nature of the isolates was identified by gram's staining. The biochemical tests were carried out to confirm the isolates.

DNase test:

DNA hydrolysis test is used to determine the ability of an organism to hydrolyze DNA and utilize it as a source of carbon and energy for growth.DNase base agar medium were prepared, sterilized and poured onto plates.Dry the surface of agar plates before use.Each plate may be divided into sections by drawing lines on the bottom of the plate.Use heavy inoculums and draw a line 3-4 cm long from the rim to the center of DNase test agar plate.Incubate the plate at 37 c for 18-24 hours. After incubation, flood the plate 1N Hydrochloric Acid.Leave the plate to stand for a few minutes to allow the reagent to absorb into the plate. Decant excess hydrochloric acid and then examine the plate within 5 minutes against a dark background. Observe the results.

Isolation of *Candida spp.*:

Urine Samples was cultured on Potato dextrose agar plates.Then incubated at 37 °C and examined for its growth at 24-48 hours. The culture Plates was examined for the appearance, size, color and morphology of the colonies.

Identification of *candida spp.*:

Gram stain were identified *Candida spp.* and use lacto phenol cotton blue to stain Candida. Finally examined under 40 x and 100 x with oil immersion microscopic lens. Germ Tube test carried out to confirm the Candida

Media used for screening:

- MacConkey agar was used for the isolation of bacterial strains.
- Mannitol Salt Agar was used for the isolation of *Staphylococcus aureus*.
- Potato Dextrose agar and chrom agar was used for the isolation of the fungal strains *Candida spp.*.
- Muller Hinton agar was used for the antimicrobial studies.

Antibiotic susceptibility test:

The antimicrobial sensitivity pattern of gram positive bacteria and yeast *candida spp.*, was performed by Kirby Bauer Disc diffusion method following Clinical and laboratory standards institute (CLSI 2017) recommendation.

The antibiotics discs (Hi-Media, India) used were Nitrofurantoin (300 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Tetracycline (33 µg), Erythromycin (15 µg), clindamycin (2 µg), Co-Trimoxazole (25 µg), Linezolid (30 µg), Piperacillin (100 µg), Ampicillin (10 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Amikacin (30 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Cefazolin (30 µg), Oxacillin (1 µg), Amphotericin-B (20 µg), Itraconazole (30 µg), Fluconazole (25 µg), Voriconazole (1 µg).

On MHA medium, 0.5 McFarland standardized isolates were swabbed and incubated at 37°C for 24 hours. The zone of inhibition was measured and compared with CLSI guidelines. The organisms reported as either sensitive, intermediate sensitive or resistant to antimicrobial agents tested. *E.coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *K. pneumonia* ATCC 70063, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *Candida albicans* ATCC were used as control strains. These control strain were used to check the microbial inhibition properties with *Ocimum sanctum* plant extracts.

Phenotypic screening of methicillin resistance amongst study isolates:

The cefoxitin disc diffusion method was employed for the phenotypic detection of MRSA by using 30 µg of cefoxitin disc (HiMedia Labs, India) along with other antibiotics with antibiotic susceptibility testing by standard technique described by Bauer et al. The zone of inhibition for each test strain was measured and the results were interpreted according to CLSI guidelines,

2015. *S. aureus* ATCC 25923 and MRSA ATCC43300 strains were used for quality control.

Collection of plant materials:

Ocimum sanctum plants were collected from botanical gardern, Tiruvannamalai, Tamil Nadu, India. The fresh leaves, stems and seeds were taken from plants and used for extraction process. The leaves, stems and seeds were threshed into small pieces and were thoroughly washed 5 or more times with distilled water. These materials were dried under shade for 5 to 6 days and mechanically grinded to make a fine powered and stored in airtight container at room temperature in dark until used.

Preparation of plant extract:

The powered material was weighed on electronic balance. These materials subjected to solvent extraction with low polarity (hexane) and high polarity (ethanol) for 2 to 3 days. Then filtered by Whatman No 1 filter paper. Then the filtrate was evaporating at constant temperature (50 C) by using Rotary Evaporator. These extract was dissolved 1:3 in dimethyl sulphoxide (DMSO) as neutral solvent to make final concentration. These residues were obtained and stored under refrigerated in vials for further process. The final yield of plants were obtained by using following formula,

$$\text{Yield\%} = \frac{\text{weight of the dry extract} \times 100}{\text{weight of the dry powder (g)}}$$

$$\text{Percentage Yield \%} = \frac{\text{weight of product after evaporation} \times 100}{100}$$

$$\text{Loss on drying \%} = \frac{\text{Extractive Value Total weight used} \times 100}{\text{Loss on Drying}}$$

Disc preparation (Arun kumarsatharsivam et al., 2010):

Wattman No:1 filter paper disc (6mm) were prepared. The discs were sterilized by autoclave at 121°C. After sterilization the moisture discs were rinsed 20µl of the hexane extractand ethanol extract of *Ocimum sanctum* and allowed to dry at room temperature.

Sterility check:

The hexane extract and the ethanol extract of *Ocimum sanctum* were streaked separately on Muller Hinton agar and Nutrient agar to check the purity. After 24 hours of incubation the plates were used for bioassay. Nutrient agar and Muller hinton agar was prepared and poured in petriplates and allowed to get solidify.The hexane extracts of *O. sanctum* and ethanol extracts of *O. sanctum* were streaked in the agar plates and incubate at 37°C for 24 hours. After incubation the plates were observed for any contamination.The extracts were found to be sterile. The extracts were then distributed in sterile vials and stored in deep freezer at -20°C until further use. The extracts were powered form were obtained and stored at 4°C until use.The contaminated extracts were discarded.

Screening for phytochemical properties:

To assess the presence of the microbial growth inhibiting bioactive compounds of Ocimum ethanolic, hexane, DMSO and warer extract of plants. The screening of Quinones, phenols, steroids, terpenoids, flavonoids, saponines, cardiac glycosides and anthraquinone glycosides. The results were analysed and useful for further studies. Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001).

Spot overlay method:

To assess the inhibition activity of the *Ocimum sanctum* extract the spot overlay method carried. The MHA prepared swab with culture and a drop of *Ocimum* extract with water, *Ocimum sanctum* with ethanol, *Ocimum sanctum* with hexane and *Ocimum sanctum* with DMSO placed in each plates and observed for the results.

Screening antioxidant property:

The reducing antioxidant property of plant hexane, DMSO, ethanolic extract of *Ocimum* determined by Oyaizu method. Different concentration of plant extract in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, pH 6.6) and potassium free cyanide (2.5 ml %). The mixture incubated for 20 mins at 50°C. 2.5 ml of Tricholoroacetic acid was added to mixture and centrifuged for 10 mins at 300 rpm. The supernatant were mixed with distilled water and FeCl₃. The absorbance measured at 700 nm in UV-Vis spectrophotometer. The reaction mixture indicates increase the reducing properties.

Thin layer chromatography:

For separation detection of the bioactive phytochemical compounds of *Ocimum* by Thin layer chromatography technique. The *Ocimum* extracts were applied in spot form using capillary pipette on precoated silica gel plate of TLC in the distance of 1.5 cm. The plates were developed in Methanol: Acetic acid: DH₂O (3:1:1) as mobile phase solvent in chamber. The TLC plates were air dried and removed. The solvent moved 15 cm from origin. The color component detected on TLC plate in visible light. The TLC plates were placed in iodine chamber and examined under visible light. The TLC plates recorded by photography. The movement of the compounds calculated with Rf value.

$$Rf = \frac{\text{distance from origin to analyte } xa}{\text{distance from origin to solvent front } xt}$$

Gas chromatography and mass spectrophotometer:

Gas chromatography plays a role in separation and introduces target substance into an ms system by directly injecting analytes into a chromatographic column after injecting and heating of plant crude extract (*O. sanctum*- leaves and stem).

Basic procedure of GC-MS:

The GC -MS were subjected at the council of sathyabama institute of science and technology in Chennai for the determination of bioactive volatile compounds. GC-MS analysis of the sample carried out using 6890 GC with 5973 I MSD. Helium was used as the carrier gas, and the temperature programming were set with initial oven temperature at 400°C subjected at the council for 3 min and the final temperature of the oven

were at 4800 °C with a rate at 100°C .a 2 μ l sample was injected with split less mode .mass spectra were recorded over 35-650 Amu range with electron impact ionization energy 70ev .the total running time for a sample is 20 min. the chemical components from the hexonic extract of plants were identified by comparing the rentention time of chromatographic peak using QUADRA pole detector .

Antimicrobial activity:

Microorganisms used for antimicrobial activity:

The sample collected from UTI patients have the large number of gram positive cocci and *Candida spp.*. So those organisms used for the antimicrobial studies and MIC (MBC and MFC) study.

Determination of the antimicrobial activity:

Assay of antimicrobial activity of *O. sanctum* plant extract was done by agar

Well diffusion method, MIC (MBC and MFC).

Screening of antibacterial activity:

Well diffusion assay:

The susceptibility of UTI isolates to the extracts was determined by agar well diffusion method (**Anjana Sharma, Rani Verma and Padmini Ramteke, 2009**) with a slight modification. Agar well diffusion method was adopted to assess the antibacterial activity of *Ocimum sanctum*- leaves, stems, seeds extracts against pathogens. For the test, Muller-Hinton agar plates were swabbed with test organism by using 8mm borer make a gentle well on MHA plate. Each well was loaded with 250 μ g/ml of corresponding concentration of sample extracts. After incubation for 24 hrs, the antibacterial efficiency of the extract was determined by measuring the zone of inhibition formed around the well.

Screening of anticandidal activity by well diffusion assay:

In case of yeast, *Candida* spp., Sabouraud's Dextrose Agar (SDA) used a medium and antifungal efficacy was evaluated by same agar well diffusion assay method. The diameter of inhibition zone was measured after 72 hrs incubation at 37°C.

Minimal inhibitory concentration:

Minimum inhibitory concentration is defined as the lowest concentration of an antimicrobial that will inhibited the visible growth of a microorganisms after overnight incubation and minimal bactericidal concentration as the lowest concentration of antimicrobial that will prevent the growth of an organisms after sub culturing on antibiotic free medium. Mics are used by diagnostic laboratories mainly to confirm the resistance but most often as a research tool to determine the in vitro activity of new antimicrobials properties present in medicinally valuable plants.

Determination of minimum inhibitory concneteration (mic):

Macrodilution method:

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate in test tubes. To 0.5 ml of varying concentrations of the extracts (5, 25, 50, 75, 100, 125, 150, 175 and 200 mg/ml) in test tubes, Nutrient broth (2 ml) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. A tube containing Nutrient broth only was seeded with the test organisms, as described above, to serve as controls. The culture tubes were then incubated at 37°C at 24 hrs for bacteria and at 48 hrs for yeast or fungi. After incubation the tubes were then examined for microbial growth by observing for turbidity.

Microdilution method:

The MIC values were defined as the lowest concentration of extracts that inhibited growth. Extract MIC a value was evaluated using a microtiter dilution assay according to **Elshikhet al.**, with a slight modification: assays were carried out under aseptic conditions in 96 well microtiter plates (**Nunc, Roskilde, Denmark**).

- The first column of each microtiter plate was filled with 100 µL of test materials (from 1 mg/mL extract stock solution), and the 2nd to 10th wells were filled with 50 µL of MHB. A two-fold serial dilution (throughout 2nd to 10th wells) was achieved by transferring 50 µL of test material wells in the first column to subsequent wells of each row, so that each well had 50 µL of test material in serially descending concentrations.

- From wells in the 10th columns, 50 µL solutions were removed. The working solution of extracts was diluted across the 96-wells using a two-fold serial dilution to give final testing concentrations of 1,000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.9, and 1.95 µg/mL.
 - Each microtiter plate had a set of 2 controls: (a) test organisms without test extract as a (positive control; 11th wells), and (b) test extract without test organisms (12th wells), as a control for contamination during plate preparation. Aliquots (20 µL) of bacterial, yeast or fungal suspensions (test organisms) were added to each well.
 - The plates were incubated in a temperature-controlled incubator at 37°C for 24 h for bacterial and at 48 hrs for yeast or fungi. After the incubation period, were observe the results.

Determination of minimum bactericidal concentration (mbc):

The bactericidal activities of the extracts obtained from *Ocimum sanctum* (leaves, stems and seeds extracts) were tested. The number of the bacteria in the initial microorganism suspensions was counted by the surface plate method. After the determination of MIC, the number of bacteria was counted in each of the tubes of broth that showed no visible turbidity after overnight incubation, and was compared with the number of bacteria in the initial microorganism suspension. According to NCCLS (1997), the lowest concentration of the extract solution that allowed less than 0.1% of the original inoculum to survive was taken to be the minimum bactericidal concentration.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by spot method. Nutrient agar plates only were also spotting with the test organisms and plant extract to serve as negative and positive controls respectively. All the plates were then incubated at 37°C for 24hrs. After incubation the concentration at which no visible growth was seen was noted as the Minimum Bactericidal concentration (MBC).

Determination of minimum fungicidal concentration (mfc):

To determine the MFC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile PDA by spot method. PDA plates only were also spotting with the test organisms and plant extract to serve as negative and positive controls respectively. All the plates were then incubated

at 37°C for 24 - 48 hrs. After incubation the concentration at which no visible growth was seen was noted as the Minimum Fungicidal concentration (MFC).

Result

In the present investigation was made to prove the validity of our traditional medicine has been undertaken to evaluate the phytochemical and antimicrobial activity of *Ocimum sanctum* against UTI caused by Gram Positive Coccii and *Candida spp.*

The Gram Positive Coccii and *Candida spp* were isolated from the urine sample of UTI patient. The samples were grown on CLED media and Macconkey media for the identification. Gram Positive Coccii in cluster and Diplococci were identified by gram staining. Biochemical test like indole production test, methyl red test, vogesproskaver's test, citrate utilization test, urease production test, triple sugar iron test, catalase, oxidase, Mannitol motility agar test, Bile esculin agar test and DNase test were performed to confirm the *Staphylococcus sp.* and *Enterococcus sp.* it shows on the **Chart 1** and **Table 1**.

Staphylococcus aureus were grown in MSA media, it produce golden yellow colonies. *Staphylococcus aureus* and *Enterococcus* were grown in blood agar media, it produce haemolytic colonies. The results were shown in **Table 2**.

The identification of isolated fungi by means of lactophenol cotton blue staining and germ tube test to confirm the *candida spp*. The organisms were further screened by their colony morphology PDA and CHROM agar. The results were shown in the **Table 3**.

In antibiotic sensitivity test, among the 41 isolates, highest resistance were seen for Co-Trimoxazole followed by Linezolid, Clindamycin, Piperacillin, Cefoxitin, Vancomycin, Cefazolin, Nitrofurantoin and Teicoplanin. All isolates showed 100% sensitive to Ciprofloxacin, Erythromycin, Tetracycline and Cefotaxime. The results were shown in **Chart 2** and **Table 4 and 5**.

The *S. aureus* isolates were screened for methicillin resistance by cefoxitin disc diffusion method. To phenotypically confirm *S.aureus* and methicillin resistance. 13 of the 21 *S.aureus* isolates were resistant to methicillin (MRSA).The results were shown in **Chart 3** and **Table 6**.

The *Ocimum sanctum* (leaves, stem and seed) were powdered and used for microbial growth inhibition properties. The **Figure 10** shows the *Ocimum sanctum* plant and powder.

Sequential extraction of plant:

The leaves, stem and seed of *Ocimum sanctum* were prepared by dissolving 100g of plant powder in 300 ml of respective solvents, such as hexane and ethanol. It can be filtered by whatmann no.1 filter paper and are kept at room temperature for evaporation or artificial evaporator is used for evaporation process and the extracts were shown as **Figure 11** and **Table 7**. The amount of yield percentage was obtained from this plant extract are:

The dry weight of <i>Ocimum sanctum</i> leaves hexane extract was	=0.745 g
The dry weight of <i>Ocimum sanctum</i> leaves ethanol extract was	=2.156 g
The dry weight of <i>Ocimum sanctum</i> stem hexane extract was	=0.501 g
The dry weight of <i>Ocimum sanctum</i> stem ethanol extract was	=3.721g
The dry weight of <i>Ocimum sanctum</i> seed hexane extract was	=0.746 g
The dry weight of <i>Ocimum sanctum</i> seed ethanol extract was	=4.079g

Ocimum sanctum extracts (leaves, stem) were screened for phytochemical potential. The results were shown in **Table 8**.

The screening of microbial growth inhibition properties of *O. sanctum* extract against uropathogenic organisms by spot overlay method. The screening of antimicrobial activity of *O. sanctum* against ATCC strains. The results were shown in **Figure 12** and **Table 9**.

The antioxidant potential of *O. sanctum* extracts of screened. It shows high reducing power. The results were shown in **Table 10 A. and 10 B.**

O. sanctum extract subjected to thin layer chromatography to analysis is microbial growth inhibition properties. To confirm the presence of *O. sanctum* as greenish yellow coloured spots on TLC plates were observed after the spraying of ninhydrin reagent. The **Figure 13 and Chart 4** shows the TLC plate.

The bioactive volatile compounds of *Ocimum sanctum* were confirmed by using GC-MS. The results were shown in **Chart 5 and Table 11**.

The screening of microbial growth inhibition of *O. sanctum* towards the commercial antibiotic resistance bacterial microbes by using well diffusion assay. The microbial growth inhibitions of *O. sanctum* were high and stable

compared to the standard antibiotic disc. The results were shown in **Chart 6** and **Table 12**.

The screening of microbial growth inhibition of *O. sanctum* towards the commercial antibiotic resistance fungal microbes (*Candida sp.*) by using well diffusion assay. The microbial growth inhibitions of *O. sanctum* were high and stable compared to the standard antibiotic disc. The results were shown in **Chart 7** and **Table 13**.

The MIC (MBC and MFC) carried for the minimum inhibitory concentration of *O. sanctum* towards antibiotic resistance bacterial and fungal. The growth inhibition of *O. sanctum* is great when compared to the antibiotics. The results were shown in **Table 14** and **15**.

The present investigation showed that our traditional medicine *O. sanctum* shows the maximum level of microbial growth inhibition properties against the uropathogenic organisms than the chemical drugs. Further more studies are needed in future on *Ocimum sanctum* to increase the best efficiency this product obtained from the plant which compared to the standard antibiotics.

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Table 1: characteristics of clinical (uti) bacterial isolates

SL NO.	Isolates	Gram Staining	Biochemical identification										
			I	MR/ VP	C	U	TSI	MM	B	DNase	CA	O	CO
1	172	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
2	109	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
3	107	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
4	85	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
5	56	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
6	55	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
7	16	GPC	-	+/+	+	+	+A/G-	+NM	-	-	+	-	-
8	5	GPC	-	+/+	+	+	+A/G-	+NM	-	-	+	-	-
9	27	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
10	34	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
11	32	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
12	20Lf	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
13	131	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
14	136	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
15	46	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
16	89	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
17	115	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
18	117	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-

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19	118	GPC	-	+/+	+	+	+A/G-	+NM	-	-	+	-	-
20	124	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
21	162	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
22	156	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
23	188	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
24	175	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
25	149-1	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
26	44-2	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
27	159	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
28	173	GPC	-	+/+	+	+	+A/G-	+NM	-	-	+	-	-
29	181	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
30	39-2	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
31	39-1	GPC	-	+/+	+	+	+A/G-	+NM	-	-	+	-	-
32	N ₂ Pale	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
33	40	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
34	6s	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
35	2Lf	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
36	1 Tiny	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
37	5s	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
38	41	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
39	61	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+
40	177	GPC	-	-/+	-	-	+A/G-	+NM	+	-	-	-	-
41	6N	GPC	-	+/+	+	+	+A/G-	+NM	-	+	+	-	+

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GPC - Gram positive cocci; I – Indole; MR – Methyl Red; VP – Voges Proskauer’s; C – Citrate; U – Urease; TSI – Triple Sugar Iron; MM – Mannitol Motility; B – Bile Esculin; DNase – Deoxyribonuclease; CO – Coagulase; O – Oxidase; CA – Catalase.

Table 2: colony morphology of bacterial isolates

S. No	Sample	Selective medium	Colony morphology	Organisms
1.	Urine	MSA	Yellow colonies	<i>Staphylococcus aureus</i>
2.	Urine	MSA	Red colonies	<i>Staphylococcus saprophyticus</i>
3.	Urine	BEA	Black colonies	<i>Enterococcus</i>

MSA – Mannitol Salt Agar; BEA – Bile Esculin Agar

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Table 3: colony morphology and microscopic morphology of fungal isolates

S NO.	sample/ isolates	medium	colony morphology	microscopic morphology	organisms
1	Urine/A7	PDA	Creamy white, smooth colonies	Ovoid or spherical budding cell which produce pseudomycelia	<i>Candida albicans</i>
2	Urine/A14	PDA	Cream colored with a slightly mycelia border	Oval blastspores which produce true hyphae	<i>Candida tropicalis</i>
3	Urine/A9	PDA	Off-White, Dull and smooth to lobed	Ovoid or spherical cell which produce pseudohyphae	<i>Candida krusei</i>
4	Urine/A4	PDA	Creamy, shiny and smooth or wrinkled	Ovoid or spherical cell which produce pseudohyphae	<i>Candida parapsilosis</i>

Table 4(a): Antibiotic sensitivity test for uti bacterial isolates (staphylococcus)

S. NO.	Name of the antibiotics	Staphylococcus (n=26)
1	Nitrofurantoin	11(42.3%)
2	Co- Trimoxazole	4(15.3%)
3	Ciprofloxacin	11(42.3%)
4	Tetracycline	5(19.2%)
5	Erythromycin	14(53.8%)
6	Clindamycin	20(76.9%)
7	Gentamycin	11(42.3%)
8	Cefoxitin	18(69.2%)
9	Cefotaxime	5(19.2%)

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10	Cefazolin	16(61.5%)
11	Oxacillin	14(53.8%)
12	Linezolid	12(46.1%)

Table 4(b): Antibiotic sensitivity test for uti bacterial isolates (enterococcus)

S. NO.	Name of the antibiotics	Enterococcus (n=15)
1	Nitrofurantoin	10(66.6%)
2	Co- Trimoxazole	13(86.6%)
3	Ciprofloxacin	6(40%)
4	Tetracycline	6(40%)
5	Erythromycin	7(46.6%)
6	Vancomycin	9(60%)

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7	Gentamycin	4(26.6%)
8	Piperacillin	11(73.3%)
9	Teicoplanin	9(60%)
10	Linezolid	12(80%)

Table 5: Antibiotic sensitivity test against fungal isolates

S. NO.	Name of antibiotics	candida sp
1	Amphotericin-B	3 (27%)
2	Itraconazole	9 (81.8%)
3	Fluconazole	9 (81.8%)
4	Voriconazole	3 (27%)

Table 6: Phenotypic confirmation of MRSA

Name of the organisms	total isolates	MRSA	MSSA
<i>S. aureus</i>	21	13	8

Table 7: yield % obtained in ocimum sanctum

1. The amount of yield obtained by hexane extraction method

$$\text{Yield \% for hexane extract of Ocimum leaves} = \frac{0.745 \times 100}{100} \\ = 0.745 \text{ g}$$

$$\text{Yield \% for hexane extract of ocimum stem} = \frac{2.156 \times 100}{100}$$

$$\text{Yield \% for hexane extract of ocimum seed} = \frac{0.501 \times 100}{100} \\ = 0.501 \text{ g}$$

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2. The amount of yield obtained by ethanol extraction method:

$$\begin{aligned}\text{Yield \% for ethanol extract of ocimum leaves} &= \frac{3.721 \times 100}{100} \\ &= 3.721\text{g}\end{aligned}$$

$$\begin{aligned}\text{Yield \% for ethanol extract of ocimum stem} &= \frac{0.746 \times 100}{100} \\ &= 0.746\text{g}\end{aligned}$$

$$\begin{aligned}\text{Yield \% for ethanol extract of ocimum seed} &= \frac{4.079 \times 100}{100} \\ &= 4.079\text{g}\end{aligned}$$

S.NO	SOLVENT USED	TYPE OF EXTRACT	YI ELD% (g)
1.	Hexane solvent	Leaf	0. 745 g
		Stem	2. 156g
		Seed	0. 501g

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2.	Ethanol solvent	Leaf	3. 721g
		Stem	0. 746g
		Seed	4. 079g

Table 8: Preliminary phytochemical analysis of leaves and stem extract of *o. sanctum*

S. NO.	PHYTOCHEMICAL CONSTITUTES	LEAVES HEXANE EXTRACT	STEM HEXANE EXTRACT
1	Tannins	+	-
2	Phlobatannins	+	+
3	Saponins	+	-
4	Flavonoids	+	-

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5	Steroids	+	+
6	Terpenoids	+	+
7	Cardiac Glycosides	+	+
8	Alkaloids	+	+
9	Quinones	+	+

(+) Positive; (-) Negative

TABLE 9: ACTIVITY OF *O. SANCTUM* AGAINST ATCC STRAINS

S.NO	LIST OF ATCC ORGANISMS	ACTIVITY OF <i>OCIMUM SANCTUM</i> AGAINST ATCC AND ZONE SIZE IN MM.					
		LH	SH	SEH	LE	SE	SEE
1.	<i>E.coli</i>	12	13	-	12	-	-

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2.	<i>Pseudomonas</i>	15	13	-	-	-	-
3.	<i>Staphylococcus</i>	12	10	11	15	-	13
5.	<i>Klebsiella</i>	12	16	17	-	11	-
6.	<i>Candida</i>	18	15	15	18	14	14

Table 10 (A): Reducing antioxidant power of ethanol leaf extract of *ocimum sanctum*

S. NO.	LEAF EXTRACT μ L	OCIMUM (nm)
1	Ethanol (control)	0.00
2	1	0.018
3	2	0.074
4	3	0.004
5	4	0.001
6	5	0.002
7	6	0.001
8	7	0.018

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9	8	0.003
10	9	0.002
11	10	0.014

Table 10 (B): Reducing antioxidant power of hexane leaf extract of *ocimum sanctum*

S. NO.	LEAF EXTRACT μ L	OCIMUM (nm)
1	Hexane (control)	0.00
2	1	0.010
3	2	0.034
4	3	0.020
5	4	0.031
6	5	0.033
7	6	0.036
8	7	0.036
9	8	0.039
10	9	0.040
11	10	0.041

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Table 12: antibacterial activity of o. sanctum

S. NO .	Isolates	Organisms	AST-R	antibacterial activity- zone of inhibition (mm)					
				LH	SH	SEH	LE	SE	SEE
1	172	<i>S. aureus</i>	E, GEN	11	-	-	-	-	-
2	109	<i>S. aureus</i>	CD, E, GEN	12	11	-	-	-	-
3	107	<i>S. aureus</i>	NIT, LZ, CD, CX, CTX, OX	13	13	-	-	-	-
4	6N	<i>S. aureus</i>	NIT, LZ, CD, CX, CTX, OX, MRSA, CIP, E	18	16	13	22	15	15
5	56	<i>Enterococcus</i>	COT, TEI	-	12	13	-	-	12
6	16	<i>S. saprophyticus</i>	NIT, LZ, CD, CIP, CX, CTX, OX, E, COT, CZ	13	-	14	-	-	-

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7	55	<i>Enterococcus</i>	COT, TEI	-	13	14	-	-	13
8	34	<i>Enterococcus</i>	NIT, GEN, CIP, TE, LZ, TEI, PI, COT	12	11	15	-	-	-
9	32	<i>S. aurues</i>	NIT, LZ, CD, CX, CTX, CZ, OX	12	-	-	-	-	-
10	20Lf	<i>Enterococcus</i>	CIP, E, VA	20	17	16	-	14	15
11	136	<i>Enterococcus</i>	NIT, CIP, TE, LZ, TEI, PI, E, COT, VA	18	19	21	17	17	16
12	46	<i>Enterococcus</i>	NIT, LZ, TEI, PI, E, COT, VA	-	12	-	14	-	-
13	115	<i>Enterococcus</i>	NIT, LZ, PI, COT, VA	11	-	15	16	14	11
14	117	<i>Enterococcus</i>	NIT, LZ, PI	13	-	-	15	13	14
15	124	<i>S. aurues</i>	NIT, CIP, LZ, CD, CX, OX, CZ	21	16	17	18	15	14
16	162	<i>Enterococcus</i>	CIP, TE, LZ, VA, PI, COT	16	16	-	14	14	-

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17	156	<i>S. aurues</i>	GEN, CIP, OX	11	-	-	14	15	-	-
18	175	<i>S. aurues</i>	CD, CZ	11	-	10	-	-	-	-
19	149-1	<i>Enterococcus</i>	NIT, GEN, CIP, TE, LZ, PI, E, COT, VA	-	-	-	-	13	-	-
20	159	<i>S. aurues</i>	GEN, CIP, TE, LZ, CD, CX, CTX, E, COT, OX, LZ	-	11	-	15	-	-	-
21	181	<i>Enterococcus</i>	NIT, CIP, GEN, TE, LZ, TEI, PI, E, CO	-	16	-	15	13	16	
22	39-2	<i>S. aurues</i>	MRSA, CD, E, GEN, OX, CX, CIP	14	-	-	-	-	-	-
23	N ₂ pale	<i>S. aurues</i>	CD, E, GEN, CZ	11	-	12	-	-	-	-
24	2Lf pale	<i>S. aurues</i>	NIT, CIP, LZ, CD, OX, CX, E	-	-	12	-	11	-	-
25	1 Tiny	<i>S. aurues</i>	OX, E, GEN, CIP, CX	-	-	-	13	-	-	-

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26	5S	<i>S. aurues</i>	OX, CD, E, CZ, CIP, GEN, CX	15	12	14	14	-	-
27	41gpc	<i>Enterococcus</i>	E, COT, TEI, VA, LZ	20	20	17	18	-	-
28	177	<i>Enterococcus</i>	NIT, TE, LZ, TEI, PI, COT, VA	20	23	22	18	17	18

NIT-Nitrofurantoin; GEN- Gentamycin; CIP- Ciprofloxacin; TE-Tetracycline; E- Erythromycin; C- Clindamycin; COT- Co-Trimoxazole; LZ- Linezolid; PI- Piperacillin; VA- Vancomycin; TEI- Teicoplanin; CX- Cefoxitin; CTX- Cefotaxime; CZ- Cefazolin; OX- Oxacillin.

Table 13: Antifungal activity of *o. sanctum*

S. NO	ISOLATES	ORGANISMS	AST-R	ANTIFUNGAL ACTIVITY- ZONE OF INHIBITION (mm)					
				LH	SH	SHE	LE	SE	SEE
1	80y	<i>C. albicans</i>	AMP, IT, FLU	14	14	14	14	-	13

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2	107y	<i>C. albicans</i>	AMP, IT, FLU	13	-	13	14	-	-
3	A14	<i>C. tropicalis</i>	AMP, IT, FLU	22	19	23	23	20	16
4	A5	<i>C. albicans</i>	IT, FLU	18	16	15	13	-	13
5	A6	<i>C. albicans</i>	IT, FLU	-	-	17	14	-	12
6	A15	<i>C. albicans</i>	IT, FLU	15	-	-	16	-	-
7	A7	<i>C. albicans</i>	IT, FLU	19	16	17	16	16	15

8	21y	<i>C. albicans</i>	AMP, IT, FLU	-	-	11	-	15	-
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LH – Leaves Hexane; SH- Stem Hexane; SEH- Seed Hexane; LE-Leaves Ethanol; SE-Stem Ethanol; SEE-Seed Ethanol

TABLE 14: Minimum inhibitory concentration of *ocimum sanctum* against bacterial isolates
a) Macrodilution method:

S. NO.	NAME OF THE EXTRACT	ANTIBACTERIAL ACTIVITY OF MIC
1	Hexane leaf extract	150µg/ml
2	Hexane stem extract	175µg/ml
3	Hexane seed extract	200µg/ml
4	Ethanol leaf extract	175µg/ml
5	Ethanol stem extract	200µg/ml
6	Ethanol seed extract	200µg/ml

b)Microdilution method:

S. NO.	NAME OF THE EXTRACT	ANTIBACTERIAL ACTIVITY OF MIC
1	Hexane leaf extract	250µg/ml
2	Hexane stem extract	250µg/ml
3	Hexane seed extract	500µg/ml
4	Ethanol leaf extract	250µg/ml
5	Ethanol stem extract	500µg/ml
6	Ethanol seed extract	250µg/ml

TABLE 15: MINIMUM INHIBITORY CONCENTRATION OF *OCIMUM SANCTUM* AGAINST FUNGAL ISOLATES

a) Macrodilution method:

	NAME OF THE EXTRACT	ANTIFUNGAL ACTIVITY OF MIC
1	Hexane leaf extract	175µg/ml
2	Hexane stem extract	200µg/ml
3	Hexane seed extract	175µg/ml
4	Ethanol leaf extract	200µg/ml
5	Ethanol stem extract	200µg/ml
6	Ethanol seed extract	175µg/ml

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b) Microdilution method:

S. NO.	NAME OF THE EXTRACT	ANTIFUNGAL ACTIVITY OF MIC
1	Hexane leaf extract	250µg/ml
2	Hexane stem extract	500µg/ml
3	Hexane seed extract	250µg/ml
4	Ethanol leaf extract	250µg/ml
5	Ethanol stem extract	500µg/ml
6	Ethanol seed extract	500µg/ml

Discussion

The multidrug resistant strains of UTI have become a cause of major health concerns and novel antimicrobial agents are required to tackle this problem. The situation forced the researchers to search for the new and effective antimicrobial agents to replace the current regiments.

The indiscriminate use of commercial antimicrobial drugs can cause multidrug resistance in human pathogenic microorganisms. The extract of *Ocimum sanctum* shows the broad spectrum of antimicrobial activity, anticytotoxic activity, antiproliferative and antidiabetic activity. It is used from olden days as a holy medicine.

Natural products are in great demand for their extensive biological properties and bioactive components which had been proved to be useful against large number of causative agents of diseases. Studies on the natural products of plant origin such as *Ocimum* proved that the released secondary metabolites have strong antimicrobial activity.

The colony morphology, gram staining shows that the golden yellow colonies on MSA medium, purple color cocci and diplococci were observed on gram staining. The biochemical test results as indole negative, methyl red positive and negative, vogesproskaver positive, citrate negative, urease positive and negative, acid production were identified the TSI agar catalase list shows that hydrogen peroxide splits into oxygen and forms effervesance with the organism. The organisms have ability to split H_2O_2 to O_2 it shows catalase positive, oxidase negative, coagulase positive and negative, DNase positive and negative, Mannitol fermented and non motile, Bile esculin negative and positive, hence *Staphylococcus sp.* and *Enterococcus sp.* were respectively. **Similar to our study(Bizani and Brandelli 2002, Saleem et al, 2009)** identified basis of morphological cultural and biochemical character.

The phytochemical properties and antioxidant properties of *Ocimum sanctum* shows high efficacy of antimicrobial activity. The *Ocimum sanctum* have rich source of bioactive phytoconstituents. The *ocimum* evaluated for the transformation of Fe in the presence of reducing power. The reducing power increased with an their reducing ability when the contraction of extracts was increased and the ability to reduce the Fe may increase in sample. **Similar to our study,** The reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) TO Fe (II) in the presence of the sample extracts. The reducing power of plant extracts are reducing increased with an increase in samples, increasing their reducing

ability when the contraction of extracts was increased. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds, which is also related to the presence of reducing agent. In addition, the number and position of hydroxyl group of phenolic compounds also ruled their antioxidant activity (**Sawaddiwong et al, 2014**).

Thin layer chromatography is important for separation and detection of phytoactive components present in plant extract. The *Ocimum sanctum* showed Rf value **0.16** and **0.63**. It revealed the presence of active bioactive compounds against the pathogen responsible for UTI. **Similar to our study** the *O. sanctum* ethanolic extracts showed more number of spots, with Rf value values 0.06-0.94, than the *O.sanctum* aqueous extracts, conferring Rf values 0.29-0.84. The TLC chromatogram revealed the presence of two components (Rf values: 0.13 and 0.63) in *O. sanctum* leaf ethanolic extract, and such components were found active against bacterial pathogens. The chromatographic variation, in TLC study, might be due to the difference in polarity of solvent system, variations in the methods of extraction and the solvents used in the extraction process, stage of maturity of the leaves (**Reddy et al, 2015**).

The GC-MS is the most efficient method for the detection of bioactive components present in the plant extract. The *O. sanctum* shows **16.872** to **48.284** peak level of bioactive compounds. It revealed that the presence of active components in *O. sanctum* have the ability to treat the UTI pathogens.

The antimicrobial activity of *O. sanctum* extracts produce highest zone of inhibition **22mm** (Leaf Ethanol extract), **21mm** (Leaf Hexane extract), on *Staphylococcus sp.* and **23mm** (Stem Hexane extract) on *Enterococcus sp.* moderate zone of inhibition 18mm (Leaf Ethanol) on *Staphylococcus sp.* and **19mm** (Stem Hexane extract) on *Enterococcus sp.* and low zone of inhibition **14mm** (Leaf Hexane extract extract) on *Staphylococcus sp.* and **14mm** (Leaf Ethanol extract and Stem Ethanol extract) on *Enterococcus sp.* **Similar to our study**, The results indicated that table 6, 7,8 and 9 in disc diffusion and tables 10, 11, 12 and 13 in poison plate all plants extracts showed antimicrobial activities toward the gram positive bacteria *S. aureus* as well as gram negative bacteria *E. coli* and *C. albicans*. The methanol extract of all the plants extracts showed more effective result against bacteria and fungi, with the two plants *O. sanctum* effective result in inhibition zones in both methods. Thus, it was evident that in the plant the organic extracts were more effective (**Gomathinayagam Subramanian et al., 2014**).

Anticandidal activity of *O. sanctum* extracts shows maximum zone of inhibition as 16mm to 22mm against *C. tropicalis* and least zone of inhibition as 15mm to 19mm against *C. albicans*. **Similar to our study**, the ethanolic and ethyl acetate extracts of tulsi leaves showed half the amount of inhibitory zone against *C. albicans* (13mm) when compared with betel leaf and the equal amount with standard drug fluconazole. The low candidal activity of the tulsi may be improved via the use of different solvents and different extraction procedures, considering the polarity of the active compound (**Basireddy Siva reddy, et al., 2019**).

The MIC (MBC and MFC) of *O. sanctum* results shows better choice of inhibition **150 μ g/ ml** in bacterial isolates and **175 μ g/ ml** in fungal isolates using hexane and ethanolic extracts. The higher MIC values obtained from the extract of *O. sanctum* attributed to the use of crude extracts of the leaves when compared with the purified form of standard drugs. **Similar to our study**, the efficacy of these leaf extracts was tested by obtained their MIC values. It showed that the MIC value of fluconazole (62.5 μ g/ml) was twice better than those of ethylacetate extract of mature betel leaves (125 μ g/ml). They showed the higher MIC values. Tulsi leaves showed higher MIC values (2000 μ g/ml) than standard drugs (**Nayankara et al., 2014**).

In the study the *O. sanctum* extracts used against multidrug resistant UTI pathogens like *Staphylococcus aureus*, *Enterococcus* and *Candida spp.* This probably explains the use of these plants by indigenous people such as number infections since generations. The *O. sanctum* plant studied here had shown that they are potentially rich in antimicrobial compounds and also have extensively used by the tribal's. The millenaries use of this folk medicine suggests that they represent an economic and safe alternative for the treatment of UTI. The antimicrobial efficacy of *O. sanctum* indicates that the plant possesses potent antimicrobial properties as well as *Ocimum sanctum* is widespread in India. It can be recommended as an easily available, cost effective and renewable source. It is the best alternative and non antibiotic agent against the combat UTI. Further more studies are needed in future for improving the effect of *Ocimum sanctum* against standard commercial antibiotic drugs.

Conclusion

The present study clearly showed that our traditional medicine *Ocimum sanctum* have several bioactive phytoconstituents: phenols, phylobatannins, terpenoids, saponin, quinones, flavanoids, steroids and alkaloids and is said to be a rich source of phytochemical constituents. The extracts of *Ocimum*

sanctum have former microbial growth inhibition property. It can kill uropathogens especially *Staphylococcus aureus*, *Enterococcus spp.* and *Candida spp.* The antimicrobial efficacy of *Ocimum sanctum* indicates that the plant possesses potent antimicrobial properties as well as *O. sanctum* is widespread in India. It can be recommended as an easily available, cost effective and renewable source of antimicrobial agent instead of synthetic chemicals. It is the best alternative and non antibiotic agents in order to combat UTI. This would help to reduce synthetic drugs to which *Candida* have evoled resistance by promoting these traditional medicines. Further more studies are needed on *Ocimum* by using different solvents would increase the efficacy of this plant product compared to standard commercial antibiotic drugs.

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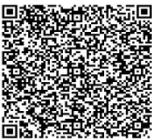
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Population Ecology

N. Uma Maheswari and R.Vishali

PG and Research Department of Microbiology,
STET Women's College (Autonomous), Sundarakkottai,
Mannargudi, Thiruvarur (Dt), Tamil Nadu, India.
Email ID: *umasamyamf@gmail.com*

Introduction

Ecology is the study of interactions between organisms and their environment. Population ecology is the study of the processes that affect the distribution and abundance of animal and plant populations. A population is a group of organisms of the same species occupying a given space at the same time. The geographic boundaries of a population are easy to establish for some species but more difficult for others. For example, plants or animals occupying islands have a geographic range defined by the perimeter of the island. In contrast, some species are dispersed across vast expenses, and the boundaries of local populations are more difficult to determine.

Characteristics of Population Ecology

Ecologists use various terms when understanding and discussing populations of organisms. A population is all of one kind of species residing in a particular location. Population size represents the total number of individuals in a habitat. Population density refers to how many individuals reside in a particular area.

Population Size The population size for unitary organisms, is represented by the letter N, and it equals the total number of individuals in a population. The larger a population is, the greater its generic variation and therefore its potential for long-term survival. Increased population size can, however, lead to other issues, such as overuse of resources leading to a population crash.

Population Density refers to the number of individuals in a particular area. A low-density area would have more organisms spread out. High-density areas would have more individuals living closer together, leading to greater resource competition.

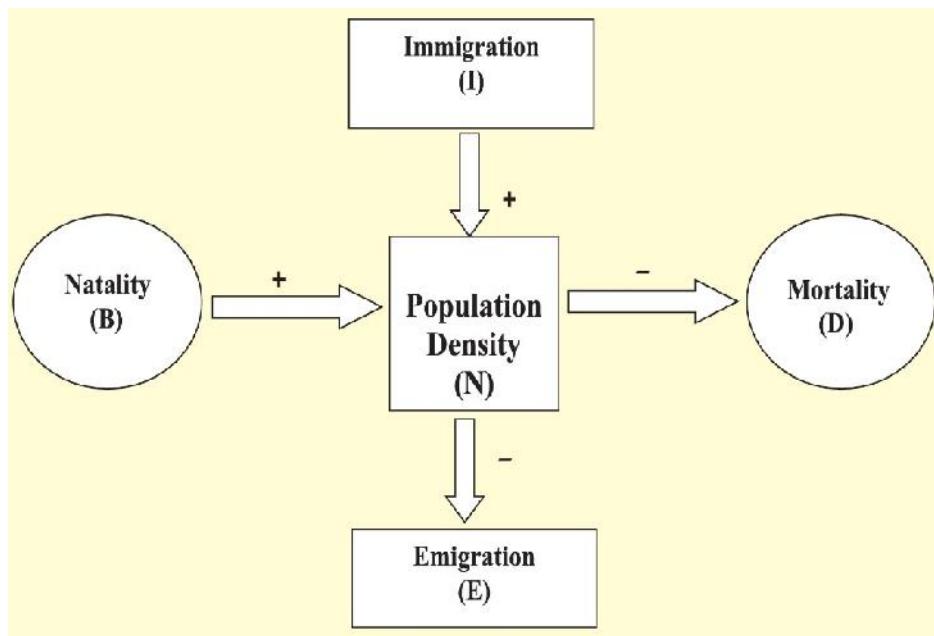


Fig 1: Population Density

Natality

Natality is a broad term that covers production of new individuals (birth, hatching, germination, fission). Fertility is a physiological term indicating that an organism is capable of breeding. Fecundity is an ecological term describing the number of offspring produced during some time period; usually expressed as number of offspring per reproductively active female per unit time.

We distinguish between realized and potential fecundities:

- Realized fecundity rate for humans may be 1 birth per 15 years per female;
- Potential fecundity rate for humans may be 1 birth per 11 months per female of childbearing age.

Consider the evolutionary forces that led to differences in annual natality rate among organisms:

- Oyster — 55 million to 114 million eggs
- Many fish — thousands of eggs
- Frogs — hundreds of eggs
- Birds — 1 to 20 eggs
- Mammals — rarely >10, often only 1 or 2

General patterns? Consider the relationship between natality and the amount of parental care given to the offspring..

Mortality

Mortality is expressed a probability of dying or as a rate; informative when examined for specific time intervals and age classes.

Related to longevity, both potential and realized:

For example, realized longevity for spotted owls is about 8 to 12 years whereas potential longevity(in captivity) is >24 years

Survival and Mortality are converses: $m = 1 - s$; $s = 1 - m$

Survival (or mortality) can be estimated indirectly if we know the abundance of successive age groups in a population.

For example, if a population of bluegill sunfish has:

292 age-II fish 147 age-III fish 54 age-IV fish

then apparent survival between age classes II and III = $147/292 = 0.50$; between age classes III and IV = $54/147 = 0.37$.

Survivorship Curves

Plotting number of individuals remaining alive versus age we generate a survivorship curve, which is standardized to number of survivors per 1,000 births.

There are three general types: • Type I — low morality until older age classes; • Type II — constant mortality that is independent of age; • Type III — high mortality of young, then low mortality.

In nature, survivorship curves never fit these exactly; however, humans in developed nations and Dall sheep are well represented by a Type I curve; some birds and plants have a Type II curve; some fishes, marine invertebrates, and parasites often have a Type III.

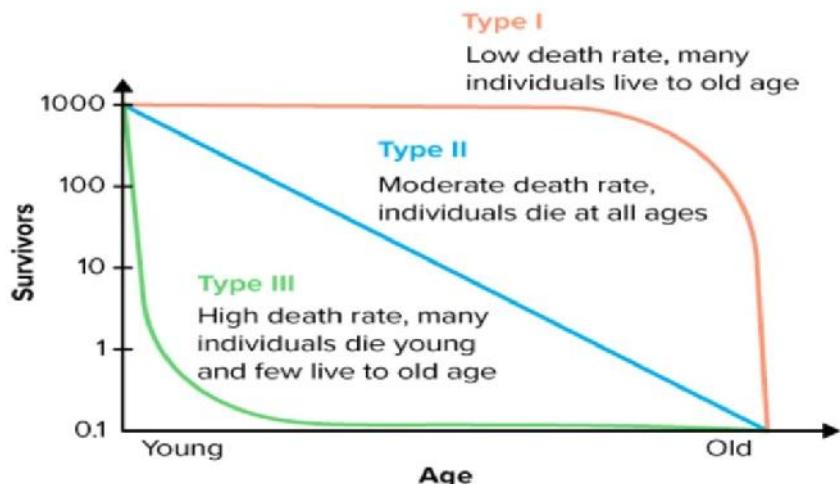


Fig 2: Survivorship Curves

Immigration and Emigration

Immigration and Emigration are permanent movements into and out of a population, and are seldom measured; typically, they are assumed to be equal (?).

Dispersal is defined as leaving an area permanently, often a natal area. Thought to prevent inbreeding and enhance gene flow between populations.

A problem in conservation biology is how to design parks and reserves so immigration and emigration among reserves in a fragmented landscape is facilitated.

Population Dispersion: Yields helpful information about how species interact with each other. Researchers can learn more about populations by studying the way they are distributed or dispersed.

Population distribution describes how individuals of a species are spread out, whether they live in close proximity to each other or far apart, or clustered into groups.

- ❖ *Uniform dispersion* refers to organisms that live in a specific territory. One example would be penguins. Penguins live in territories, and within those territories the birds space themselves out relatively uniformly.

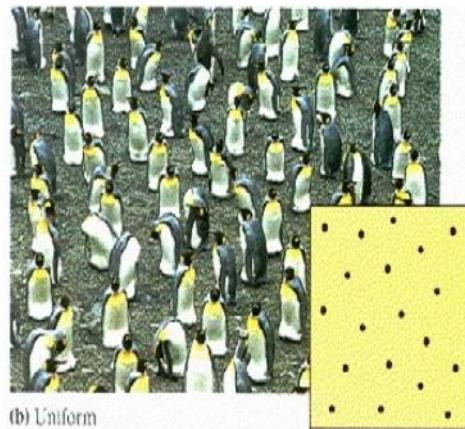


Fig 3: Uniform dispersion

- ❖ *Random dispersion* refers to the spread of individuals such as wind-dispersed seeds, which fall randomly after travelling



Fig 4 : Random dispersion

- ❖ *Clustered or clumped dispersion* refers to a straight drop of seeds to the ground, rather than being carried, or to groups of animals living together, such as herds or schools. Schools of fish exhibit this manner of dispersion.

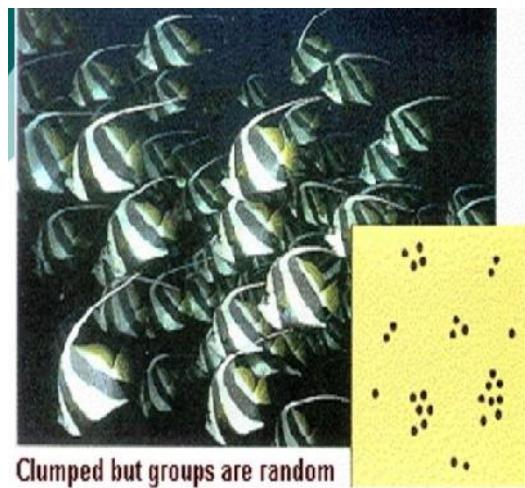


Fig 5: Clustered or clumped dispersion

Age and Stage structure: The age structure describes the number of individuals in each age class as a ratio of one class to another. Age classes can be specific categories, such as years months, or life history stages, such as eggs, larvae, pupae and instars.

A stable age distribution results where the ratio of one age group to the next remains the same and the shape of the age pyramid does not change over time. the shape stays constant because the birth and death rates for each age class are constant.

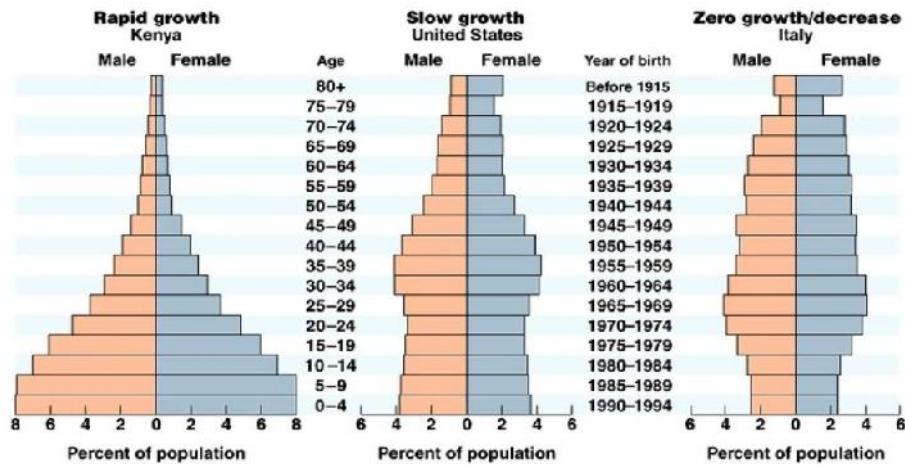


Fig 6: Age and structure

Population growth

A population that is increasing at its intrinsic rate will undergo a geometric increase in population number and will follow the characteristic geometric curve. This can only happen in reality if the population does not run out of resources. Although population numbers increase rapidly, the per capita rate of increase (r) remains constant. The geometric increase occurs because as more individuals are added to the population, will be greater than in the previous time interval.

Individuals are added to a population through birth (B) and immigration (I) and are removed as a result of death (D) and emigration (E). If the gains exceed the losses (i.e. if $B + I > D + E$), the population will grow in numbers. The immigration to the original population at time t (N_t) and subtracting the number of deaths and emigrates to give a new population size at the time $t+1$ (N_{t+1}). This is represented by the equation:

$$N_{t+1} = N_t + B + I - D - E$$

In closed populations, experiencing no exchange of individuals, population growth will depend on birth and death rates. Population can be regulated by density. That is their growth rate depends on the size of the population and how close it is to the maximum that the habitat can support. Unregulated and regulated growth are described in more detail below.

For a particular set of conditions, an individual has a maximum potential for reproduction which is its intrinsic natural rate of increase, r . Its name is slightly misleading because its value will be different in different environments as death and birth rates differ. The intrinsic rate of increase is the theoretical maximum that may be reached in a given environment if the population is not resource limited. Populations with finite resources may have positive, negative or zero values of r , where the population is, respectively, increasing, decreasing or static. The intrinsic rate of increase is related to the basic reproductive rate R_0 , such that:

$$r = \ln R_0 / T$$

The parameter r is usually applied to a closed population, where no immigration or emigration occurs, and represents the difference between instantaneous birth and death rates per individual.

Exponential growth

Exponential growth, a population's per capita (per individual) growth rate stays the same regardless of population size, making the population grow faster and faster as it gets larger.

In nature, populations may grow exponentially for some period, but they will ultimately be limited by resource availability.

Exponential growth produces a **J – Shaped curve**.

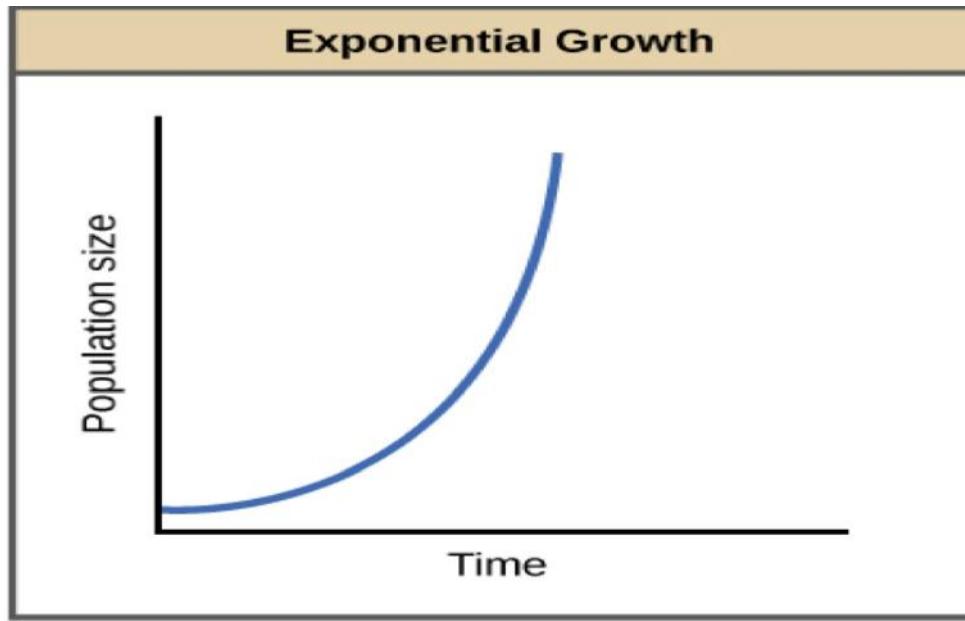


Fig 7: Exponential Growth

Logistic growth

In Logistic growth, a population's per capita growth rate gets smaller and smaller as population size approaches a maximum imposed by limited resources in the environment, known as the carrying capacity.

Logistic growth produces a **S – Shaped curve**

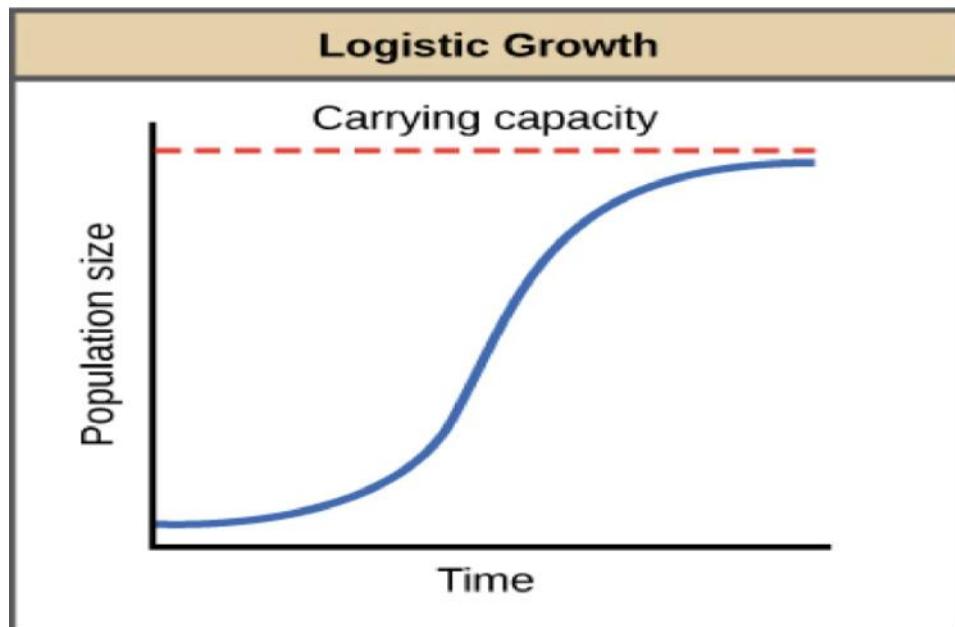


Fig 8: Logistic growth

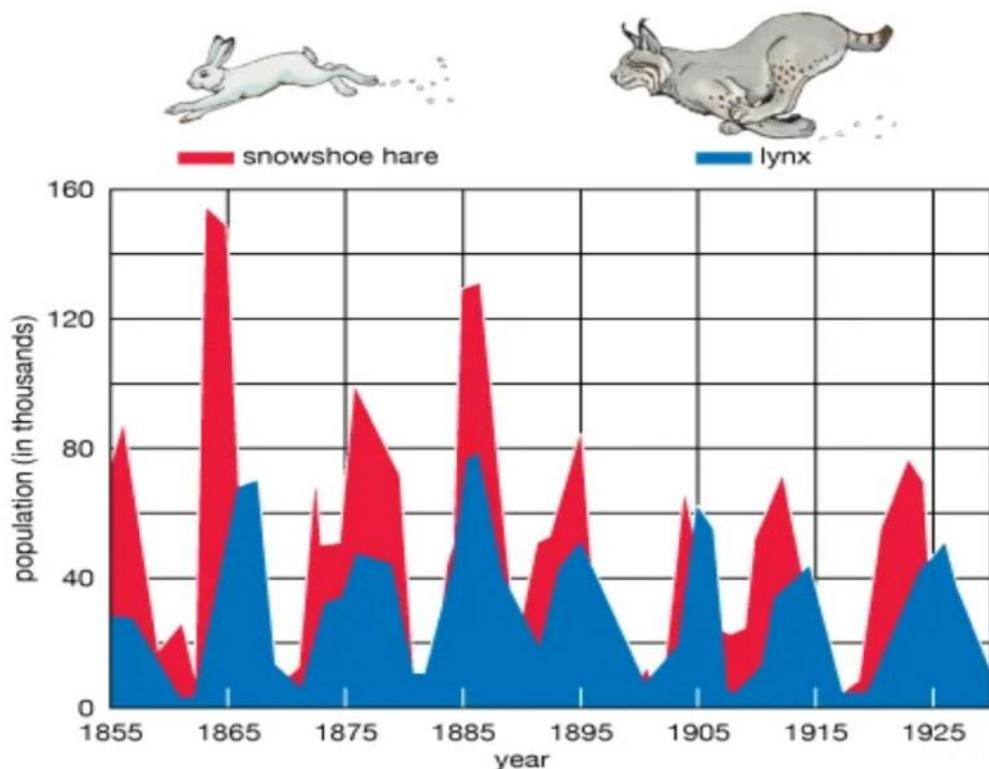
Population fluctuations

Population may fluctuate for a number of reasons:

A time lag between a change in density and its effect on the population size or delayed density dependence. The population can overshoot the carrying capacity and then show gradually diminishing, damped oscillations before eventually stabilizing at equilibrium. This delayed density dependence may also produce cycles in predator and prey abundance;

Overcompensating density dependence. This can lead to damped oscillations stable limit cycles or chaotic fluctuations that appear random;

Environmental stochasticity. This is a nondeterministic, unpredictable variation in the environmental conditions, resulting in a changing equilibrium density

**Fig 9: Population Fluctuations**

Conclusion

In general, the population size and its growth rate are instable parameters, which are highly sensitive to the effects of abiotic, biotic and anthropogenic factors. For this reason people should realize all the features of the population, which is somehow maintain, to ensure its sustainable long term existence.

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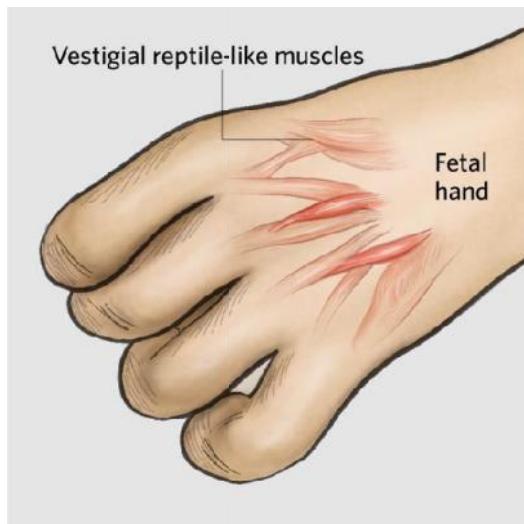
Reptile-like muscles in fetuses

K.Dhivya Dharshini & M. Kannahi

PG and Research Department of Microbiology, STET Women's College
(Autonomous), Sundarakottai, Mannargudi (Tk),
Thiruvarur (Dt), Tamilnadu, India.
Contact – 9626457136, 6369512389
Email Id: dhivyakarunakarn2105@gmail.com, kannahiamf@gmail.com

Introduction

Last October, researchers reported that muscles typically seen in reptiles and other animals—but not people—were present in the limbs of human embryos. Using a combination of immunostaining, tissue clearing, and microscopy, the team generated high-resolution 3-D images of upper and lower limb muscles in tissue samples from preserved 8- to 14-week-old embryos and fetuses. These structures, which disappear before birth, may be anatomical remnants of our evolutionary ancestors that disappear during the early stages of development, the authors suggest. They only examined 13 images, however, so experts caution that it's a preliminary finding that needs to be replicated in a larger sample.



A brief review:

In the last few years, a cluster of anatomical discoveries has been reported which overturned the long existing dogmas about the structure and function of human body. First to come was the discovery that established the existence of a lymphatic system pertaining to the central nervous system (CNS). CNS was believed to be anatomically immune privileged owing to the absence of any lymphatics and presence of the blood-brain barrier around it, but latest research has established beyond any reasonable doubt that true lymphatic channels carry immune cells in meninges thus challenging the existing theory. Studies also supported the presence of a 'Glymphatic system' (created by the perivascular spaces lined with the leptomeninges and a sheath of glial cells) in the CNS draining interstitial metabolic waste from CNS. The second discovery unraveled the previously unknown parts of the human mesentery in adult and established that it is a continuous entity all along the intra-abdominal gut tube against the previous notion that it is fragmented in the adult humans. A very recently reported third discovery demonstrated a previously unknown tissue component-'interstitium'-a networked collagen bound fluid-filled space existent in a number of human organs. All these structures bear considerable applied importance towards the pathogenesis, prognostic and diagnostic investigations and management of human diseases. This article attempts to present a brief review of all three remarkable discoveries and emphasizes their applied importance within the realm of medical sciences.

Do fetuses have muscles?

Only a small number of primary muscle fibers develop during the embryonic period, which will serve as templates for the formation of secondary muscle fibers during the fetal stage.

Diogo pulled 13 3D images from the embryonic image database, representing embryos and fetuses between roughly 7 and 13 gestational weeks old. His team found that, at about week 7 of gestation, human fetuses have hands and feet that contain about 30 muscles each, but the number dwindles to just 20 about six weeks later.

Medical Scan Reveals Babies In The Womb Have Lizard-Like Muscles In Their Hands

A medical scan recently revealed that babies in the womb actually have lizard-like muscles in their hands.

It all goes back to evolution and scientists- as well as biologists- dating these "remnants of our evolutionary history" back as far as 250 million years

ago. This is when biologists noted that reptiles transitioned from their reptilian selves to mammals. Biologists are still not sure why human fetuses develop with lizard-like features and then lose them before birth.

New 3D scans show how a baby's head changes shape during birth:

It almost doesn't make sense, but one theory is that the step that makes our thumbs dextrous since thumbs require an extra muscle unlike the rest of our fingers. You might have noticed that earlier in the article we mentioned that babies will lose "most" of all the lizard-like muscle development in their hands before birth.

This is because of the fact that there have been cases of both children and adults presenting extra muscles in their fingers and hands but doctors and scientists say that those instances are extremely rare. Furthermore, when biologists looked at the 3D medical scans of the embryos and fetuses at seven to 13 weeks' gestation time, they found that these cases of adults and children presenting with extra hand and finger muscles didn't match up with all of the muscles (scientifically referred to as dorsometacarpales) as the unborn babies in medical scans.

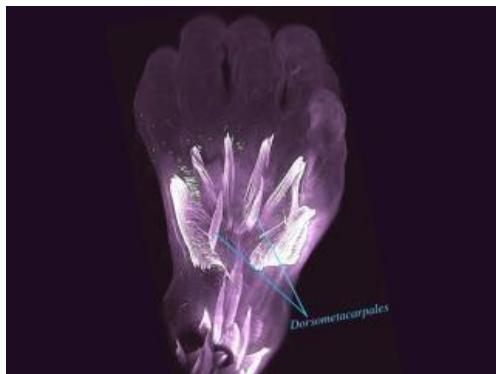
Dr. Rui Diogo's statement:

Dr. Rui Diogo from Howard University stated that adults and children with existing dorsometacarpales may help doctors and biologists understand more about babies who are born with limb deformities.

"We have a lot of muscles going to the thumb, very precise thumb movements but we lost a lot of muscles that are going to the other digits," Dr. Diogo said. "These muscles were lost 250 million years ago. "No adult mammal, no rat, no dog has those muscles. It's impressive."

Tiny 'Lizard-Like' Muscles Found in Developing Embryos Vanish Before Birth:

Detailed 3D images of embryos reveal that some muscles form and then vanish during early human development.



The hand of a 10-week-old human embryo with atavistic (relating to an ancestor) muscles called dorsometacarpales labeled. (Image credit: Rui Diogo, Natalia Siomava and Yorick Gitton)

In the womb, developing humans grow extra muscles in their hands and feet that later disappear without a trace, scientists have discovered.

The temporary tissues, the researchers found, may be leftovers from our evolutionary ancestors.

The mysterious muscles can be found in limbed animals with more dexterous digits than ours, explained study co-author Rui Diogo, an evolutionary biologist and hominid paleobiologist at Howard University in Washington, D.C. Many of the muscles crop up in lizards, which sport fantastically wiggly toes, while a couple of them appear in mammals like chimpanzees, known for their flexible feet. However, in humans, the tissues either fuse to other muscles or shrink away to nothing before birth, according to the small study, published Oct. 1 in the journal [Development](#).

The authors suggest that some of the transient muscles may have vanished from our adult ancestors more than 250 million years ago, as mammals began evolving from mammal-like reptiles. Given the study's small sample size, though, it remains to be seen whether these muscles appear in all human embryos and what that may mean for human evolutionary history.

The muscles "were present [in the developing embryos] and then they were not, but then in-between, there's something that's not known," he said. "What is inducing this disappearance of the muscles?"

Looking under the skin:

Though Chédotal was not involved in the current research, data from his lab fueled the investigation into fetal muscle development. In 2017, Chédotal and his colleagues published a collection of detailed 3D snapshots of human embryos and fetuses the likes of which had not been seen before. The team used a technique called "whole-mount immunostaining" to render the skin of their samples transparent and highlight specific kinds of cells within the tissue. Using antibodies that latch onto myosin, a protein found only in muscles, the researchers captured various stages of human muscle development in high-resolution.

As an anatomist, Diogo had the skill to spot unusual muscles lurking in the images of fetal hands and feet, Chédotal said. Diogo pulled 13 3D images from the embryonic image database, representing embryos and fetuses between roughly 7 and 13 gestational weeks old. His team found that, at about week 7 of gestation, human fetuses have hands and feet that contain about 30 muscles each, but the number dwindles to just 20 about six weeks later.

For example, a muscle in the hand known as "contrahens 5" connects to the pinky finger and pulls the digit down and toward the midline of the hand. The muscle appears in adult monkeys and developing human embryos, but the researchers observed that around gestational week 10, the tissue begins to degrade and completely disintegrates before week 11. In the feet, muscles that lie between the metatarsal bones in the feet and pull the toes together fully form and then break down by week 9.

Though some muscles appeared to degrade or fuse into other muscles as early as week 7, some persisted well into week 11, "which is strikingly late for developmental atavisms," Diogo said in a statement.

"These are muscles that we know were present in our ancestors ... and they are still there," Diogo said of the transient structures. The muscular remnants are known as atavisms — anatomical structures that have been lost in certain organisms but might appear during embryonic development or in adults as variations or anomalies.

Although humans normally lose one-third of their atavistic limb muscles before birth, according to the study, on rare occasions, a muscle or two persists through the pruning and hangs around into adulthood, Diogo said. The lingering muscles often go unnoticed, neither causing problems nor granting their owner super-nimble digits, but appear to be significantly more common in individuals with developmental delays, such as those with Down syndrome or

Edwards syndrome. It may be that people are more likely to retain atavistic muscles when they experience arrested or delayed development in the womb, the authors suggest.

The study provides the "first precise atlas" of embryonic limb development in humans, Delphine Duprez, a developmental biologist at the Institute of Biology Paris-Seine, told Live Science in an email. However, she added that the results have yet to be verified and may prove difficult to confirm, given that it remains "difficult to study muscle development in human embryos as compared to animal models."

In hopes of easing embryonic research, Chédotal and his lab members are continuing to build up their database of images. Now, they can tag up to eight tissue types with different antibodies at one time, meaning they can show how arteries, nerves and muscles interact in early human development. Muscles need to be hooked up to nerves and well supplied with blood to survive, so the detailed data may allow scientists like Diogo to piece together exactly when, why and how muscles disappear in the womb, he said.

The growing database, which is already available for public use, will eventually be adapted so that it is compatible with virtual reality and other platforms that enable users to interact with 3D images, Chédotal added. He hopes that the database will prove useful to everyone from acclaimed researchers to medical students, who up until now have studied fetal development from decades-old illustrations in textbooks, he said.

Diogo plans to use images from the database to study how the human head, arteries and nerves develop in utero. Beyond unearthing new details of human evolutionary history, Diogo said he aims to help medical professionals predict exactly what lies beneath their patients' skin. If researchers could predict which anatomical variations might be present in a particular patient, he suggested, doctors could be better prepared for surgery and generally deliver superior care.

Ancient reptilian limb muscles found in human fetuses reveal evolutionary remnants:

A compelling new imaging study has looked at the development of human limb muscles in embryos across the early weeks of gestation. The research discovered lizard-like muscles temporarily develop before ultimately disappearing by birth. It is suggested these extra reptilian muscles represent distant traces of our evolutionary history.

Current Research in Life Sciences

In the field of biological science an atavism is an ancient trait that can be seen in a modern organism. Perhaps the most well-known human atavism is the temporary presence of a tail. For a very short time while in the womb, a fetus develops a tail, but by the eighth week of gestation this tail has generally disappeared, its temporary existence a sign of our ancient ancestral form.

Thanks to technological advances, evolutionary biologists are now able to track human fetal development with unprecedented detail.

"It used to be that we had more understanding of the early development of fishes, frogs, chicken and mice than in our own species, but these new techniques allow us to see human development in much greater detail.

The team examined a number of 3D images tracking embryo development between weeks seven and 13 of gestation. The focus was particularly on muscle development in the hands and feet. Strikingly, the researchers found that there are 30 separate muscles in each limb by around week seven of gestation. By week 13 this number has dropped to around 20 individual muscles, with some of these atavistic muscles either fusing with others, or simply degrading and disappearing entirely.

"What is fascinating is that we observed various muscles that have never been described in human prenatal development, and that some of these atavistic muscles were seen even in 11.5- weeks-old fetuses, which is strikingly late for developmental atavisms," says Diogo.

This new discovery certainly raises quite a few questions that future research will hopefully be able to answer. The small sample size of the study, only 15 babies, means further verification in larger datasets is necessary before it can be concluded this is a universal developmental process. It is also unclear exactly what happens to these muscles to cause them to ultimately disappear.

The hypothesis that they are atavistic muscles points to an evolutionary divergence about 250 million years ago. No trace of these specific muscles can be found in any living adult mammal, but these extra limb muscles can be identified in some reptiles, such as lizards featuring ultra-flexible feet.

Diogo notes that in very rare instances these muscles can be detected in adult humans. Sometimes they are found in healthy adults with no noticeable effects, but other times they can be directly identified to be the result of congenital malformations.

"This reinforces the idea that both muscle variations and pathologies can be related to delayed or arrested embryonic development, in this case perhaps a delay or decrease of muscle apoptosis, and helps to explain why these muscles are occasionally found in adult people," says Diogo. "It provides a fascinating, powerful example of evolution at play."

Human embryos have extra hand muscles found in lizards but not most adults:

Muscles in the back of a 10-week-old human embryo's hand called dorsometacarpales (the two smallest horizontal muscles highlighted at center) will be lost or fuse with other muscles during development.

Human embryos are more muscle-bound than adult humans, new microscope images cataloging early development show.

For instance, at seven weeks of gestation, embryonic hands have about 30 muscles. Adults have about 19. Many of the muscles are lost, and some fuse with others, adopting the adult arrangement by 13 weeks of gestation, researchers report October 1 in *Development*.

Muscles in the feet, legs, trunk, arms and head also appear and disappear during development, researchers discovered after analyzing detailed 3-D images of human embryos and fetuses up to 13 weeks of gestation.

These appearing and disappearing, or atavistic, muscles are remnants of evolution, says biologist Rui Diogo of Howard University in Washington, D.C. Such atavistic muscles are built as a base from which to start paring down to the final set of muscles that people are born with, he says. "Losing and specializing, that's what happens in human evolution."

Other animals have kept some of those muscles. Adult chimpanzees and human embryos have epitrochleoanconeus muscles in their forearms, but most adult humans don't. Human's mammalian ancestors also lost dorsometacarpales muscles from the back of the hand about 250 million years ago as mammals and reptiles split on the evolutionary tree. Lizards still have those muscles, and they appear in human embryos, but then are lost or fuse with other muscles during development and aren't found in most adults.

Sometimes, people retain some of the usually lost muscles, resulting in harmless anatomical variations. For example, about 13 percent of people in one study had epitrochleoanconeus muscles in their forearms.

Conclusion

The unprecedented resolution of the 3D images revealed the transient presence of several of such atavistic muscles.

The team then compared their observations with few earlier studies that focused on the development of arm and leg muscles in humans, in order to provide information summarizing the timing of appearance, as well as the splitting, fusion and/or loss of each of these muscles.

“It used to be that we had more understanding of the early development of fishes, frogs, chicken and mice than in our own species, but these new techniques allow us to see human development in much greater detail,” said Dr. Diogo, a researcher in the Department of Anatomy at the Howard University College of Medicine.

“What is fascinating is that we observed various muscles that have never been described in human prenatal development, and that some of these atavistic muscles were seen even in 11.5- weeks old fetuses, which is strikingly late for developmental atavisms.”

“Interestingly, some of the atavistic muscles are found on rare occasions in adults, either as anatomical variations without any noticeable effect for the healthy individual, or as the result of congenital malformations,” he said.

“This reinforces the idea that both muscle variations and pathologies can be related to delayed or arrested embryonic development, in this case perhaps a delay or decrease of muscle apoptosis, and helps to explain why these muscles are occasionally found in adult people.”

“It provides a fascinating, powerful example of evolution at play.”

“We hope that our work will not only contribute to the understanding of limb muscle development in humans and in tetrapods in general, but also pave the way for, and stimulate, other researchers to undertake deeper and broader discussions on the links between the upper and lower limbs, between atavisms, variations and anomalies, and between phylogeny and evolution,” the scientists concluded.

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Insect Pest Management

S.Keerthana and M.Kannahi

P.G and Research Department of Microbiology,
STET Women's College (Autonomous), Sundarakottai,
Mannargudi (TK), Thiruvarur (Dt), Tamil Nadu, India
Contact: 9361023865, 6369512389

Email ID: 2004025skeerthana@gmail.com, kannahiamf@gmail.com

Introduction

Insect pests cause enormous loss to litchi through direct and indirect invasion on various plant parts. Use of chemicals has been one of the conventional methods to reduce these losses, however, now-a-days due to various unwarranted side effects, pest management is relied upon many other options along with pesticides. The integration of all these options is called IPM (Integrated Pest Management). Integrated Pest Management is a strategy to manage pests on the basis of a systems approach that looks at the whole orchard ecosystem. This includes understanding how the pests interact with their plant hosts, with the general climatic conditions, plant health and nutrition and with each other. When implementing an IPM system, growers should select ways to reduce overall pest levels in their orchard and ensure that the management of pests is compatible with their other crop management strategies. It is important that growers realize that IPM system is updated from time to time in response to biological changes that occur in their orchard and new techniques or technologies are introduced as soon as additional relevant information becomes available.

Integrated pest management

There are many insects that are present in the orchard. All of them do not cause economic damage. A few of them (key pest) may cause damage to that extent where initiation of action becomes essential. Do not apply pesticide just by seeing a few insect in the orchard. Identify other climatic and cultural factors that may reduce pest numbers or damage. There are many natural enemies of insect pests; these are called predators and parasite. Recognize these beneficial insects, which are found in unsprayed orchards and reduce pest damage in orchards.

Visit your orchard even if there is no fruit, exclusively for pest record. Monitor the incidence of pests and beneficial insects to determine the abundance, life cycles and levels of damage. If possible, keep a pest record register. Record and summarize the monitoring information so that relationships and seasonal trends of pests' damage and beneficial insects can be recognized within the orchard.

Keep your orchard vigorous. Make attempts to optimized the nutrition of orchard because pest attacks are usually reduced in vigorously growing healthy trees

Realize the centre opening, skirting and hygiene like pruning operations can reduce some pest problems (e.g. scale/ mite) by altering the density of the tree, but may increase others (e.g. shoot borer) by encouraging flush growth.

Important insect pests and management

Nearly 42 insect species and mite pests have been reported to attack litchi trees and fruits in different stages of growth; nevertheless, litchi is relatively free of any serious pests. Fruit borers, bugs, nut borer, leaf rollers and mites are the important pests affecting litchi production.

Erinose mite

Erinose mite is a major litchi pest. The incidence of litchi mite is seen during March which remains active up to June-July. Severe infestation has been observed in Bihar during March-September and its population decline from November-February. However, mites remain active throughout the year in one stage or the other. It is found active on litchi trees from January to October and under hibernation in adult stage under the hairy and velvety growth (erignum) from November to December on the under surface of the leaf.

The adults start multiplying from the end of March and the peak activity is noticed around July. The female adults lay eggs singly at the base of the hair on the lower surface of the leaves. The eggs hatch within 2-3 days and newly emerged nymphs feed on soft leaves

Both nymphs and adults damage the leaves, inflorescence and young developing fruits. They puncture and lacerate the tissues of the leaves with their stout rostrum and suck the cell sap. As a result of its infestation, undersurfaces of the infested leaves show abnormal growth of epidermal cells in the form of hair like velvety growth of chocolate brown colour. In some cases, the mites cause galls or wart-like swellings or depressions on the upper surface of the infested leaves.

Chocolate-brown velvety growth on the ventral surface of leaves indicates the presence of this pest. The attacked leaves become thick, curl, wither and ultimately fall off. The infestation generally begins from the lower portion of the trees and gradually extends upwards. In addition to leaves, the mite also infests the newly formed leaf bud; inflorescence and epicarp of the newly formed fruit.

The attacked leaf buds fail to bear flower or fruit. The growth of green patch on the epicarp indicates the mite infestation on fruits. Young plants and plantlets in nurseries are very susceptible and could even be killed if leaf drop is excessive. Dispersal of the erineum mite from one orchard to the other usually takes place through old infested litchi leaves or by winds. The current of the wind carries the erineum along with the mite from one orchard to the other and initiates infestation in uninfested orchards. Planting material obtained as layers may also be source of infestation if they have been taken from tree with the mites. Later infestations occur when the mites are moved around the orchard by direct contact between trees or carried around by orchard workers, wind and bees.

Management

-) Litchi mite control measures must be preventive. Once the mite is established, it is almost impossible to eradicate, hence depending upon infestation it is recommended that:
-) Layers should be prepared only from non-infested plants.
-) Layer saplings may be sprayed with 0.05 per cent dimethoate when they leave the nursery. Prior to planting out, the operation should be repeated twice at 10-14 day intervals.
-) The leaves should be checked regularly for symptoms over summer and autumn. All trees in an orchard are not to be flushed or infested at the same time. Therefore, branches infested with the mite should be cut off and burnt.
-) After harvesting in June, infested branches must be removed.
-) In September-October, trees must be treated just prior to vegetative flushing with 0.05 per cent dimethoate either alone or in combination with 0.12 per cent dicofol. Spraying should be repeated two weeks later and monthly thereafter until new growth is free of all symptoms of infestation. Infested leaves should be gathered and burnt or buried deeply into the ground.

-) In December-January, just before flush/flower buds, the affected shoots must be removed and spraying of 0.15% kelethane may be done. In the month of Feb., two sprays, one each before and after flowering have been found useful.

Bark eating caterpillar

Litchi is damaged to a considerable extent by the bark-eating caterpillars, which attack trees of all ages, particularly the older ones, lowering their vitality. They bore into trunk, main stems and thick branches of litchi trees. They have a wide range of host plants including litchi. The old, shady and neglected orchards are more prone to attack by this pest. When severely infested, the entire branch or tree may die.

The female moth lays eggs in cuts and crevices in the bark in cluster in early June. Egg hatches in 8 to 10 days and newly emerged caterpillars come out. The newly emerged caterpillars start nibbling at the bark. The attack by this pest is characterized by the presence of long-winding, thick, blackish or brownish ribbon-like masses composed of small chips of wood and excreta, both of which intermix with the help of adhesive material secreted by the caterpillar. After 2-3 days, larvae bore into the trunk or main branches usually at the forking place and make tunnel downwards.

There is only one larva in each hole, and there may be 2-16 holes in each tree, depending upon the intensity of infestation and age of the tree. By continuously devouring the tissues, it tunnels through the stem and branches. The caterpillars remain within the bored holes during day and come out at night to feed upon the bark. The attacked trees show the presence of windings and silken galleries full of frass and faecal matter. As a result of feeding in the trunk or main branches, the translocation of sap is disrupted and in case of severe infestation, the growth of tree is arrested. Fruiting capacity of tree is adversely affected. Severe injury weakens the stem, resulting in drying of the branches and finally of the tree itself.

Management

-) The caterpillars can be killed by inserting an iron spoke into the tunnels.
-) This insect has also been successfully controlled by injecting kerosene oil into the tunnel by means of a syringe and then sealing the opening of the tunnel with mud.

-) Another method of control is dipping a small piece of cotton in any of the fumigants, like carbon bisulphide, chlorosal or even petrol and introducing it into the tunnel and sealing the opening with clay or mud.
-) Remove the webs from tree trunks and put emulsion of DDVP (0.05%) in each hole and plug them with mud. Mix chlorpyrphos 2 ml per litre of water and apply the bark eating caterpillar infested area with a brush at 15 days interval.
-) As a preventive measure, spraying of the attacked trunk and branches with 0.05%DDVP may be done.

Litchi Leaf Roller

The incidence of leaf roller is reported during July to February. The number of larvae is the highest during December to February, preceding flowering season of litchi. The breeding season of the leaf roller on litchi leaves is from August to February when new leaf flush is available and restricted breeding takes place during off-season (March to July) on alternate hosts such as kath-jamun (*Eugenia jambolana*) and chhota amaltas (*Cassia tora*) growing around litchi orchards. It may attack flower also. The female moth lays eggs under the surface of newly emerged tender leaves which hatch within 2-8 days. The last instar larvae pupate in larval clip, a small portion of the leaf on the margin, both anteriorly and posteriorly and conceal themselves by bending and sealing the clipped piece of the leaf

The symptoms of leaf injury by the larvae are manifested through rolling of tender leaves and feeding inside. As a result of larval injuries, the infested twigs distort and wither. Litchi trees whose foliage is attacked by the larvae, very poor flowering or incase of younger tree, no flowering is seen in the season. Thus, the crop yield gets reduced considerably.

Management

-) The damage caused by leaf rollers is tolerated as long as it is restricted to the foliage and unlikely to affect flower initiation.
-) The rolled leaves that contain larvae may be removed manually during light infestations.
-) If necessary, carbaryl 2g/l can be applied when 20 per cent of leaf flushes are infested to minimize damage to young trees or at critical periods of leaf growth in older trees.

Litchi fruit borer

This pest is known as litchi fruit borer or litchi stem end borer or litchi seed borer. It is apparently found infesting litchi most often, and elicits the greatest economic effects. Female lays eggs singly on the under surface of the leaf or near the calyx of litchi fruits. During winter months, the leaf buds are preferred for oviposition.

Newly hatched larvae are milky white, slender with distinct light brown head. The newly emerged larvae start boring into the fruits and feed on its pulp. The infested fruits do not attain normal size and can be identified by the formation of black spot near pedicel. Larvae do not enter much deeper into the pulp but feed below the calyx about 15mm deep. When fully grown, they come out of the fruit and pupate on the leaf surface. In this way, the larvae cause direct damage to litchi fruits. The full grown larva starts spinning cocoon, which is usually formed on the old litchi leaf.

Females clearly prefer fruits over shoots for oviposition. If no fruits are available, they are constrained to lay their eggs on shoots. The survival rate is higher on shoots than in fruit. During July, they cause indirect damage by making mines in young shoots. The young larvae make mine in the lamina and bore into mid-rib of young leaves and tunnel through it, as a result branches wither and drop. The pest has now established itself as one of the major pests of litchi in India particularly in Bihar and Uttar Pradesh.

Management

-) Moths can be excluded by enclosing the fruit panicles in nylon mesh bags, but is uneconomic in areas with high labour costs.
-) NRC on Litchi, Muzaffarpur has recommended the use of *Trichogramma chilonis*@5000eggs/ha and use of pheromone trap, however, this pheromone is not giving consistent results in all the situations.
-) Fallen fruit should be removed to reduce the build-up of moths and ploughing may be done after fruit harvesting.
-) Bearing trees should be inspected during early flush development and sprayed if necessary. The leaf flush before flower initiation is very important as it supplies the carbohydrates needed for fruit development. Young, non-bearing trees do not need to be sprayed. This also allows the parasitoids to build up in the orchard.

-) Fruits may be inspected weekly from fruit set to detect eggs of borers, which are very small and almost invisible to the naked eye. Infested fruit should be picked and destroyed at infestation levels of 1 to 2 per cent. When the pest becomes more active, spraying 0.05 per cent fenitrothion or dicholorvos or carbaryl 2g/l may be done.
-) Permethrin is applied weekly, up to two weeks before harvest. Cypermethrin, deltamethrin or fenthion during early fruit set is recommended to prevent damage later in the season.
-) Affected shoots may be removed and all agronomic efforts such as ploughing and nutrient management should be done so that flushing takes place before the month of September.
-) Neem based products may be applied at the time of new shoot emergence to avoid heavy population of the pest.
-) Monocrotophos 0.05 per cent may be applied in the case of severe shoot damage.

Shoot borer

The shoot borer has been observed causing serious damage to new flush of litchi throughout the country. This pest is usually active from August to October. Female moths lay eggs on tender leaves. After hatching, the larvae first bore into the veins of young leaves and later into the soft stems of shoots situated close to the apex, working their way downwards. New shoots are damaged by the caterpillars by tunneling from growing tip downward. As a result, the shoots droop and finally dry and wither. Larvae also bore into the inflorescence stalk. When full grown, these caterpillars come out and enter into the slits and cracks in the bark of the tree, dried inflorescence or cracks or crevices in the soil for pupation. Upper andlower parts of the plants are more infested than middle parts. The affected plants become stunted. Young trees up to the age of 8-10 years are more susceptible.

Management

-) The attacked shoots may be clipped off and destroyed.
-) Spraying of Carbaryl (0.2%) or Quinalphos (0.05%) or Fenitrothion 0.047% or 0.05% Malathion at fortnightly intervals from the commencement of new flush gives effective control of the pest. A total of 2-3 sprays may be done depending on the intensity of infestation.

Fruit Sucking Moths

Fruit-piercing moth attacks many fruit crops including litchi in night. Fruit-piercing adult moth is the damaging stage and the larvae are not harmful. The mouth parts of the moth are long and strong enough to penetrate through tough-skinned litchi fruit. Once the moth has punctured the skin of the fruit, it feeds upon the juices of the fruit. Fruit flesh damaged by this moth becomes soft and mushy. Damage caused by this pest is not only a result of the direct feeding but also by the fungal and bacterial infections that develop at the wound site. When moths are abundant green fruit is also attacked, causing premature ripening and dropping of fruits. Incidence of damage by this moth is normally low, however when outbreaks occur, most of the crop is affected.

Management

-) Capture and destruction of moths is done an hour after sunset when there is sufficient darkness with the aid of torches or a strong flashlight. However, this method is not very effective but recommended only if no other control means is not possible.
-) This moth prefers darkness and avoids light, therefore the illumination of orchards with the help of lanterns and lights has been tested as a possible means of deterring moth attack but not very effective.
-) Bagging or screening of fruits may be done by covering the fruits with brown paper or transparent oil paper bags. Although this method is labour intensive, it is most practical when fruits are easily accessible and have compact bunches.
-) Smoking of the orchard masks the odour of mature and ripening fruit that attracts the moth. Containers full of inflammable material, oil, tar and some green plant trimmings to enhance the smoke are placed within the orchard at a rate of 2 to 4 per acre. The smoking process is started a half an hour before dusk and continued for 2 to 3 hours after nightfall. This period represents the time in which the moths are seeking their night time feeding grounds. If the smell of the orchard is masked, the moths choose various wild hosts and remain on them throughout the night to feed.
-) Regular collection and proper disposal of all attacked and spoiled fruit. These procedures dissipate the odour emanating from the spoiled fruit so they cannot serve as an attractant for the moths

-) The effectiveness of chemical control is variable and remains ineffective in most of the cases.

Litchi Bugs

There are many species of bugs that attack litchi. Bug (*Tessaratoma javanica*) is the most destructive . It lays globular and off pink eggs, mostly in bunch of fourteen on lower surface of leaves. Newly emerged nymph is dirty white and soft bodied insect but colour changes to yellow red after few days. Both adults and nymph feed mostly on tender plant parts such as growing buds, leaf petioles, fruit stalks and tender branches of litchi tree. Excessive feeding causes drying of growing buds and tender shoots and ultimately fruit drop. The bugs when feed on the developing fruit, it causes the fruits to fall a couple of days later.

Management

-) This pest is combated by shaking the trees in winter, collecting and dropping them into kerosene.
-) The eggs of *T. javanica* are in group and visible which can be removed and destroyed.
-) There are natural enemies which parasitize 70 to 90 per cent of eggs laid late in the season. The adults are attacked by several fungi, birds and red ants may also be used as biological means of control.
-) If chemicals are used, the timing of sprays is critical because the bugs vary in their susceptibility to insecticide at different times of the year, depending on body fat content and its nature. Many of these bugs may be controlled with dimethoate and fenthion.

Leaf Eating Weevils

Grey weevil is a polyphagus pest. Adult has long snout with grey color, though poor flier but very active feeder on the leaves of litchi. It attacks leaves, shoot and flower. Adult weevils congregate on the tender leaves and nibble irregular holes on the leaves and sometimes consume the entire leaf leaving the midrib only.Another weevil recorded recently at NRC Litchi, so far not properly identified, feeds on tender leaves. The damage of this weevil is more severe at the time of shoot emergence.

Management

- ✓ The grubs of these weevils feed on organic matter in the soil below the canopy, hence, ploughing and exposing these grubs reduces the problem.
- ✓ Hand picking of the adult weevils reduces their problem to some extent.
- ✓ When severe damage is seen, spraying of Carbaryl 2ml/l may be done

Litchi nut borer

Litchi nut borer is a polyphagous pest and infests as many as 33 fruit crops. The scale-like eggs are laid singly on the fruits. Newly hatched larva feeds on the fruit skin and then tunnels towards the seed. When this occurs in green fruit, the fruit drops, although larva is most likely to be able to develop in the fallen fruit. Ripening fruit generally does not fall, and the larva often drowns in the juice if the skin is penetrated in the equatorial region where the flesh is thickest. The rind tissue around the entry hole may appear to be scalded and such damage is sometimes wrongly attributed to fruit fly. Mature fruit damaged by nut borer may stain other fruits in a cluster or those hanging below. One larva can cause perhaps 10% more damage through this secondary staining effect. In immature fruit, the young larva bores directly into the seed which is completely eaten. A single larva may damage two or three fruit, if the fruits are small.

Management

- ✓ Examine 5 fruit panicles on 20 trees widely spaced throughout the crop, commencing when green fruit are 20 mm long. Spray if more than 5 out of 100 panicles are infested with live, unhatched and unparasitised eggs. Check developing fruit weekly for larval entry holes and or frass. Infestation levels increase as the fruit mature due to immigration of moths from alternative hosts. Oozing juice from maturing fruit may also be an indication of nut borer infestations.
- ✓ The nut borer is attacked by various species of egg, larval and pupal parasitoids; however, these do not always keep borers below economic thresholds.
- ✓ Spray with carbaryl 2 g/l when 5% of panicles have fruit with fresh, unparasitised eggs. Full cover spray 40 days before harvest or two sprays at fortnight apart commencing when the fruit are 10 mm in diameter are recommended.
- ✓ Alternatively, the panicles can be covered with paper bags.

Semilooper

In addition to above, foliage defoliating semi-looper (castor semilooper/stick worms) have been reported occasionally. These semiloopers attack tender leaves en mass and defoliate the new shoots.

Management

- ✓ Spray of Quanoalphos or chlorpyrifos @ 1.5-2.0 ml per litre water.
- ✓ Alternatively delta-Sypermethrin @ 1 ml per litre can be sprayed.

Conclusion

In summary, the widespread use of insecticides is ineffective and economically wasteful in the long run. Many insecticides do in fact accomplish the intended task of controlling pest populations. However, their detrimental health and environmental effects make them an inadequate long term solution. In addition, most synthetic and natural pesticides are susceptible to ineffectiveness due to resistance buildup in insects. Thus the only viable solution for the future is integrated pest management. The economic benefits and reduced social costs of these systems present a logical answer to the pest control problem.

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Trends in Economic Zoology- Sericulture

N. Uma Maheswari and N.M. Farhana Barveen

PG and Research Department of Microbiology
STET Women's (Autonomous) College, Sundarakkottai,
Mannargudi, Thiruvarur, TamilNadu, India.
Email id: umasamyamf@gmail.com

Introduction

Sericulture is the term which denotes the commercial production of silk through silkworm rearing. Pure or true silk is obtained from cocoons of lepidopterous insects, popularly known as silkworm or silkmoths. It is an agro based industry. The Silkworm has been domesticated for so long that it cannot survive without human care. Silk is a nature's gift eco friendly, biodegradable, and self sustaining material. It is commercial fiber of animal origin other than wool. Today more than 29 countries in the world are practicing sericulture and producing different kinds of silk. India stands second in silk production next to China.

Types of silkworm

A large number of wild and semi domesticated silkmoths belong to the family Saturiinidae. There are four kinds of natural silk which are commercially known and produced.

1. Chinese or Mulberry silkworm

The bulk of the commercial silk produced in the world comes from this variety and often silk generally refers to mulberry silk. Mulberry silk is comes from the silkworm *Bombyx mori*, belonging to the family Bombycidae. It is found on mulberry leaves in India. The silk obtained from the silkworm is white or yellow in colour.



Figure 1: Mulberry silkworm

2. Muga silkworm

Muga silk is comes from the silkworm *Antheraea assamensis*.It is a wild and semi domesticated silkworm feed on the aromatic leaves of som and soaluplants.The golden yellow colour silk is prerogative of India and the pride of Assam State.The cocoons are amber or white and the caterpillars feed on machillus, cinnamon, etc. The muga silk, high value products used in products like sarees, mekahals, chaddars, etc



Figure 2: Muga silkworm

3. Eri silkworm

Eri silk is comes from the silkworm, *philosamia ricini* that feeds mainly on castor leaves.Eri silkworm also known as Endi or Errandii.Eri is a multivoltine silk spun with open ended cocoons unlike other varieties of silk.the adult moth is stout and dark and the wings darkish brown and white.it lays 120-200 eggs in clusters which hatch in 7-10 days.The caterpillar has a green body with brown head and the body has small tubercles bearing short hairs.



Figure 3: Eri silkworm

4. Tassar silkworm

Tassar Silk is comes from the silkworm *Antheraea paphia* B which mainly thrive on food plants asan and arjun.Tassar silkworm is copperish colour,coarse silkmainly used for furnishing and interiors.it is less lusture than mulberry silk,but has own feel and appeal. Eggs laid on tender leaves of the host trees and a moth lays about 200 eggs; egg stage last for 8 to 10 days.The Caterpillars are stout green with red spiracles and the larval period is 35-70 days.pupal stage is 25-50 days.The moth is stout with yellowish or brown wings with an eyespot on each Wing.



Figure 4: Tassar silkworm

Table 1: Tabulation for different types of silkworm

Species of silkmoth	Silk Producing States	Preferred Food (Leaves)	Type Of Silk
<i>Bombyx mori</i>	Karnataka, Andhra Pradesh and Tamil Nadu	Mulberry	Mulberry Silk
<i>Antheraea assamensis</i>	Assam, Meghalaya, Nagaland, Arunachala Pradesh and Manipur	Champa	Muga Silk
<i>Antheraea mylitta</i>	West Bengal, Bihar and Jharkand	Arjun	Tassar Silk
<i>Attacus ricini</i>	Assam, Meghalaya, Nagaland, Arunachala Pradesh and Manipur	Castor	Eri Silk

Life cycle of silkworm

The life cycle of silk moth starts when a female silk moth lays eggs. The caterpillar or larvae are hatched from the eggs of the silk moth. The silkworms feed on mulberry leaves and give rise to pupa. In the pupa stage, a weave is netted around by the silkworm to hold itself. After that it swings its head, spinning a fibre made of a protein and becomes a silk fibre. Several caterpillars form a protective layer around pupa and this covering is known as the cocoon. The silk thread (yarn) is obtained from the silk moth's cocoon. The life cycle of the silkworm is explained below in detail.

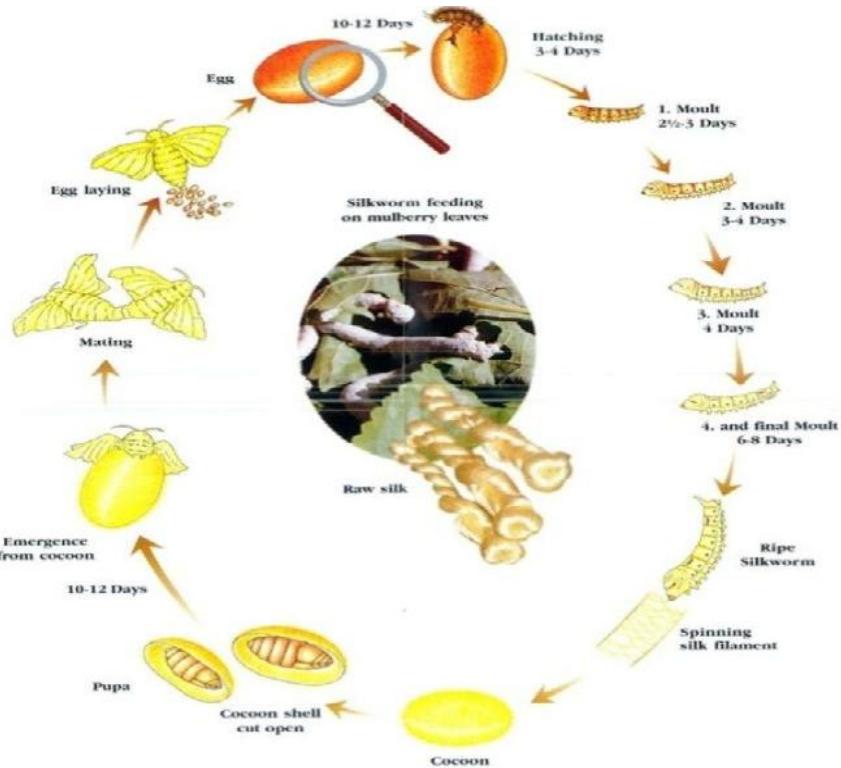


Figure 5: Life cycle of *Bombyx mori*

Stage 1: Eggs

An egg is the first stage of lifecycle of silkworm. After Fertilization, each female moth lays about 300-500 eggs which is mostly in a size of small dots. Eggs laid in clusters upon the leaves of the mulberry tree. The eggs are smooth and spherical in shape. They are first yellowish white in colour and later it becomes darker. After laying eggs, the female moth does not take food and dies within 4-5 days.

Stage 2: Caterpillar larva

A hairy silkworm arises after the eggs crack is also known as caterpillar. It is a tiny creature, about 6mm long. It has a tough and wrinkled, whitish or greyish body, which is made of twelve segments. They feed on mulberry leaves and consume a large amount of these leaves for around 30 days before going to the next stage.

Stage 3: Pupa

The caterpillar stops feeding and secretes a sticky fluid through their silk gland. The secreted fluid comes out through spinneret (a narrow pore situated on the hypopharynx). The thread becomes wrapped around the body of caterpillar forming a pupal case or covering known as the cocoon. The pupal period lasts for 10 to 12 days and the pupae cut through the cocoon and emerge into adult moth.

Stage 4: Moth

In this stage, the pupa changes into an adult moth. The female moth lays eggs after mating and thus the life cycle of silkworm begins again.

Three main components of sericulture industry

Cultivation of food plants for the silkworm.

Rearing of silkworm.

Reeling and Spinning of silk.

Cultivation of food plants for the silkworms:

The first component, is to grow the food plants for the silkworms. Mulberry leaves are widely used as food for silkworm *Bombyx mori* and the cultivation of mulberry is called as Morticulture. The favourable season for mulberry plants are June, July, November and December.



Figure 6: Mulberry plants

Rearing of silkworm:

In sericulture, the silkworm rearing process begins with the laying of eggs by the female silk moth. Typically, 300-500 eggs are obtained from one female silk moth. These eggs (laid on a paper/cardboard sheet) are then disinfected with the help of a 2% formalin solution.

A feeding bed is prepared on a rearing tray by sprinkling chopped mulberry leaves onto it. The hatched larvae are transferred into this tray via a process known as brushing. In order to maintain humidity, foam strips are soaked in water and placed on the tray.

The silkworm larvae initially have a good appetite. As they grow, their appetite slowly diminishes until their active stage. At this stage, the silkworm eats enthusiastically until its final feeding stage.

After reaching maturity, the larvae begin searching for hospitable places to begin their pupation. At this stage, the body of the silkworm shrinks and becomes translucent. These mature larvae now wrap themselves in a cocoon by secreting saliva from the two salivary glands on their heads. This saliva solidifies and becomes silk when it comes in contact with air



Figure 7: Rearing

Generally, the cocoon is spun in 2-3 days. However, some varieties of silkworms can take up to 4 days to spin their cocoons.

Reeling and Spinning of silk

Inside the cocoons, the larvae undergo metamorphosis and turn into pupae. The harvesting of silk from these cocoons is the final stage of sericulture. First, the pupae inside the cocoon are killed by boiling the cocoon and exposing it to steam and dry heat. This process is called stifling.

Now, the silk filaments are removed from the dead cocoon via a process called reeling. When the cocoons are placed in boiling water for approximately 15 minutes, the adhesion of the silk threads reduces, enabling the separation of individual filaments. These filaments are twisted into a thread with the help of a series of guides and pulleys. This silk is then re-boiled in order to improve its lustre.



Figure 8: Reeling and Spinning of silk

One thread of silk contains approximately 50 silk filaments. However, over 900 meters of filament can be obtained from a single cocoon. Thus, raw silk is obtained from the silkworm and the sericulture process is completed

Uses of Silk

Silk is mainly used in the manufacture of clothing such as shirts, trousers, ties, dresses and sarees.

Silk is extensively used for the making of various home décor furnishings engendering a lustrous, elegant and beautiful result.

Woven silk fibre is sometimes used for the construction of bicycle tires and parachutes.

Silk is used in industries and for military purposes.

It is used in the manufacture of fishing fibers, parachutes, cartridge bags, insulation coils for telephone, wireless receiver, tyres of racing cars, filter fibres, in medical dressings and as suture materials.

Disease and pest of silkworm:

Pebrine: It is the most important disease of silkworm and once infection starts it can wipe out all the worms. It is caused by a sporozoan, Nosema bombycis. The disease is found all over the world.

Muscaradine: It is a fungal disease caused by Beaveria bassiana and transmitted by spores carried by wind. All the stages of caterpillar are attacked. The body of the affected larva becomes soft but gets hard and acquires green colour after death.

Flacherie: It is a bacterial disease and the affected worms are infected by Bacillus bombysepticus.

Grasserie: the last instar Caterpillars are affected; they become swollen and look like a bag of granules, the body fluid becomes thick and cloudy and they die. The causative agent is the nuclear polyhedrosis virus.

Conclusion

Sericulture production was significant for the development of the human civilization from different aspects. Clothing is a very essential component for human shelter. Since the very early stage of human civilization, one of the reasons why clothes were often worn by people was to upgrade their social status.

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Aquaculture techniques

N. Uma Maheswari and T.Kamali

PG and Research Department of Microbiology,
STET Women's (Autonomous) College, Sundarakottai,
Mannargudi, Thiruvarur, Tamil Nadu, India.
Email id: umasamyamf@gmail.com

Introduction

Aquaculture is an industrial process of raising aquatic organisms upto final commercial production within properly partitioned aquatic areas, controlling the environmental factors and administering the life history of the organism positively and it has to be considered as an independent industry from the fisheries. On the basis of source aquaculture can classified into three categories. They are (a) Freshwater aquaculture, (b) Marine water aquaculture (c) Brackish water aquaculture.

Aquaculture is the production of aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants, and the cultivation of freshwater and marine plants and animals under controlled conditions for all or parts of their life cycles. Because of restrictions on the wild harvest of many fish species, demand for "farm-raised" options is very strong.

Aquaculture methods include

- Open aquaculture systems: Sea-cage (active feeding)
- Open aquaculture systems: Sticks, ropes, racks and cages (passing feeding)
- Semi-closed aquaculture systems
- Closed aquaculture systems

Open aquaculture systems: Sea-cage (Active feeding)

The rearing of aquatic species in enclosures in natural waterways is known as open sea-cage aquaculture. In a variety of habitats, syst including freshwater rivers, brackish estuaries, and coastal Pra marine zones, open systems are being introduced. The size of the aqu floating mesh cages varies depending on the volume of the use operation and the species being grown. Juvenile stock is taken com from hatcheries or natural populations and raised in enclosures pra until it reaches a marketable size. Finfish raised in open settings

fro are mostly carnivorous species that eat fishmeal as a diet (pellets ma comprising small schooling fish species). Yellowtail kingfish, ope southern bluefin tuna, Atlantic salmon, trout, and barramundi coa are some of the fast-growing open-water species. The growth of coa open sea-cage aquaculture has generated plenty of issues. The sin demand for fishmeal to feed carnivorous creatures is one of the as main issues. In certain cases, more than 5 kilograms of fishmeal juv is required to create just 1 kilogram of marketable fish. Other concerns include increased disease and parasite transmission Fis owing to high fish densities, the potential of escape and to interbreeding with wild species, and poor water quality due to fecal waste accumulation.



Figure 1: Open aquaculture systems sea-cage (active feeding)

Open aquaculture systems: sticks, ropes, racks and cages (Passive feeding)

Numerous shellfish species are cultured in systems that are all exposed to natural streams. Larval stages can be found in the has wild or created in hatcheries. These are then lowered into the The water column using various ways like attaching them to poles or ntal ropes or enclosing them in cages. Mussels and oysters are most common species grown using these techniques. These filter- ods feeding species are capable of obtaining nutritional needs from the water column without the use of fishmeal. Aquaculture of mussels, oysters, and other filter feeders can be considered environmentally friendly. If the water flow is sufficient, there is little influence marine ecosystems or water quality. In some ges regions, the disposal of sticks and racks may be an issue.



Figure 2: Open aquaculture systems: Sticks, ropes, racks and cages
(passive feeding)

Semi closed aquaculture systems

Semi-closed aquaculture is used when a species is grown on land and water is exchanged between the farm and a natural river. The farm is refilled with new water pumped back into the system, while wastewater is discharged into the local canal. Prawn farming is the most common type of semi-closed aquaculture in Australia, and it also makes the most extensive use of pond systems. The black tiger prawn is the most commonly farmed species, but banana, kuruma, and brown tiger prawns are also grown for the seafood sector. Vannamei prawns from Southeast Asia are becoming more widely available in markets as a low-cost alternative. Semi-closed aquaculture operations have the potential to have a substantial impact on coastal ecosystems. Ponds are frequently found near rivers when of coastal wetlands and mangroves are reclaimed for construction since they require constant water exchange. The upshot might be a significant loss of habitat, which is crucial for many species' al juvenile stages. If not handled properly, a constant discharge of water can degrade the water quality in the surrounding area. Fishmeal (pellets made up of tiny schooling fish species) is added to prawns at conversion ratios ranging from 1-3 kg of feed to 1 kg of prawns, putting pressure on wild fish populations.



Figure 3: Semi closed aquaculture systems

Closed aquaculture systems

The land-based breeding of aquatic species in raceways, tanks, and ponds is referred to as closed system aquaculture. Water is recirculated through filters and again returned to the aquaculture system using recirculation technology. This method usually keeps water quality high while minimizing contact with natural streams. The most frequent marketable species are silver perch, barramundi, yabbies, and marron, which are all grown in closed aquaculture systems. The principal marine species produced in closed systems are blacklip and greenlip abalone, which are seeing significant expansion due to demand from Asian markets. Closed system aquaculture is often regarded as one of the most environmentally friendly techniques of aquatic species rearing. Fishmeal (pellets made up of tiny schooling fish) may be used to feed carnivorous aquaculture species, which is a source of concern since it puts a strain on wild fish populations as a consequence of the right control over wastewater and the prevention of fish escape, there is very interactions with rivers.

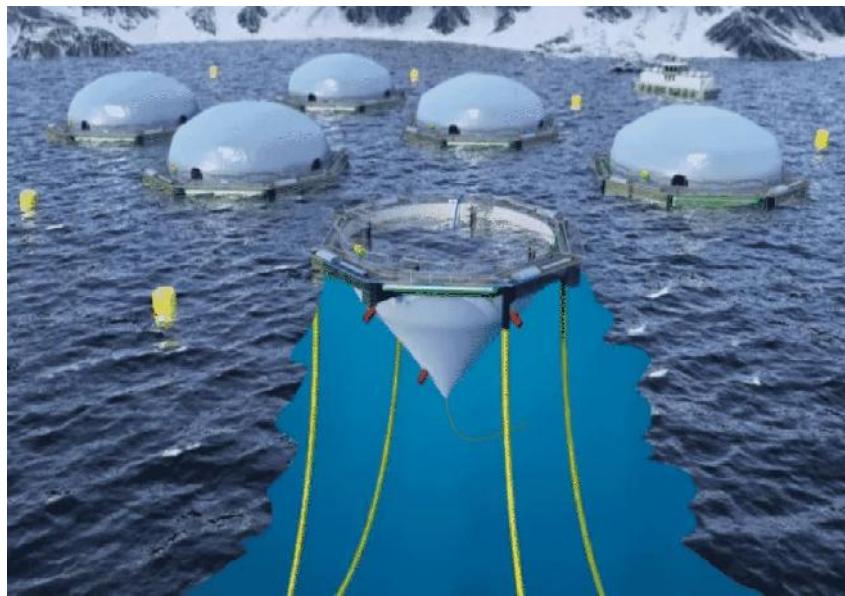


Figure 4: closed aquaculture system

Fish culture

Characteristics of cultivable fishes

The special characteristics features of cultivable fishes are:

- Fishes should have high growth rate in short period for culture
- They should accept supplementary diet.
- They should be hardy enough to resist some common diseases and infection of parasites.
- Fishes proposed for polyculture should be able to live together without interfering or attacking other fishes.
- They should have high conversion efficiency so that they can effectively utilize the food.

Types of cultivable fish

Cultivable fish are of three types

- (a) Indigenous or native fresh water fishes (Catla)
- (b) Salt water fishes acclimatized for fresh water(Mullet)
- (c) Exotic fishes or imported from other countries(Common crops)

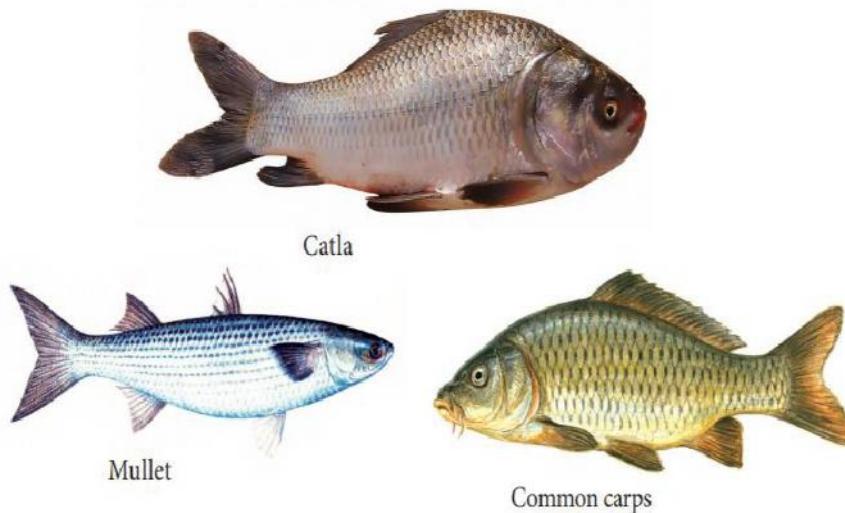


Figure 13.9 Different types of freshwater cultivable fishes

Fish pond management

The primary requisite of fish culture is the availability of land for fish pond and quality fish seeds. Although production of seeds, may be satisfactory, but rearing of those tender baby fishes in well managed Nurseries, Rearing and stocking ponds must be thoroughly known.

The above types of fish ponds are mostly found in Government managed fish farms as well as in progressive farmers. Whereas, the main objective is for fish seeds production or table- fish production through farms/ projects.

Generally, in scientific fish farming number of various sizes of ponds (as stated above) are required for rearing of various stages of fishes namely:-

- (1) Nursery pond - rearing of Spawn to Fry stage (approx. Size 4- 15 mm) for about 15 days.
- (2) Rearing pond-rearing of fry to fingerling stage (approx. Size: 16-40 mm) for about 2-3 months.
- (3) Stocking pond-rearing of fingerling (approx. Size 41- 150 mm) to marketable sizes/ adult fishes.

Of all the rearing of fishes in different types of ponds, Nursery pond management is considered as prime importance. In the present context. The State's fisheries-fish ponds being owned by farmers are non other than nursery ponds serving as both rearing and stocking purposes. As such, the fish pond management described in details below are aptly accounted and applicable for all types of ponds for our State.



Figure 5: Fish pond management

Pre-stocking pond management

The ponds need to be prepared such that the pond environment provides optimum condition for growth of the fish.

- J The pond environment should be free from predators, aquatic weeds, weed fish; it should have optimum water quality parameters and sufficient natural food should be available in semi- intensive culture systems.
- J The steps involved in pre-stocking and post-stocking management are similar in the nursery, rearing and grow-out ponds.
- J An additional step in the pre-stocking management in nursery ponds is the eradication of aquatic insects which predate on spawn and fry.

The pre-stocking pond management of drainable ponds, which can be dried, is as follows.

- Draining and drying
- Ploughing
- Liming
- Filling with water and
- Fertilization

Perennial un-drainable water bodies require the following additional pre-stocking management measures.

- Control of aquatic weeds
- Eradication of weed fish and predatory fish and animals.

Nursery ponds require eradication of aquatic insects as an additional pre-stocking management measure.



Figure 6: Pre-stocking management

Breeding Ponds

Breeding pond: The healthy and sexually mature male and female fishes are collected and introduced to this pond for breeding. Then the eggs released by the female are fertilized by the sperm. The fertilized eggs float in water as a frothy mass.

- Natural breeding
- Induced breeding

Natural breeding

For natural propagation, males and females are placed together in a breeding area such as a small pond or an enclosure where they spawn naturally. This method is usually used, for example, to produce tilapias cheaply.



Figure 7: Natural breeding

Induced breeding

Induced breeding is a technique by which ripe fishes are stimulated by pituitary hormone introduction to breed in captivity. The stimulation promotes a timely release of eggs and sperms from ripe gonads. The active factors like LH and FSH are present in fish pituitary.

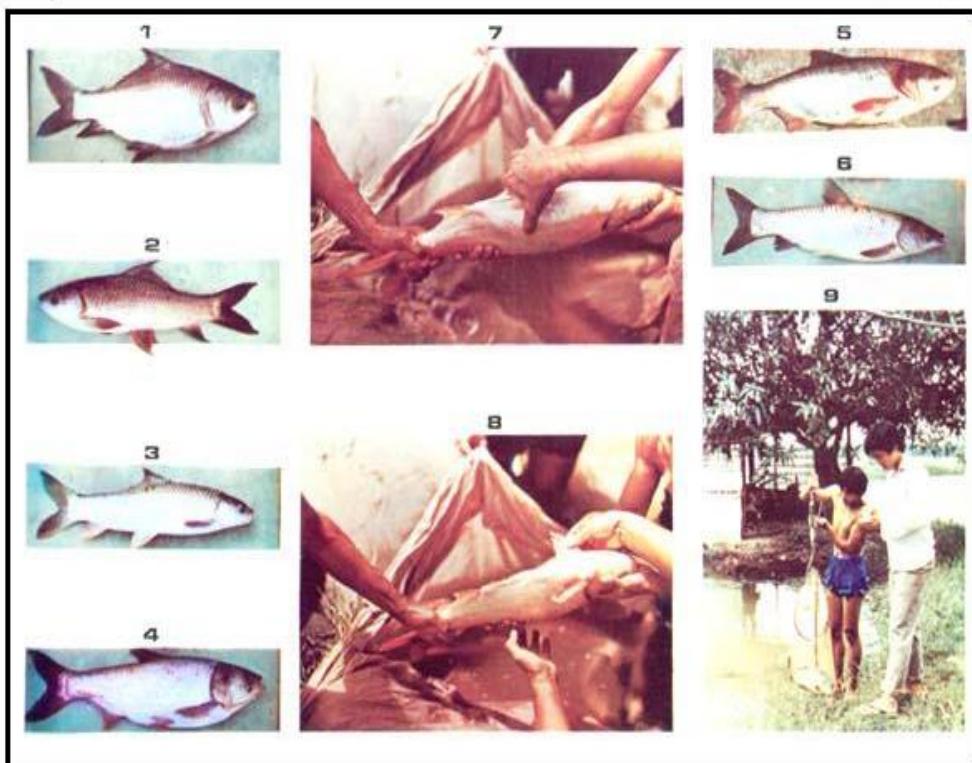


Figure 8: Induced breeding

Conclusion

Shellfish are grown on beaches or suspended in water on plastic trays, ropes or mesh bags by farmers. The shellfish raised in this manner are filter feeders, meaning they just need clean water to grow. Suspension systems are used to cultivate oysters, scallops, mussels, and clams. If the farmed species are natural to the area and the farm has enough water flow to minimize trash accumulation, shellfish production in suspended aquaculture is frequently low risk.

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Therapeutic role of *Azadirachta indica* (neem) and their active constituents

C. Srinisha and Dr.M. Kannahi

PG and Research Department of Microbiology, STETWomen's
College (Autonomous), Sundarakkottai,
Mannargudi(Tk) Thiruvarur(Dt), TamilNadu, India.
Phone no:6369512389,9361111069
EmailId: Kannahiamf@gmail.com

Abstract

Neem (*Azadirachta indica*) is a member of the Meliaceae family and its role as health-promoting effect is attributed because it is rich source of antioxidant. It has been widely used in Chinese, Ayurvedic, and Unani medicines worldwide especially in Indian Subcontinent in the treatment and prevention of various diseases. Earlier finding confirmed that neem and its constituents play role in the scavenging of free radical generation and prevention of disease pathogenesis

Biological and pharmacological activities attributed to different parts and extracts of these plants include antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, larvicidal and fungicidal activities. Others include antiulcer, spermicidal, anthelmintic, antidiabetic, anti-implantation, immunomodulating, molluscicidal, nematicidal, immunocontraceptive, insecticidal, antifeedant and insect repellent effects.

Introduction

The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutic role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties.

It is a largely accepted fact that numerous pharmacologically active drugs are derived from natural resources including medicinal plants.

It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities.

Neem ingredients are applied in Ayurveda, Unani, Homeopathy, and modern medicine for the treatment of many infectious, metabolic, or cancer diseases. Their role as anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastriculcer, antifungal, antibacterial and antitumour activities.

Although there are some previous studies about *A. indica* general uses, this review notonly focused on the main dermatological effects of *A. indica* but also on the new possibilities claimed in patents to produce dermocosmetics with enhanced attributes. Hence, weaimed to aid in the scientific selection of novel ingredients derived from plant resources and meeting the increasing consumers' demand for naturally derived ngredients, including in the cosmetic market.

Botanical description of neem

Neem tree belongs to the family Meliaceae which is found in abundance in tropical and semitropical regions like India, Bangladesh, Pakistan, and Nepal. It is a fast-growing tree with 20–23 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound, imparipinnate, with each comprising 5–15 leaflets. Its fruits are green drupes which turn goldenyellow on ripening in the months of June– August. Taxonomic position of *Azadirachta indica* (neem)isclassifiedinTable1

Taxonomic position of *Azadirachta indica*

Order	Rutales
Suborder	Rutinae
Family	Meliaceae
Subfamily	Melioideae
Genus	Azadirachta
Species	Indica

Table1

Active compounds of *Azadirachta indica*

Azadirachta indica L. shows therapeutics role in health management due to rich source of various types of ingredients. The most important active constituent is azadirachtin and the others are nimbozin, nimbolin, nimbidin, nimbol, sodium nimbinate, gedunin, salannin, and quercetin.

Leaves contain ingredients such as nimbolin, nimbanene, 6-desacetyl nimbolinene, nimbol, nimbolide, ascorbic acid, n-hexacosanol and aminoacid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbol. Quercetin and β-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties

Mechanism of action of active compounds

Neem (*Azadirachta indica*), a member of the Meliaceae family, has therapeutics implication in the diseases prevention and treatment. But the exact molecular mechanism in the prevention of pathogenesis is not understood entirely. It is considered that *Azadirachta indica* shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbozin, nimbolin, nimbol, nimbol, salannin, and quercetin. Possible mechanism of action of *Azadirachta indica* is presented as follows. Neem (*Azadirachta indica*) plants parts shows antimicrobial role through inhibitory effect on microbial growth/potentiality of cellwall breakdown. Azadirachtin, a complex tetraneortriterpenoidlimonoid present in seeds, is the key constituent responsible for both antifeedant and toxic effects..

Effect of neem as anti-inflammatory

Plants or their isolated derivatives are in the practice to treat/act as anti-inflammatory agents. A study result has confirmed that extract of *A. indica* leaves at a dose of 200 mg/kg,p.o., showed significant anti-inflammatory activity in cotton pellet granuloma assay in rats .Other study results revealed that neem leaf extract showed significant anti-inflammatory effectbut it is less efficacious than that of dexamethasone [56] and study results suggest that nimbolin suppresses the functions of macrophages and neutrophils relevant to inflammation.

Earlier finding showed immunomodulator and anti- inflammatory effect of bark and leave extracts and antipyretic and anti-inflammatory activities of oil seeds. Experimentation was made to evaluate the analgesic activity of neem seed oil on albino rats and results of thestudy showed that neem seed oil showed significant analgesic effect in the dose of 1 and 2mL/kg and oil has

dose-dependent analgesic activity. Another study was made to investigate the anti-inflammatory effect of neem seed oil (NSO) on albino rats using carrageenan induced hind pawedema and results revealed that NSO showed increased inhibition of pawedema with the progressive increase in dose from 0.25mL to 2mL/kg body weight.

At the dose of 2mL/kg body weight, NSO showed maximum (53.14%) inhibition of edema at 4th hour of carrageenan injection. Results of the study concluded that the treated animals with 100mg kg⁻¹ dose of carbontetrachloride extract (CTCE) of *Azadirachta indica* fruits kin and isolated ingredient azadiradione showed significant antinociceptive and anti-inflammatory activities.

Hepatoprotective effect

Medicinal plants and their ingredients play a pivotal role as hepatoprotective without any adverse complications. A study was performed to investigate the hepatoprotective role of azadirachtin- A in carbon tetrachloride (CCl₄) induced hepatotoxicity in rats and histologyand ultrastructure results confirmed that pretreatment with azadirachtin- A dose- depend ently reduced hepatocellular necrosis.

Further- more results of the study show that pretreatment with azadirachtin-A at the higher dose levels moderately restores the ratliver to normal. Another study wascarried outto evaluate the protective effect of active constituent of neem such as nimbolide against carbontetrachloride (CCl₄) induced liverotoxicity in rats and results suggest that nimbolide possesses hepatoprotective effect against CCl₄ induced liver damage with efficiency similar to that of silymarin standard and another study finding revealed that leaf extract was found to have protection against paracetamol-induced liver necrosis in rats.

A study assesses the hepatoprotective activity of *Azadirachta indica* (AI) leaf extract on antitubercular drugs- induced hepatotoxicity and results confirmed aqueous leaf extract significantly prevented changes in the serum levels of bilirubin, protein, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase and significantly prevented the histological changes as compared to the group receiving anti tubercular drugs.

Additionally, other results showed that ethanolic and aqueous leaf extracts of *A.indica* exhibited moderate activity over carbontetrachloride treate danimals.

Hepatoprotective effect of methanolic and aqueous extracts of *Azadirachta indica* leaves was evaluated in rats and study result established that the plant has good potential to act.

Wound healing effect

Numerous plants/ their constituents play an important role in the wound healing effect. A study was made to evaluate the wound healing activity of the extracts of leaves of *A. indica* and *T. cordifolia* using excision and incision wound models in Sprague Dawley rats and results revealed that extract of both plants significantly promoted the wound healing activity in both excision and incision wound models.

Furthermore, in incision wound, tensile strength of the healing tissue of both plants treated groups was found to be significantly higher as compared to the control group. Other results showed that leave extracts of *Azadirachta indica* promote wound healing activity through increased inflammatory response and neovascularization.

Antidiabetic activity

A study was undertaken to evaluate the 70% alcoholic neem root bark extract (NRE) in diabetes and results showed that neem root bark extract showed statistically significant results in 800 mg/kg dose.

Another experiment was performed to examine the pharmacological hypoglycemic action of *Azadirachta indica* in diabetic rats and results showed that in aglucose tolerance test with neem extract 250mg/kg demonstrated glucose levels were significantly less as compared to the control group and *Azadirachta indica* significantly reduce glucose levels at 15th day in diabetic rats. 6 Evidence- Based Complementary and Alternative Medicine Studies using in vivodiabetic murine model, *A. indica*, and *B. spectabilis* chloroform, methanolic, and aqueous extracts were investigated and results showed that *A.indica* chloroform extract and *B. spectabilis* aqueous, methanolic extracts showed a good oral glucose tolerance and significantly reduced the intestinal glucosidase activity.

Another important study suggested that leaves extracts of *Azadirachta indica* and *Andrographis paniculata* have significant antidiabetic activity and could be a potential source for treatment of diabetes mellitus.

Antimicrobial effect

Neem and its ingredients play role in the inhibition of growth of numerous microbes such as viruses, bacteria, and pathogenic fungi. The role of neem in the prevention of microbial growth is described individually as follows.

Antibacterial activity

A study was performed to evaluate antimicrobial efficacy of herbal alternatives as endodontic irrigants and compared with the standard irrigant sodium hypochlorite and finding confirmed that leaf extracts and grape seed extracts showed zones of inhibition suggesting that they had antimicrobial properties.

Furthermore, leaf extracts showed significantly greater zones of inhibition than 3% sodiumhypochlorite. The antibacterial activity of guava and neem extracts against 21 strains of foodborne pathogens was evaluated and result of the study suggested that guava and neemextracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and spoilage organisms.

Another experiment was made to evaluate the antibacterial activity of the bark, leaf, seed, and fruitextracts of *Azadirachta indica* (neem) on bacteria isolated fromadultmouthand results revealed that bark and leaf extracts showed antibacterial activity against all the testbacteria used. Furthermore, seed and fruit extracts showed antibacterial activity only at higher concentrations.

Antiviral activity

Results showed that neem bark (NBE) extract significantly blocked HSV-1 entry intocells at concentrations ranging from 50 to 100 $\mu\text{g/mL}$. Furthermore, blocking activity of NBEwas noticed when the extract was preincubated with the virus but not with the target cells suggesting a direct anti-HSV-1 property of the neem bark. Leaves extract of neem (*Azadirachta indica* A. Juss.) (NCL-11) has shown virucidal activity against coxsackie virus B-4 as suggested via virus inactivation and yield reduction assay besides interfering at an early event of its replication cycle.

Antifungal activity

Experiment was made to evaluate the efficacy of various extracts of neem leaf on seedborne fungi Aspergillus and Rhizopus and results confirmed that growth of both the fungal species was significantly inhibited and controlled with both alcoholic and water extract. Furthermore, alcoholic extract

of neem leaf was most effective as compared to aqueous extract for retarding the growth of both fungal species.

Another finding showed the antimicrobial role of aqueous extracts of neem cake in the inhibition of spore germination against three sporulating fungi such as *C. lunata*, *H. pennisetti*, and *C. gloeosporioides* f. sp. mangiferae and results of the study revealed that methanol and ethanol extract of *Azadirachta indica* showed growth inhibition against *Aspergillus flavus*, *Alternaria solani*, and Cladosporium. Aqueous extracts of various parts of neem such as neem oil and its chief principles have antifungal activities and have been reported by earlier investigators.

A study was undertaken to examine the antifungal activity of *Azadirachta indica* L.a against *Alternaria solani* Sorauer and results confirmed that ethyl acetate fraction was foundmost effective in retarding fungal growth with MIC of 0.19 mg and this fraction was also effective than fungicide (metalaxyl+mancozeb) as the fungicide has MIC of 0.78mg[87].

Antimalarial Activity.

Experiment was made to evaluate the antimalarial activity of extracts using Plasmodium berghei infected albino mice and results revealed tha neem leaf and stem bark extracts reduced the level of parasitemia in infected mice by about 51–80% and56–87%, respectively, and other studies showed that azadirachtin and other limonoids available in neem extracts are active on malaria vectors.

Another finding based on crude acetone/water (50/50) extract of leaves (IRAB) wasperformed to evaluate the activity against the asexual and the sexual forms of the malariaparasite, *Plasmodium falciparum*, in vitro and results showed that, in separate 72-hour culturesof both asexual parasites and mature gametocytes treated with IRAB (0.5 microg/mL), parasite numbers were less than 50% of the numbers in control cultures, which had 8.0% and 8.5% parasitemia, respectively.

Role of neem in dentistry

A study was made to assess the efficacy of neem based on mouth rinse regarding itsantigingivitis effect and study confirmed that *A. indica* mouth rinse is equally effective inreducing periodontal indices as chlorhexidine. Another study was carried out to evaluate the antimicrobial properties of organic extracts of neem against three bacterial strains causing dental caries and results showed that petroleum ether and chloroform extract showed strong antimicrobial activity against *S.mutans*.

Chloroform extract showed strong activity against *Streptococcus salivarius* and third strain *Fusobacterium nucleatum* was highly sensitive to both ethanol and water extract. Earlier finding confirmed that dried chewing sticks of neems showed maximum antibacterial activity against *S.mutans* as compared to *S.salivarius*, *S.mitis*, and *S.sanguis*.

Antinephrotoxicity effect

An experiment was made to investigate the effects of methanolic leaves extract of *Azadirachta indica* (MLEN) Evidence-Based Complementary and Alternative Medicine 7 on cisplatin- (CP-) induced nephrotoxicity and oxidative stress in rats and results confirmed that extract effectively rescues the kidney from CP- mediated oxidative damage.

Furthermore, PCR results for caspase-3 and caspase-9 and Bax genes showed down regulation in MLEN treated groups.

Neuroprotective effects

A study was performed to investigate the neuroprotective effects of *Azadirachta indica* leaves against cisplatin- (CP-) induced neurotoxicity and results showed that morphological findings of neem before and after CP injection implied a well-preserved brain tissue. No changes, in biochemical parameters, were observed with neem treated groups

Conclusion

Popularity of natural products or their derivatives role in diseases cure and prevention is increasing worldwide due to less sideeffect properties. Neem and its ingredients have therapeutic implication and have been traditionally used worldwide especially in Indian Subcontinent since ancient time. Clinical based studies confirmed that neem plays pivotal role in prevention of various diseases.

The role of active ingredients as chemopreventive effect has been noticed in various tumour via modulation of numerous cell signaling pathways. The detailed study should be made based on animal to know the exact mechanism of action in the diseases management.

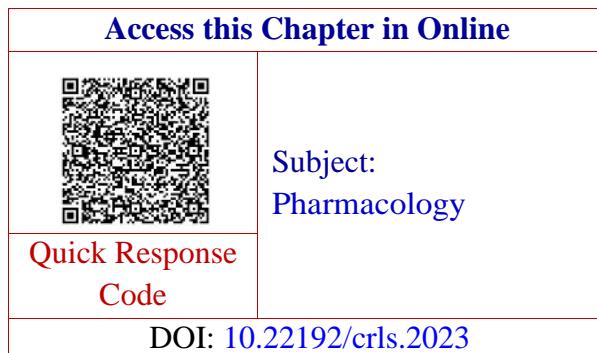
Results suggest that the ethanol extract of neem leaves showed in vitro antibacterial activity against both *Staphylococcus aureus* and MRSA with greatest zones of inhibition note dat 100% concentration.

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Agricultural importance of Algae

S.Sujitha and M.Kannahi

PG and Research Department of Microbiology

Sengamala Thayar Educational Trust Women's College
(Autonomous), Sundarakkottai, Mannargudi - 614016.

E-mail: Kannahiamf@gmail.com / 2004046sujitha@gmail.com

Mobile: +91 96557 18806

Abstract

Algae play an important role in agriculture where they are used as biofertilizer and soil stabilizers. Algae, particularly the seaweeds, are used as fertilizers, resulting in less nitrogen and phosphorous runoff than the one from the use of livestock manure. Algae are a large and diverse group of microorganisms that can carry out photosynthesis since they capture energy from sunlight. Algae play an important role in agriculture where they are used as biofertilizer and soil stabilizers. Algae, particularly the seaweeds, are used as fertilizers, resulting in less nitrogen and phosphorous runoff than the one from the use of livestock manure. This in turn, increases the quality of water flowing into rivers and oceans. These organisms are cultivated around the world and used as human food supplements. They can produce a clean and carbon-neutral food also and can be grown on abandoned lands and arid desert lands with minimal demands for fresh water. Seaweeds are an important source of iodine. Iodine levels in milk depend on what the cow producing the milk has been fed with. Feeding milk cattle with seaweeds can increase the quantity of iodine in milk, according to Fuzhou Wonderful Biological Technology. Egg-laying rate in hen is also increased by algae feed additives. In this article, we discussed the most important aspects of algae.

Key Words: Algae, seaweeds, soil stabilizers, agriculture, biofertilizer.

Introduction

Algae, particularly the seaweeds, are used as fertilizers, resulting in less nitrogen and phosphorous runoff than the one from the use of livestock manure. This in turn, increases the quality of water flowing into rivers and oceans. These organisms are cultivated around the world and used as human food supplements. Algae are ubiquitous; they occur in almost every habitable environment on earth, in soils, permanent ice, snowfields, hot springs, and hot

and cold deserts. Biochemically and physiologically, algae are similar in many aspects to other plants. They possess the same basic biochemical pathways; all possess chlorophylla and have carbohydrate, protein, and products comparable to those of higher plants. Furthermore, algae are the major primary producers of organic compounds. Since the 1950s, the use of algae has been replaced by extracts made from different species of macroalgae. Currently, these extracts have gained acceptance as "plant biostimulators". They induce physiological responses in plants, such as promoting plant growth, improving flowering and yield, stimulating the quality and nutritional content of the edible product, as well as prolonging shelf life. Furthermore, applications of different types of extracts have stimulated plants' tolerance to a wide range of abiotic stress. On the other hand, green algae and cyanobacteria are involved in the production of metabolites such as plant hormones, polysaccharides, antimicrobial compounds, among others, which play an important role in plant physiology and in the proliferation of microbial communities in the soil.

Some Big Reasons Why we Need to Start use more Algae in the Agriculture.

Improvement of soil fertility

Algae may greatly enhance soil organic carbon content by assimilating carbon dioxide. Heterocyst cells in cyanobacteria may fix atmospheric nitrogen and thereby meet the needs of soil micro and macrofauna, flora, and plants.

Soil reclamation

The difficulties in soil reclamation in arid and semi-arid regions are mostly the salinity conditions of large soil areas. Several studies have been carried out on the effect of salinity on the growth, metabolism and yield of plants and algae. Some growth regulators such as gibberellic acid (GA3) were used for improving the salt tolerance of the plants. From an economic point of view, growth regulators are expensive and are non-practical especially, when applied in large amounts. Algae play an economic role in soil reclamation increases soil fertility and improve plant conditions under certain environmental factors.

Sources of organic matter

Algae are found in a range of aquatic habitats, both freshwater and saltwater. By virtue of these characteristics, the general term "algae" includes prokaryotic organisms cyanobacteria, also known as blue-green algae as well as eukaryotic organisms.

Nitrogen Fixation

Algae, especially cyanobacteria, may be the most important nitrogen-fixing agents in many agricultural soils. Their importance as nitrogen fixers in rice fields has been studied by several investigators. The great majority of cyanobacteria that fix nitrogen are probably heterocystous; however, non-heterocystous cyanobacteria fix nitrogen as well. The nitrogen fixed by the algae is liberated and then re-assimilated by the higher plants. A large variety of cyanobacterial species are known to be nitrogen-fixing and their importance in improving soil fertility for sustainable agriculture in submerged and irrigated rice cultivation is well recognized. The use of cyanobacteria as a biofertilizer for rice fields is very promising but limited due to fluctuations in quality and quantity of inoculum and its physiological attributes in varied agro-ecological regions. The utilization efficiency of fixed nitrogen by rice plants is often low and efforts are therefore being extended to isolate suitable strains of cyanobacteria that would be prolific not only in fixing atmospheric nitrogen but also in excreting it continuously, thus making it available to the growing rice plants. Cyanobacteria are widely used in rice fields throughout Asia, where their enhancement of soil fertility by means of biological nitrogen fixation (so-called legalization) in place of N-rich fertilizers (Halperin et al., 1981), but their beneficial effects are not limited to that. The cyano-bacterium *Tolypothrix tenuis* is grown in cultures and added to rice fields.

Production of extracellular substances

Cyanobacteria excrete a great number of substances that influence plant growth and development. These micro-organisms have been reported to benefit plants by producing growth-promoting regulators (the nature of which is said to resemble gibberellin and auxin), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity.

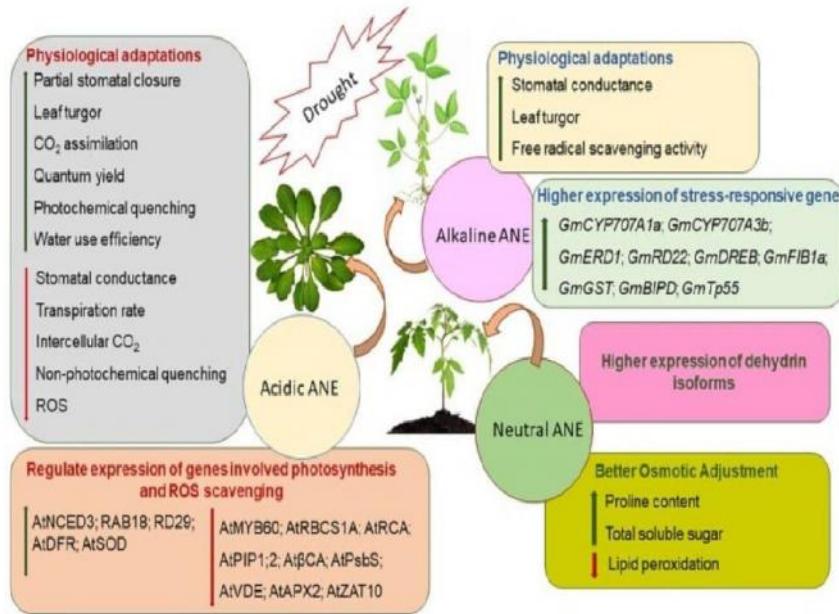


Figure 1: Production of extracellular substance

Algae as biofertilisers

One of the big challenges of the 21st century is improving the way we produce our crops. We need technologies are environmentally sound and sustainable so we can feed the growing global population. Biofertilisers are promising in this respect. They have been touted as an eco-friendly and sustainable way of replacing synthetic fertilisers, whose production is reliant on fossil fuels, which in turn contribute to greenhouse gas emissions. Algae in particular have emerged as a suitable option for restoring soil fertility as well as enhancing plant growth and productivity. Plus their use as a biofertiliser can substantially limit the use of synthetic fertilisers.

Benefits of algal fertilisers

Algal fertilisers could not only help in improving soil fertility through the increase of soil carbon and nitrogen, and through the aggregation of soil particles to improve soil structure. They also secrete extracellular polymeric substances which help bind soil particles together, as well as help soil overcome conditions of water stress. Plus algae release plant hormones, which increase plant growth and therefore yield. And they release phytochemicals, which protect plants and allow them to develop resistance toward biotic and abiotic stress.

Uses of algae in agriculture

1. The increase in the mass and size of the fruit, as well as the acceleration of the ripening phase by the application of extracts of this algae in kiwi.
2. The stimulation of the growth and consumption of calcium, potassium and copper of the plants, as well as the increase in the size, mass, firmness and fruit production in the vine cultivation by the foliar application of extracts.
3. The promotion of growth, of the content of chlorophylls, N, K, Fe, Mn and Zn in the leaves of apple plants by the foliar application of extracts of this algae (2 mL L-1) together with amino acids (0, 5 mL L-1). Furthermore, fruit production increased with the application of the extract alone and in combination with amino acids.
4. The increase in the leaf area and the content of chlorophylls, carbohydrates, nitrogen and zinc in the leaves of peach plants by foliar spray at a concentration of 4 mL L-1.
5. The increase in the total content of phenols, total flavonoids and total isothiocyanates in two broccoli cultivars by the application of extracts of these algae.
6. Stimulation of germination and reduction of the emergence time of plants in the bean crop by immersing seeds in an extract of these algae at a concentration of 0.8 mL L-1 for 15 minutes.
7. The stimulation of the growth and performance of onion plants by the application of an extract of these algae with a dose of 2.5 g m-2.
8. In contrast, algae of the order of Corallinales (Coralinas), when presenting their carbonate-rich composition, have been used as soil conditioners, since they correct the pH in acidic soils and in turn provide numerous trace elements.

Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat

The paper we discussed week presents an example of an experiment carried out using cyanobacteria isolated from the root area (rhizosphere) of wheat crops. The cyanobacterial inoculants which emerge have positive effects on plant height, dry weight and grain yield. And they increase the number of soil microorganisms that transform soil nutrients which are unavailable to plants into forms that are available to plants. Soil microorganisms also improve soil air circulation. However, in our discussion, several of the Scholars

suggested there limitations in the study. For instance, cyanobacterial fertilisers were added in combination with inorganic fertiliser. As a result, it is hard to determine whether the growth of plants was due to cyanobacteria alone or the impact of the inorganic fertiliser. However, there is evidence that cyanobacteria alone are able to increase rice and wheat grain and yield, so the problem is limited to just this paper. That said, the mechanisms behind the actions of cyanobacteria are not fully understood.

General characteristics of spirulina and effects of application in agriculture

Spirulina (*Arthrospira platensis*) is a type of blue-green algae, which has a great interest in the field of biotechnology, its pharmaceutical use and as human and animal food being highly exploited, because it is cultivated in many parts of the world for its high nutritional value. Spirulina has approximately 60–70 % of its dry mass in proteins with high bioavailability. It is the terrestrial and aquatic organism with the highest protein content and the best aminogram and digestibility, reason why it is widely used as a source of amino acids for men, animals and plants. In addition, it contains essential polyunsaturated fatty acids and vitamins, as well as xanthines, phycobiliproteins, carbohydrates, nitrogen, phosphorus, potassium, calcium, iron, manganese and zinc. It also has a high content of vitamins B12, B1, B2, B6 and E, biotin, pantothenic acid, folic acid, inositol and niacin. Also, great richness in - and β-carotenes, phycocyanin, considerable amounts of -linolenic acid (polyunsaturated fatty acid with different beneficial effects), a high concentration of phytohormones, trace elements, antioxidants and polysaccharides, therefore, it is an excellent biological complement. Furthermore, chlorophyll a, xanthophylls and lipids have been identified in these algae.

Extraction methods of the active principles of algae

To the extent that the processes from collection to extraction of the active ingredients are well adjusted, the results obtained in the field will be the best. In general, most of the extractive processes must include cell disruption to release the components of interest to the extract. Processes may include alkali extraction, acid extraction, suspension cell rupture, enzyme digestion, high pressure water extraction, extraction with chemical solvents, assisted extraction with microwaves and extraction with supercritical fluids.

Sometimes, simply, a drying followed by a spray and the powder is used to be to the ground applied. Many of these processes are carried out in most cases using low temperatures so as not to damage any metabolite. Next, the extraction processes that have been most used will be described.

(i) Extraction with alkalis

This method was developed in the 1940s and consists of the use of a base (generally potassium hydroxide), together with the application of heat. The algae used are dried at high temperatures ($>100^{\circ}\text{C}$) to facilitate storage and the product obtained generally has a high pH; all this leads to a denaturation of active ingredients that result in a drastic loss of their properties. This means that although this method was one of the most widely used, it is not one of the most feasible to obtain extracts with a large number of benefits.

(ii) Extraction with chemical solvents

In this method, a set of chemical solvents with different polarities are used for the extraction of their active ingredients, the most used being water and hydroalcoholic solutions and high temperatures are not used. The fact of not using high temperatures, or chemical solvents that drastically affect the pH, makes this one of the preferred methods since the properties of the active principles of the algae are not affected.

(iii) Extraction with supercritical fluids (CO_2)

This method does not apply either chemical solvents or high temperatures. The raw material used has to be fresh, so the production plants have to be close to the coast. In this method, the algae is to very small particles crushed and it is to high pressure subjected, to promote the extraction of the active ingredients. Since no high temperatures are applied at any stage of the process and chemical solvents are not used either, the active principles are conserved and the pH is maintained at its physiological level of approximately 4.5. The extraction process chosen is key to obtaining a product with the composition necessary to achieve the desired effects and they are chosen depending on the composition required. For example, to obtain an extract rich in auxins, alkali extraction is generally used, microwave assisted extraction has been used to obtain an extract rich in polysaccharides and if this is combined with extraction with water at high pressures, an extract rich in fucoidans is obtained. Extraction with 70 % ethanol allows obtaining an extract rich in cytokinins, while using 85 % methanol an extract rich in gibberellins is obtained and using the extraction of supercritical fluids, extracts rich in lipids, volatile metabolites, pigments, are obtained antioxidants, carotenoids, chlorophylls, vitamin E and linoleic acid.

(iv) Products made from algae

With the aim of expanding the use of algae in agriculture, a wide variety of products is produced currently.

(v) Chopped and powdered macroalgae

Algae biomass for these purposes generally comes from the exploitation of natural populations of *Ascophyllum*, *E. Macrocytis*, *Durvillea*, *Ecklonia*, *Fucus*, *Sargassum*, *Cystoseira* and *Laminaria*. It is (in the sun or in tobacco-type dryers) dried and chopped and/or ground to give flour. Generally, these are used close to the coastal areas. These flours are "dusted or dissolved in water for hydroponic planting. On the other hand, they are spread to eroded or contaminated soils, slopes, crop fields, etc., in order to fix road slopes and clearings, regenerate poor soils and with toxicity problems, treating grass sports fields and planting steep meadows, among others.

(vi) Liquid algae extracts

In general, liquid algae extracts are used for foliar application as biofertilizers, although they are also applied to the soil. Some commercial extracts contain only macroalgae, although extracts supplemented with trace elements, fishmeal and pesticides are more abundant. Extracts from microalgae (live; eg: Agroplasma) and from cyanobacteria (dead; eg: "GA Gel of algae" and Agro-organic Mediterranean) appeared on the market in the late 90's. There is a large number of commercial algae-based biostimulants, most of which are made from the *Ascophyllum nodosum* algae, examples of these products are Acadian, Fruticrop, Solu-Sea and Stimplex. In addition, commercial products made from microalgae such as Spirulina or Chlorella exist for example, CBFERT and Naturplasma, respectively or from the combination of both as the product known as Naturvita.

Renewable resource

Unlike coal, natural gas and petroleum, oil derived from algae is a renewable resource. Algae grow rapidly, doubling their biomass within hours. Strains of algae that grow well and produce high amounts of oil can produce sufficient biodiesel to replace an estimated 48 percent of imported U.S. oil for transportation, according to a study, published in Water Resources in 2011. Compared with other sources of biofuels, such as corn, algae can produce about 80 times more oil per acre. Algae, unlike some other biofuel sources, is also not a major food source.

Algae biodiesel is carbon dioxide neutral

Algae use carbon dioxide, water, nutrients (fertilizer) and solar energy to make sugars, which they further metabolize into lipids, or oil. Algae biodiesel is net carbon neutral, because the carbon dioxide produced by burning algae biodiesel is the same amount of carbon dioxide that the algae

took up to grow and produce the oil. If power plants capture their carbon dioxide, nearby built algae biofuel facilities could readily use the carbon dioxide and avoid transportation costs and accompanying greenhouse gas emissions.

Algae in agriculture

Various blue green algae such as Oscillatoria, Anabaena, Nostoc, Aulosira increase the soil fertility by fixing the atmospheric nitrogen. In view of the increasing energy demands and rising costs of chemically making nitrogenous fertilizers, much attention is now being given to nitrogen fixing bacteria and blue green algae. Many species of sea weeds are used as fertilizers in China and Japan.

Conclusion

Algae, especially microalgae and cyanobacteria are ubiquitous in the world's soils. Although they are the primary microbial photosynthetic agents of the soil, their ecological role is still not fully defined. In this study, emphasis was laid on the role of algae, especially microalgae in soil fertility and reclamation and some of their advantageous properties and beneficial effects influence the plant/soil system, such as:

Excretion of organic acids that increase P-availability and P-uptake, Provision of nitrogen by biological nitrogen fixation, Increased soil organic matter, Production and release of bioactive extracellular substances that may influence plant growth and development. These have been reported to be plant growth regulators (PGRs), vitamins, amino acids, polypeptides, antibacterial or antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity. Stabilization soil aggregation by extracellular polysaccharides of soil aggregate Concentrate metal ions present in their environment.

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A Review on Medicinal plants - A nature made medicines

Chandru. S

B.Sc. Perfusion Technology, FAHS, Meenakshi Academy of Higher Education and Research, West KK Nagar, Chennai, Tamil Nadu, India.

Email ID: *chandrus5334@gmail.com*

Introduction

- ✓ Medicinal plants use in many years it is one of the oldest traditional medicines
- ✓ Medicinal plants are plants it used in treatment of illness and improve health
- ✓ Medicinal plants used in Ayurveda, Siddha, Unani and Homeopathy systems of medicine.
- ✓ More than thousand medicinal plants are grown in India out of which
- ✓ 130 medicinal plants are available for sale
- ✓ Medicinal plants use in essential drugs and common illnesses and injury, for endemic infectious diseases and it improve the oral health and mental health.

Considering the significance of medicinal plants under current era, herbs will be a alternative way of treatment to traditional allopathic medications.

Most common medicinal plants

- Tulsi
- Spearmint
- Carom
- Giloy
- Aloe vera
- Curry leaves

- Khus
- Ashwagandha
- Sage
- Fenugreek

Tulsi

- ❖ Tulsi is most common medicinal plants in India
- ❖ It used to Indigestion, diabetes, fever, cold, cough and bronchitis
- ❖ It reduces stress reliving and detoxifying body cells
- ❖ Tulsi prevents asthma symptoms because it has anti-inflammatory and anti-allergic properties that reduce inflammation in the bronchial mucous membranes
- ❖ Tulsi has a immunomodulatory and antimicrobial property so it stimulate the immune system in the body,
- ❖ it has a antipyretic and diuretic property it induces the sweating it helps for reduces the fever
- ❖ Tulsi helps to reduce the diabetes because it has a insulin sensitivity and increase the insulin level
- ❖ Tulsi helps to reduce the diabetes complications
- ❖ It protects the pancreas cells and improve the liver, kidney, heart function (antioxidant property)

Chemical constituents

Linoleic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and -caryophyllene,

Spearmint

- ❖ Spearmint is world's oldest medicine
- ❖ Spearmint rich of the Vitamins, Anti-oxidants
- ❖ It protects the sore throat, cramps, Arthritis, Diarrhea, Fatigue, and flatulence
- ❖ Spearmint has a anti-fungal and antioxidant properties helps cure scars and acne

Uses

- Digestive disorders including gas, indigestion, nausea, diarrhea, upper gastrointestinal tract spasms, irritable bowel syndrome bile duct and gallbladder swelling (inflammation), and gallstones.

Contents

The nutritional value Trusted Source of 100 grams

- ❖ Energy – 44 kilocalories
- ❖ Carbohydrates – 8.41 grams
- ❖ Fat – 0.73 grams
- ❖ Protein – 3.29 grams
- ❖ Iron – 11.87 milligrams
- ❖ Manganese – 1.118 milligrams
- ❖ Copper – 0.240 milligrams
- ❖ Potassium – 458 milligrams
- ❖ Riboflavin – 0.175 milligrams
- ❖ Pyridoxine – 0.158 milligrams
- ❖ Vitamin C – 13.3 milligrams
- ❖ Cholesterol – 0 milligrams
- ❖ Pantothenic acid (vitamin B5) – 0.061 milligrams
- ❖ Vitamin B6 – 0.041 milligrams
- ❖ Folate (vitamin B9) – 3 µg
- ❖ Vitamin C – 4.6 milligrams

- ❖ Mints stimulate the salivary gland it promotes digestion and increase the digestion enzyme
- It has an antiseptic and antibacterial properties so it kills or inhibit the bad bacteria

- ❖ Spearmint improves hemoglobin levels and promotes brain function
- ❖ It causes the soothing effect it
 - Acts as a good relaxant and relieves chest congestion
- ❖ Good total phenolic and flavonoid contents” and “excellent antioxidant activity.”

Carom

- ❖ Contains Antioxidants, Vitamins, minerals, fiber
- ❖ Exhibit antibacterial and antifungal properties.
- ❖ It use for avoid vomiting sensation
- ❖ Used Indigestion, Ulcers, Acidity, Arthritis, Bad Cholesterol, Blood pressure and even common cold or cough.
- ❖ Relieve severe indigestion problems for pregnant women.
- ❖ It is the source of Fiber, antioxidants and micronutrients
- ❖ carom seeds increase the gastric acid flow it improve the gastric function
- ❖ it helps to prevent the chronic indigestion
- ❖ Carom seeds has a anti-inflammatory properties and it help to decrease inflammation in the body.
- ❖ They are an excellent antidote for infection and cold because anti-bacterial, anti-biotic, and anti-fungal.
- ❖ Contains Thymol, oleic acid, linoleic acid, gamma-terpinene), p-cymene, palmitic acid, and xylene. Giloy
- ❖ Giloy has been used in Ayurveda for centuries
- ❖ Giloy help to treatment of Diabetes, Arthritis, , Indigestion, combats respiratory problems and also maintain healthy heart conditions
- ❖ Giloy increase the platelet count
- ❖ Giloy roots help to relieve breathing in asthma patient
- ❖ its high nutritional content and the alkaloids
- ❖ Its effective against various disorders, such as diabetes, cancer, neurological problems, fever,

- ❖ Giloy has a antipyretic herb property it increases the platelet count in dengue it reduces the complications
- ❖ it is highly antioxidant and reduces the toxins in the body
- ❖ Giloy increase the glucose level in blood and manage of diabetes
- ❖ It has a antioxidant and anti-inflammatory properties it reduces the diabetes complications (ulcer,wound,kidney damage)
- ❖ Giloy increase the certain digestion enzyme, it improves the digestion and metabolism, reduces acidity, and improves liver function.
- ❖ It used cure all pandemic diseases, like fever, infection, virus, digestion, improve immunity and also help to treat depression and anxiety disorders.
- ❖ It's Management of pain and inflammation in arthritis

Aloe vera

- ❖ *Aloe vera* is powerful antioxidant, aloe vera contains several components it helps to inhibit the growth of bacteria that can cause the infection
- ❖ *Aloe vera* improve the wound healing
- ❖ It used in topical medicine and it use in treating sores, and particularly burns, including sunburn.
- ❖ Tooth decay and gum disease are common health problems Aloe vera reduces the problem by inhibiting plaque-producing bacteria.
- ❖ Aloe vera is used in the treatment of diabetes to improve blood sugar management, it is used in type 2 diabetes, it has its effect on glycemic control.
- ❖ it reduces the pain in inflammation of mouth
- ❖ it contains vitamins A (beta-carotene), C and E, which are antioxidants. It also contains vitamin B12, folic acid, and choline
- ❖ aloe vera inhibit the bacteria and fungi, because it presents in antiseptic agent (Lupeol, salicylic acid, urea nitrogen, cinnamic acid, phenol and sulfur.)
- ❖ Minerals: It provides calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc
- ❖ It contains Auxins and gibberellins hormones it helps for wound healing

- ❖ aloe Vera contains Anthraquinone it caused the laxative effect it improves the water in intestine and increase the mucus secretion and increased intestinal peristalsis

Curry leaves

- ❖ Curry leaves improve the hair growth and reduces the hair fall
- ❖ curry leaves are good for skin and relieve the skin eruption and burns
- ❖ curry leaves help in your weight loss and it cleanses your body from harmful chemicals
- ❖ It contains many vitamins and good source of iron and calcium, improve the immunity
- ❖ Curry leaves take in empty stomach it can help in deal with diarrhea, constipation and dysentery.
- ❖ It helps for relieve such as nausea,
- ❖ It contains many vitamins and good source of iron and calcium, improve the immunity
- ❖ curry leaves are stimulating the digestive enzyme it help in breaking down the food inside your stomach and gut
- ❖ curry leaves is good for heart health as it contains antioxidant property, this property help to improve the good cholesterol and it regulates the bad cholesterol
- ❖ Curry leaves contain vitamin a it is good for eye health
- ❖ Curry leaves contain vitamin A, vitamin B, vitamin C, vitamin B2, calcium, and iron,
- ❖ Curry leaves has a rich iron content it increase the hemoglobin and red cells,it prevent the anemia

Conclusion

This review concludes that medicinal plants will open a new alternative way of treatmentin modern medicine.

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Angiosperm or Flowering plants

N. Uma Maheswari and S. Kamatchi

PG and Research Department of microbiology

STET Women's (Autonomous) College, Sundarakottai-614016

Mannargudi, Thiruvarur, Tamilnadu, India

Email Id: umasamyamf@gmail.com

Introduction

Angiosperm, also called flowering plant, any of about 300,000 species of flowering plants, the largest and most diverse group within the kingdom Plantae. Angiosperms represent approximately 80 percent of all the known green plants now living. The angiosperms are vascular seed plants in which the ovule (egg) is fertilized and develops into a seed in an enclosed hollow ovary. The ovary itself is usually enclosed in a flower, that part of the angiospermous plant that contains the male or female reproductive organs or both. Fruits are derived from the maturing floral organs of the angiospermous plant and are therefore characteristic of angiosperms. By contrast, in gymnosperms (e.g., conifers and cycads), the other large group of vascular seed plants, the seeds do not develop enclosed within an ovary but are usually borne exposed on the surfaces of reproductive structures, such as cones.

Salient features of Angiosperms

1. the sporophyte which is the dominant plant in the life-cycle is differentiated into roots, stem and leaves.
2. the highest degree of perfection of the vascular system with true vessels in the xylem and companion cells in the phloem
3. the organization of the microsporophylls (stamens) and megasporophylls (carpels) into a structure called the flower, which is typical only of the angiosperms.
4. the presence of four microsporangia (pollen sacs) per microsporophyll
5. the ovules are always on the basal region of the megasporophyll.
6. production of two kinds of spores, microspores (pollen grains) and megasporangia. angiosperms thus are heterosporous.

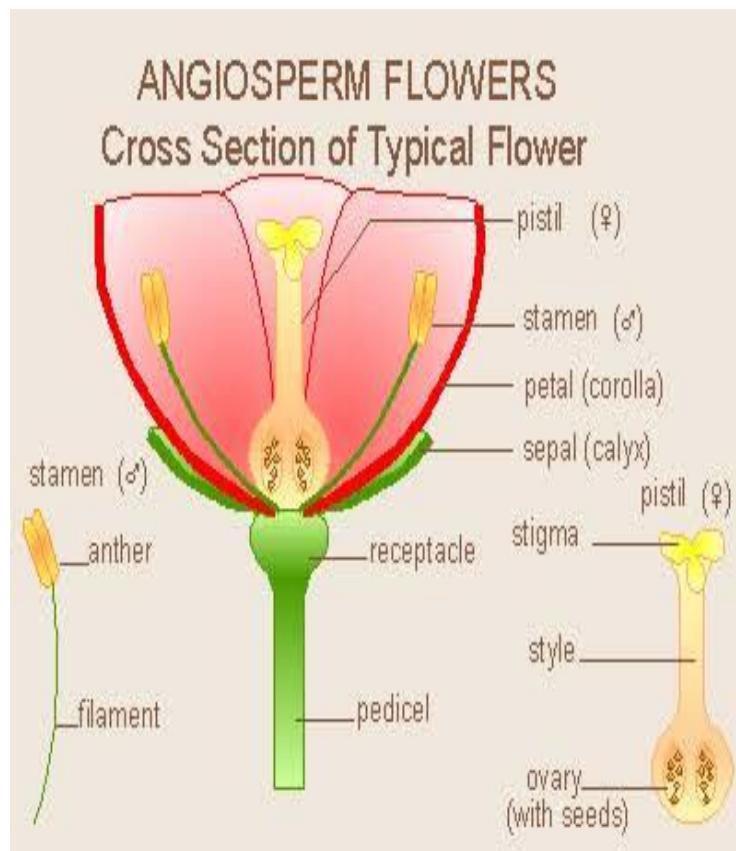
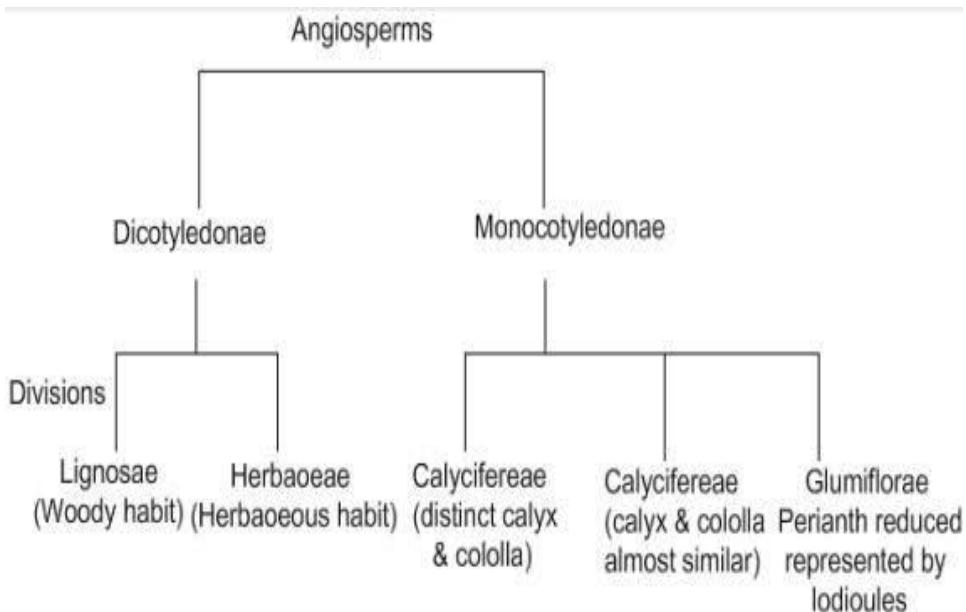


Figure: 1

7. presence of a single functional megasporangium which is permanently retained within the nucellus or megasporangium.
8. adaptation of flower to insect pollination.
9. pollination consists of the transference of pollen grains from anther to stigma.
10. spore dimorphism having resulted in the production of gametophytes, male and female.
11. extreme reduction in size, duration of existence and complexity of the structure of the gametophytes which are entirely parasitic.
12. the male gametophytes has reached the limits of reduction. it consists only of the pollen grain and the pollen tube contains the tube nucleus and two male gametes or nuclei. the male cells (gametes) are non-ciliated.

13. the female gametophyte lacks any extensive development of vegetative tissue it consists of three egg apparatus cells three antipodal cells and two polar nuclei in the centre of the embryo sac
14. the non motile male cells or nuclei are carried bodily to the neighborhood of egg apparatus by the tube
15. the seed or seeds remain enclosed in the ripened ovary called the fruit
16. the phenomenon of double fertilization or fusion is the characteristic of the
17. the endosperm develops after fertilization it is triploid
18. the angiosperms are completely adapted to life on land.



Figure; 2

Classification of Angiosperms

One of the earliest classification systems is what we now refer to as an **artificial classification system**. This means that plants are grouped based on similar characteristics, not on their genetic makeup.

Under an artificial classification system, an angiosperm could be placed in a group based on the color of its flowers, the shape of its foliage, or the size of its fruit. In this type of system, a lily and an orchid might be placed in the same group based on flower color, even though we know that lilies and orchids

are not closely genetically related (aside from both being part of the angiosperm group).

This is clearly not the most scientific means of classifying angiosperms, but it can be a useful method for identifying unknown plants based on their features - which in ancient times was probably the most important reason to classify plants anyway.

Figure: 3



Even though these are all tulips, an artificial classification system could place them into different groups based on flower color.

Natural Classification System

Natural classification systems attempt to group angiosperms based on more scientific factors, such as their chemistry, preferred growth locations, anatomical features, and other similarly scientific features. Natural classification is much more like the taxonomy you might be familiar with from biology class.

Taxonomy considers the same factors that natural classification systems do, and breaks them into clearly defined classifications and groupings based on those characteristics. Instead of grouping the plants based on a singular factor, like habitat, as a natural classification system might, taxonomy takes it a step further by factoring in multiple features and placing the organisms into a clearly defined hierarchy.

This is in noticeable contrast to artificial classification systems, which lack truly scientific descriptors and groupings. We use natural classification systems today to help us identify the genus, species, and family groupings for

plants. While natural classification systems are more scientific than artificial ones, they are still not the most scientifically precise way to classify angiosperms.

Figure: 4



Natural classification systems consider features like anatomy when grouping plants.

In some systems habit and habitat have been considered for this purpose:

- (i) Theophrastus (370 – 285 BC), a Greek philosopher, in his book *Historia Plantarum* classified about 480 plants into four groups on the basis of their habit-herbs, undershrub's, shrubs and trees.
- (ii) Otto Brunfels (1464-1534) for the first time classified plants into Perfecti and Imperfect based on the presence or absence of flowers.
- (iii) Andrea Caesalpino (1519-1603), an Italian botanist and Physician, in his book *De Plantis* classified about 1500 plants on the basis of habit (herbs and trees) and then subdivided them on the basis of fruits and seeds which they produced.

(iv) Joseph Pitton de Tournefort (1656-1708), a French Botanist and Physician, in his book *Elements de botanique* divided flowering plants into herbs and trees. He further sub-divided them on the basis of several morphological features, such as petal bearing or non-petal bearing flowers, simple or compound flowers (now referred to as polypetalous and gamopetalous), flowers regular or irregular.

(v) John Ray (1627-1705), an English naturalist, in his book *Methods Plantarum Nova* (1682) for the first time divided herbs, shrubs and trees into dicotyledons and monocotyledons on the basis of two or one cotyledons. Broadly he divided the plants as under:

(vi) Carolus Linnaeus (also called Carl Linnaeus) (1707-1778), a Swedish naturalist in his book *Species Plantarum* (1753) classified 7300 species of plants into 24 classes, mainly on the basis of number, union and length of stamens. For example, he described the classes as Monandria (1 Stamen), Diandria (with 2 stamens), Triandria (with 3 stamens) and so on Polyandria (with 20 or more stamens). This system is commonly known as sexual

System of classification

[II] Natural Systems:

In these systems the organisms are classified on the basis of their natural affinities (i.e. the basic similarities in the morphology) rather than on a single character for determining the affinities.

(i) A.L. de Jussieu (1748-1836) published a natural system of classification of plants in his book *Genera Plantarumsecundus ordines Naturales Disposita*. He grouped all plants into 15 classes which were further divided into 100 orders (now called families).

He divided the plants into three main groups, i.e. Acotyledones (plants without cotyledons, e.g., algae, fungi, mossesetc.) Monocotyledones (plants with one cotyledon) and Dicotyledones (plants with two Cotyledons). He mainly emphasized on the number of cotyledons and their presence or absence, number of petals and their presence or absence, and position of stamens.

(ii) A.P. de Candolie (1778-1841) a French botanist published *Theorieelementaire de la Botanique* in which he classified about 58,000 species into 161 families. He divided plants into two major groups i.e. cellulaires (non-vascular plants) and vasculares (vascular plants).

(iii) Bentham and Hooker's Classification:

The most important and the last of the natural systems of classification of seed plants was proposed by two British taxonomists George Bentham (1800-1884), a self trained botanist, and Joseph Dalton Hooker (1817-1911), the first director of the Royal Botanical Garden, Kew (England).

They recorded precise description of most of the plants known at that time. Their monumental work which took about quarter of a century for completion was described in three volumes of *Genera Plantarum*, published in Latin during July 1862 and April 1883. Bentham and Hooker's system of classification is still used and followed in several herbaria of the world. It is supposed to be the best system for the students to identify plants in the laboratory.

Salient Features of Bentham and Hooker's system:

1. It is a classification of only the “seed plants” or phanerogams.
2. They described 97,205 species of seed plants belonging to 7,569 genera of 202 families starting from Ranunculaceae up to Gramineae.
3. They classified all the seed plants into 3 groups or classes i.e. Dicotyledons (165 families), gymnosperms (3 families) and monocotyledons (34 families).
4. They included disputed orders among Ordines Anomali which they could not place satisfactorily.

Monocotyledons were described after the dicotyledones.

6. The dicotyledons were divided into 3 Divisions (Polypetalae, Gamopetalae and Monochlamydeae) and 14 series. Each series again divided into cohorts (modern orders) and cohorts into orders (modern families).
7. The authors did not mention anything about the origin of the angiosperms.
8. Creation of the Disciflorae, a taxon not described by the earlier taxonomists.
9. Among the Monochlamydeae, major taxa, like the series, were divided on the basis of terrestrial and aquatic habits.
10. Polypetalae carries 82 families, 2610 genera & 31,874 species. Gamopetalae carries 45 families 2619 genera & 34,556 species.

Monochlamydae includes 36 families, 801 genera & 11,784 species. Similarly Monocotyledons consist 34 families, 1495 genera and 18,576 species.

Merits of Bentham and Hooker's System:

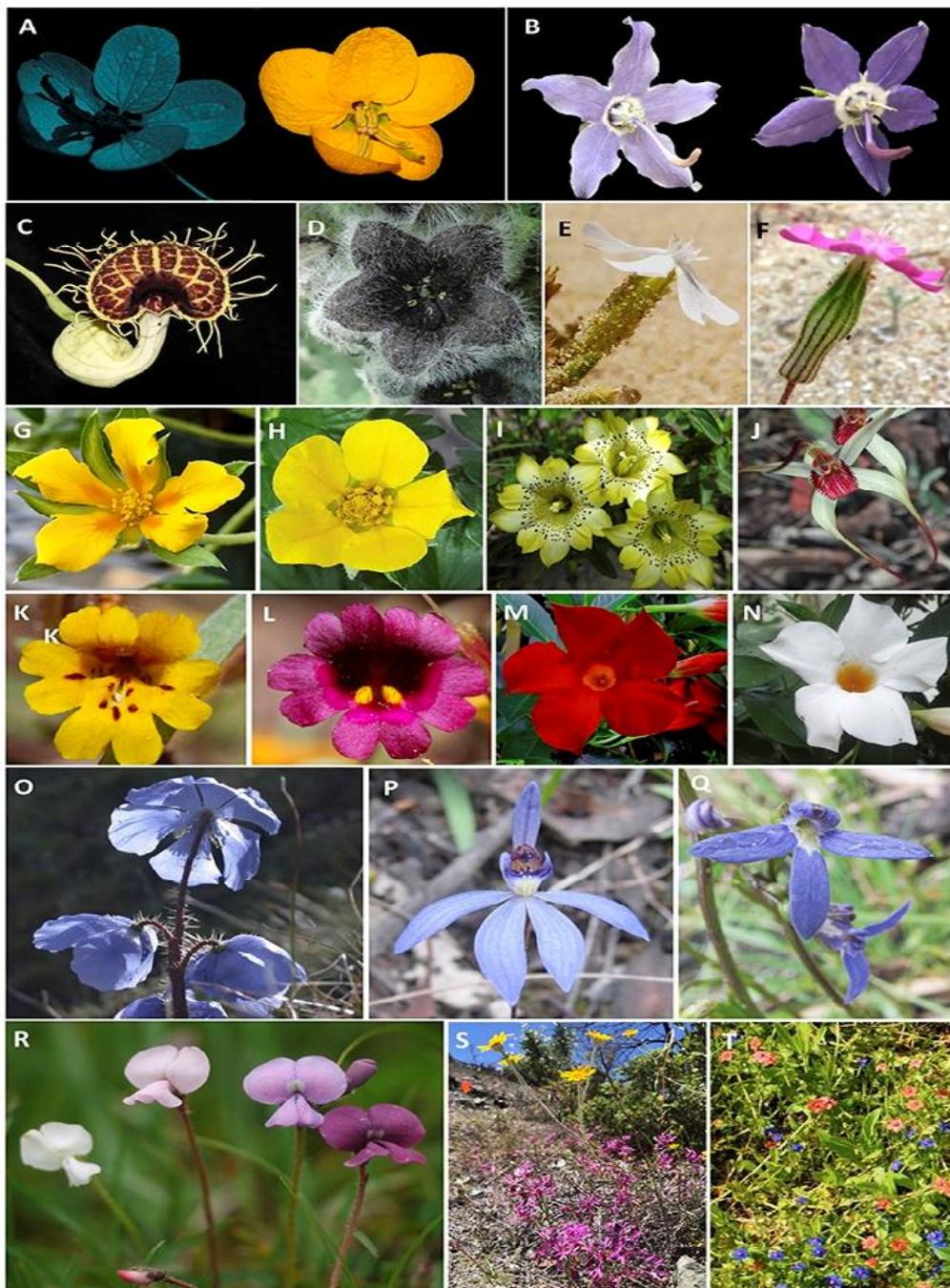
1. Each plant has been described either from the actual specimen or preserved herbarium sheets so that the descriptions are detailed as well as quite accurate.
2. The system is highly practical and is useful to students of systematic botany for easy identification of species.
3. The flora describes geographical distribution of species and genera.
4. The generic descriptions are complete, accurate and based on direct observations.
5. Larger genera have been divided into sub genera, each with specific number of species

Figure: 5

ECONOMIC IMPORTANCE OF ANGIOSPERMS

- **As medicine:** Organic compounds synthesized (alkaloids, glycosides) by plants are used in treating various ailments. E.g. Artemisinin used in treating malaria
- **As food:** They are consumed directly as food e.g. cereals, pulses (legumes), fruits, vegetables, roots and tubers, or indirectly in food and beverage industry e.g. essences (vanilla), malted cereals for beer.
- **As fibers:** They provide fibres for clothing, ropes, etc. For e.g. Cotton (*Gossypium* sp.), Jute (*Crochchorus capsularis* and *C. olitorius*), Sisal hemp (*Agave sisalana*), Linen (*Linum usitatissimum*).
- **As Constructional material:** Wood of various angiosperms is used for construction of buildings, railways, ships, and furniture. E.g. Teak (*Tectona grandis*).
- **As Rubber:** The latex of few angiosperms used in various industrial processes e.g., tyre-making from the rubber plant (*Hevea* sp.)
- **As Essential oils:** The essential oil obtained from flowers, fruits, seeds, leaves, etc. are used in cosmetic industry.
- **As Horticulture:** Ornamental, landscaping and restoration of ecosystem.

Different types of angiosperm flowers



Conclusion

Pollination is a vital process of nature that is very well known, but is extremely important in the food growing process, and without it, we would be in trouble for the fruit seeds to develop, pollen has to be transferred between two flowers of the same species, which then fertilizes the flower and allows the production of healthy seeds on the plant.

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Reproduction and pollination system in plants

U. Lathifa and Dr. M. Kannahi

PG and Research Department of Microbiology

Sengamala Thayar Educationanl Trust Women's College (Autonomous),

sundarakottai, Mannargudi, Thiruvarur, Tamil Nadu, India

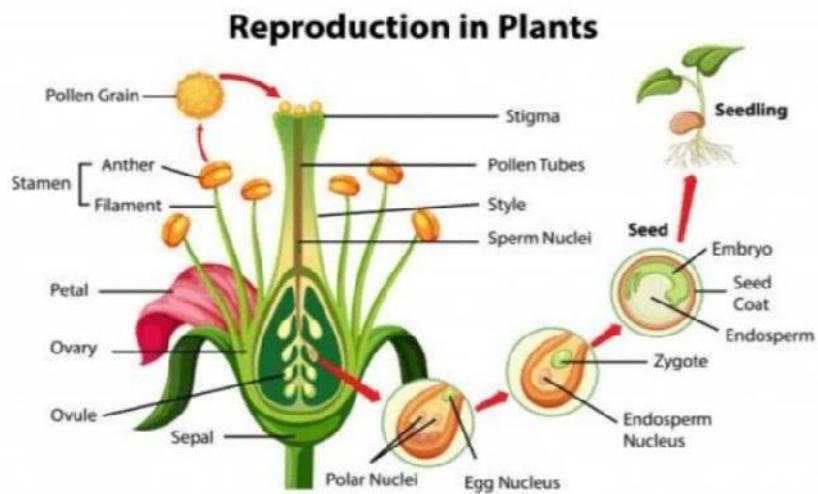
Email id: *Kannahiamf@gmail.com*

Introduction

Mode of reproduction determine the genetic constitution of crop plants, that is whether the plants are normally homozygous or heterozygous. This,in turn, determines the goal of a breeding programme. If the crop plants are naturally homozygous.,e.g.,as in self pollinators like wheat, a homozygous line would be desired as a variety. But if the plants are heterozygous population has to be developed as a variety. Consequently, the breeding methods have to be vastly different for the two groups of crop plants. A knowledge of the mode of reproduction of crop plants is also important for making artificial hybrids. Production of hybrids between diverse and desirable parents is the basis for almost all the modern breeding programmes

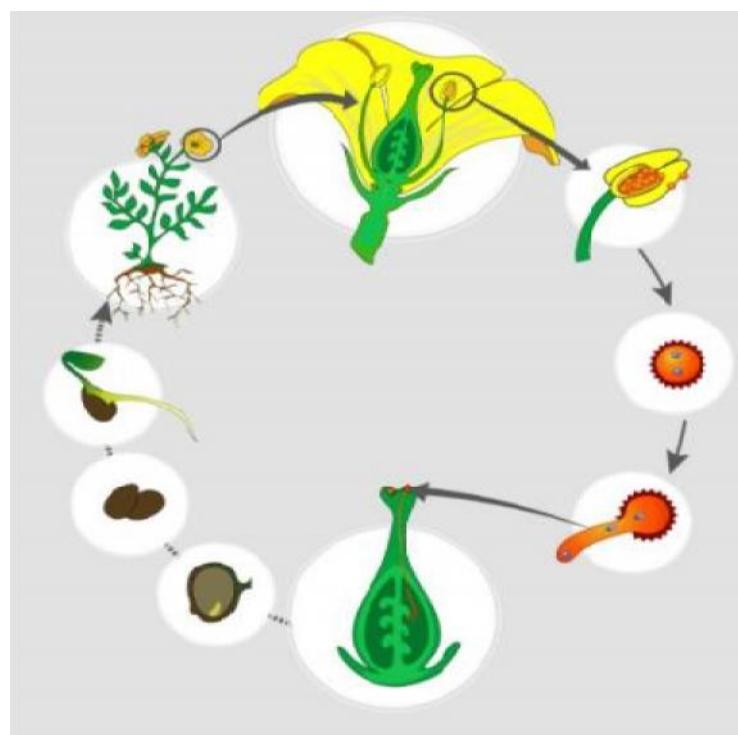
Reproduction

Plant reproduction is the production of new offspring in plants, which can be accomplished by sexual or asexual reproduction. Sexual reproduction produces offspring by the fusion of gametes resulting in offspring genetically different from either parent. Asexual reproduction produces new individuals without the fusion of gametes, resulting in clonal plants that are genetically identical to the parent plant and each other, unless mutation occur.



Sexual reproduction

In sexual reproduction, two parents are involved in producing a new individual. Offspring is produced by the fusion of gametes (sex cells) from each parent.



Animals like dog, cats, lions, giraffe, humans, etc. all reproduce sexually.

Advantages of sexual reproduction

- Leads to variations.
- Variations which are desirable often show hybrid vigour.
- High adaptability of individuals to changing environmental conditions.
- Variations provide a basis for evolutionary changes.

Disadvantages of sexual reproduction

- Fusion is difficult if two individuals are isolated.
- Some variations may have undesirable qualities.
- Population growth is slow.

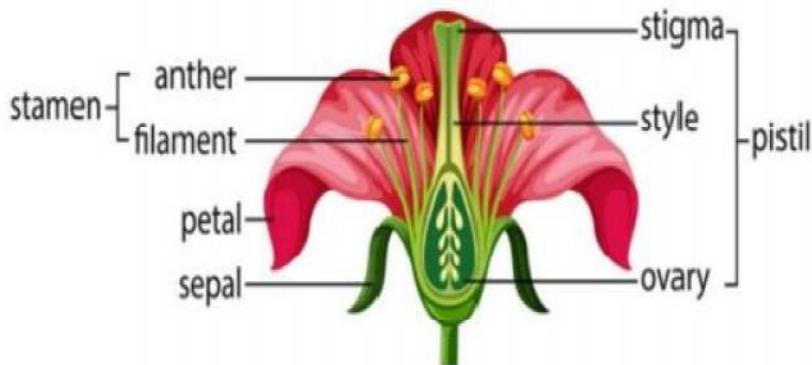
Flowers

Flower, sometimes known as a bloom or blossom, is the reproductive structure found in flowering plants, the majority of the flowering plants reproduce sexually. The flower is the reproductive part of a plant i.e., both male and female gametes are produced by flowers. Sexual reproduction in plants takes place in flowers. The complete flower typically consists of four parts:

➤ Petals

- Sepals
- Stamen (male reproductive part)
- Pistil/Carpel (female reproductive part)
- Stamen (male reproductive part) consists of anther and filament.
- The anther is a sac-like structure that produces and stores pollen.
- The filament supports the anther.
- The pistil (female reproductive part) comprises three parts- stigma, style, and ovary.
- Stigma is the topmost part of a flower.
- The style is the long tube which connects the stigma to the ovary.

- The ovary contains a lot of ovules. It is the part of the plant where the seed formation takes place.



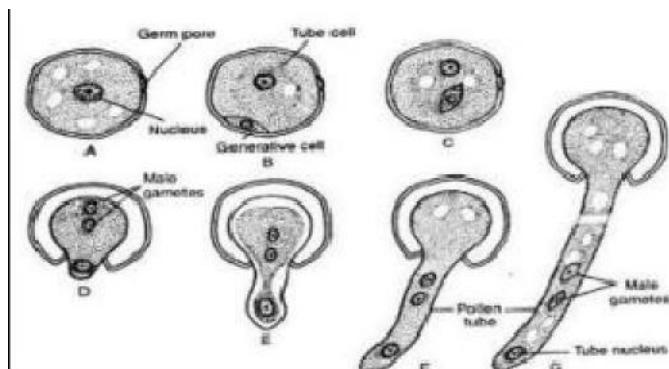
Sporogenesis

Sporogenesis is the production of spores in biology. The term is also used to refer to the process of reproduction via spores. Reproductive spores were found to be formed in eukaryotic organisms, such as plants, algae and fungi, during their normal reproductive life cycle.

1. Microsporogenesis
2. Megasporogenesis

Microsporogenesis

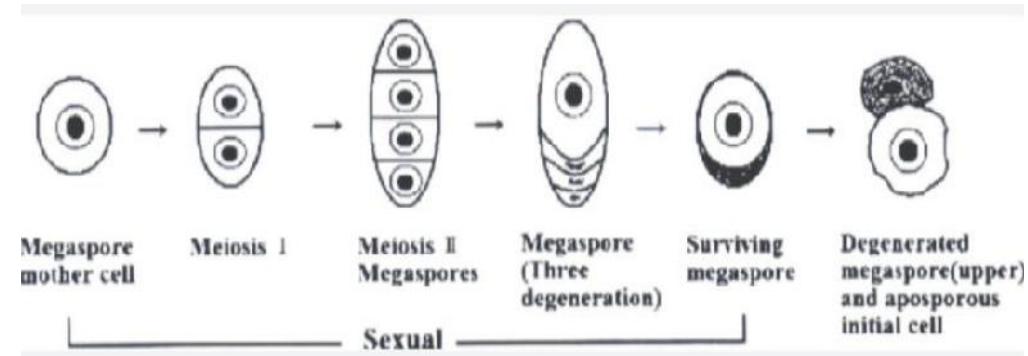
Microsporogenesis is the meiotic or reduction division process that produces pollen grains (or microspores) inside the pollen sacs (or microsporangium) of flowering plants.



Each microspore, which is the male gametophyte's first cell that creates male gametes.

Megasporogenesis

Megasporogenesis refers to the development of megaspores from the megasporocyte, the cell that undergoes meiosis. Meiosis of the megasporocyte nucleus results in the formation of four haploid megasporule nuclei.



Gametogenesis

The production of male and female gametes in the microspores and megaspores, respectively, is known as gametogenesis

1. Microgametogenesis
2. Megagametogenesis

Microgametogenesis

Microgametogenesis is the process in plant reproduction where a microgametophyte develops in a pollen grain to the three-celled stage of its development. In flowering plants it occurs with a microspore mother cell inside the anther of the plant.

Megagametogenesis

Megagametogenesis is the process of maturation of the female gametophyte, or megagametophyte, in plants. During the process of megagametogenesis, the megasporule, which arises from megasporogenesis, develops into the embryo sac, which is where the female gamete is housed.

Fertilization

In plants, fertilization is a process of sexual reproduction, which occurs after pollination and germination.

Fertilization can be defined as the fusion of the male gametes (pollen) with the female gametes (ovum) to form a diploid zygote. It is a physicochemical process which occurs after the pollination of the carpel. The complete series of this process takes place in the zygote to develop into a seed.

Fertilization Process

Step 1: Pollination

In general, male gametes are contained in pollen, which is carried by wind, water, or wildlife (both insects and animals) to reach female gametes. The pollen is deposited on a plant's stigma, which is part of the pistil (the elongated part of a flower extending from the ovary). This process is called pollination.

Types of Pollination

All plants having flowers completely rely on pollination method for reproduction. There are 2 types of pollination –

1. *Self Pollination*
2. *Cross-Pollination*

1. Self Pollination (Autogamy)

It is referred to as the primary type of pollination as it includes a single flower. Self-pollination occurs when pollen grains fall directly from anther into the stigma of the flower.

Advantage

- Self-pollination ensures that recessive characters are eliminated.
- The wastage of the pollen grain is very less compared to cross-pollination
- In the process of self-pollination, the purity of the race is maintained, as there is no diversity in the genes
- In self-pollination, there is no involvement of external factors like wind, water, and other pollinating agents.
- Self-pollination ensures that even a smaller quantity of produced pollen grains from plants have a good success rate in pollination.

Disadvantages

- The major disadvantage of Self-pollination is there is no mixing up of genes. Due to which:
 - The vigour and vitality of the race are reduced
 - The immunity to diseases is reduced in the resultant offsprings.

2. Cross pollination (Allogamy)

It refers to a complex type of pollination that allows the transfer of pollen grains from the anther of the flower into the stigma of another flower. This method leads to an increase in genetic diversity as different flowers will share and combine their genetic information to create unique offspring.

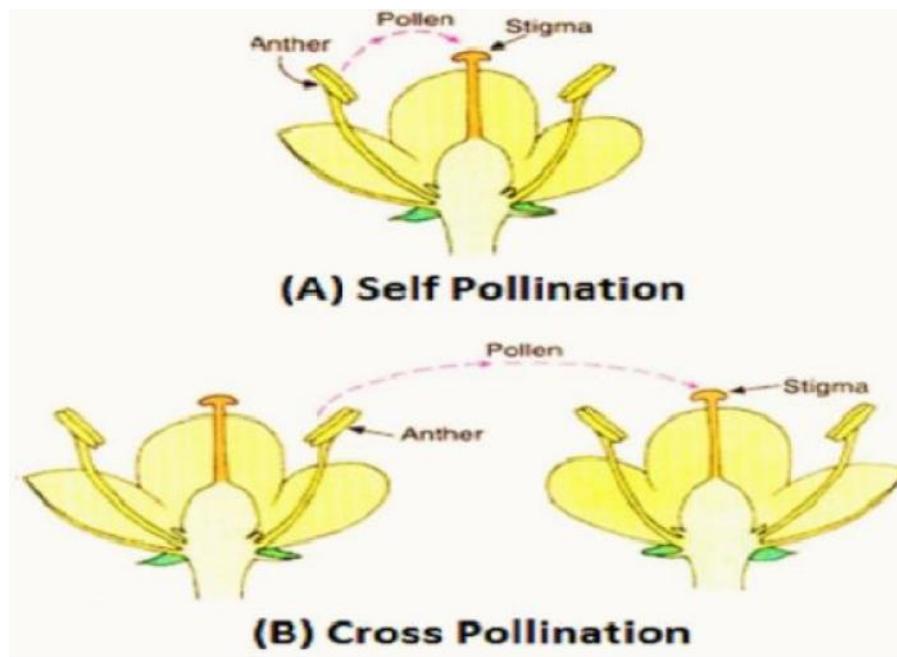
1. pollinated by wind-Anemophily
- 2 . pollinated by animals – zoophily
- 3 .pollinated by insects - entomophily
4. pollinated by water - hydrophily

Advantage

- The produced seeds are good in vigour and vitality.
- All unisexual plants can reproduce through the process of Cross-pollination.
- The recessive characters in the lineage are eliminated as a result of genetic recombination.
- This process improves the immunity of the offsprings towards the diseases and other environmental factors.
- Cross-pollination introduces new genes into a sequence of species and this is mainly due to the fertilization between genetically different gametes.

Disadvantages

- In this process, there is a great wastage of pollen grains.
- Due to genetic recombination during meiosis, there are chances of eliminations of good qualities and additions of unwanted characteristics in offspring.



Step 2: Germination

Within a few minutes, pollen tubes begin growing, or germinating, toward the egg cell. These tubes will provide a path for the sperm carried in the pollen to reach the egg.

Step 3: Penetration of the Ovule

The pollen tubes penetrate the ovule, which contains the female gametes.

Step 4: Fertilization

Sperm travel down the pollen tubes and fertilize an egg. Most angiosperms undergo double fertilization, where both an egg and the polar nuclei in the embryonic sac are fertilized.

Types of Plant Fertilization

There are three primary types of plant fertilization:

1. **Porogamy**- Porogamy occurs when a pollen tube enters the ovule through a micropyle, or minute opening in the ovule; this is the most common form of plant fertilization.

2. **Chalazogamy**- In chalazogamy, pollen tubes enter the ovule through the chalaza, located opposite the micropyle,

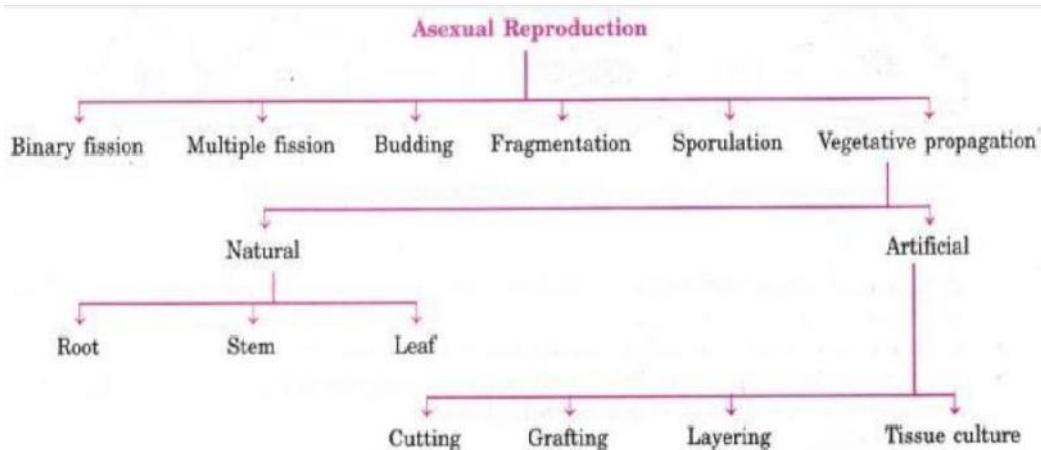
3.Mesogamy- In mesogamy, these tubes enter the ovule through its integument (outermost layer or layers).

Post fertilization changes

- 1.The ovule developed in the seed
- 2.The integuments of the ovule developed into seed coat.
- 3.The Ovary enlarges and develops into a fruit.

Asexual reproduction

Asexual reproduction in plants occurs through budding, fragmentation, vegetative propagation, and spore formation. No flowers are required for this method. The plants produced by asexual reproduction thrive well in stable environments.



Types Of Asexual Reproduction In Plants

Binary fission

Binary fission ("division in half") is a kind of asexual reproduction. It is the most common form of reproduction in lower plants such as bacteria. In this method, the nucleus splits or divides into two and then the cell splits across the middle, forming two small identical cells called the daughter cells. Vegetative

Multiple fission

The process of asexual reproduction in which many daughter cells are produced from the parent cell instead of two daughter cells is called multiple fission. During this process, the nucleus is repeatedly divided to generate a large number of nuclei.

Budding

Budding is the mode of asexual reproduction wherein a new plant is developed from an outgrowth known as the bud. A bud is generally formed due to cell division at one particular site Propagation

Fragmentation

Fragmentation is a very common type of vegetative reproduction in plants. Fragmentation occurs when a shoot that is rooted becomes detached from the main group. In plants, there are different other mechanisms. There are several other known mechanisms of natural fragmentation in plants. Vegetative

Sporulation

Sporulation is an asexual reproduction method in which a parent plant creates hundreds of reproductive units called spores, which germinate under favourable conditions and produce young organisms with numerous vegetative hyphae.

Vegetative propagation

It is any form of asexual reproduction occurring in plants, in which new plants are produced from the vegetative parts of the plants, i.e. roots, stems or buds. Vegetative propagation in plants can occur both by naturally or also can be artificially induced by horticulturists.

The most common techniques of vegetative propagation are:

1.**stem**-Runners are the stems which usually grow in a horizontal form above the ground.

2.**roots**-A new plant is developed from modified roots called tubers.

Example: Sweet Potato

3.**leaves**-- In some plants, detached leaves from the parent plant can be used to grow a new plant. They exhibit growth of small plants, called plantlets, on the edge of their leaves.

Example : Bryophyllum.

Natural Methods

Natural methods of asexual reproduction include self-propagation. The different ways in which a plant self propagates are mentioned below:

Tuber: potato (Solanum tuberosum) et.

Bulb: Onions (Allium cepa), garlic(Allium sativum) etc.

Rhizome :Ginger (Zingiber official), turmeric (Curcuma longa) etc

Corm :Bunda(Colocasia antiquorum), arwi(Colocasia esculenta)

Artificial Methods

Following are the artificial methods of asexual reproduction in plants:

Cutting

In this method, a part of a plant is cut along with the node and is buried in the soil.The cutting is watered regularly.this is the cheapest method of vegetative propagation in plants.

Grafting

In this method, the parts of two different plants are joined together such that they continue to grow as a single plant.The rooted plant is known as the stock. The other plant is known as the graft.

Layering

It is the method in which a stem attached to a plant is lowered in the ground and covered with soil. The stem grows roots while attached to the parent plant and then detaches as an independent plant.

Micropagation

This is the method of producing a large number of plants from an explant under laboratory conditions within a short time interval. This facilitates the growth of rare and endangered plant species that are difficult to grow under natural conditions.

Advantages of Asexual Reproduction

Following are the **advantages of asexual reproduction:**

-]) Mates are not required.
-]) The process of reproduction is rapid.
-]) An enormous number of organisms can be produced in very less time.
-]) Positive genetic influences pass on to successive generations.
-]) It occurs in various environments.

Disadvantages of Asexual Reproduction

- | The major disadvantages of asexual reproduction are:
- | Lack of diversity. Since the offsprings are genetically identical to the parent they are more susceptible to the same diseases and nutrient deficiencies as the parent. All the negative mutations persist for generations.
- | Since only one organism is involved, the diversity among the organisms is limited.
- | They are unable to adapt to the changing environment.
- | A single change in the environment would eliminate the entire species.

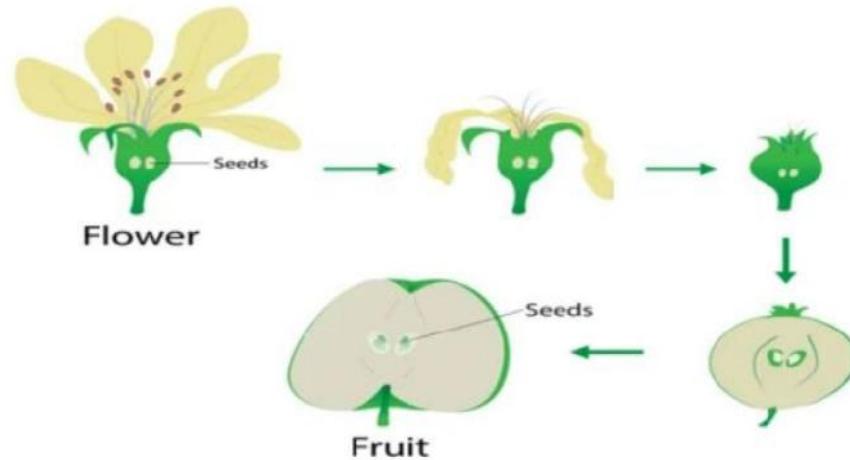
Formation of fruits

The pericarp is the wall of the ovary that develops as the wall of the fruits. The pericarp of the fruits might be fleshy as in guava, mango, etc. or might be dry as in mustard, walnut, etc. The pericarp is further differentiated into three layers, namely:

Epicarp: Outermost layer, forms the peel.

Mesocarp: Middle layer, fleshy, edible portion of the fruits

Endocarp: Innermost layer, the inner rough portion where the seed is accommodated.



Types of Fruits

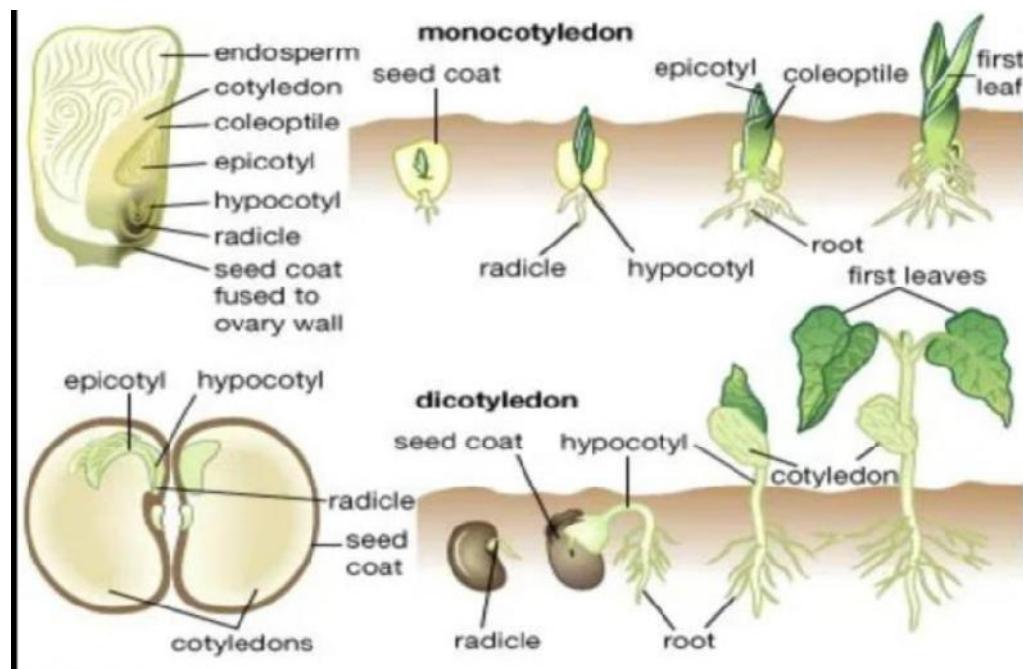
Based on the number of ovaries and the number of flowers involved in the fruit formation, fruits are classified into three major groups namely:

- 1.simple fruit.
2. Aggregate fruit.
- 3.Composite fruit.

Seed formation

Seed is formed when fertilised ovule divides by mitosis. It stores food and has the potential to develop into a new plant under optimal conditions.based on the number of cotyledons in the seed the angiosperm is divided into two groups

1. **Dicotyledon** - seeds with two cotyledons e. g. Pea, bean and castor
2. **Monocotyledons**-seeds with one cotyledon e. g. Maize, rice, wheat and onion.



Conclusion

The process of fertilization happens when male gametes land on female gametes. In the absence of fertilization, seeds and fruits cannot be produced. Therefore, it has been proved that plants cannot reproduce and produce fruit without pollination.

Acknowledgment

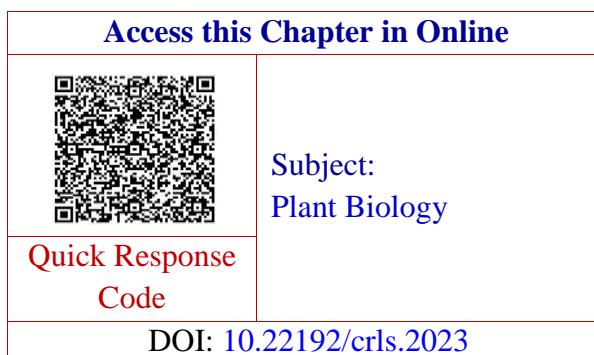
The author express our science thanks to Dr.V.Dhivaharan, Dean, Department of Lifesciences, stet women's college, sundarakkottai, Mannargudi, Thiruvarur dt, Tamilnadu, India for providing us all the facilities and encouragement for completing this study successfully.

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Corona Virus

N. Uma maheswari, K. Jayabharathi and S.Udhaya

PG and Research Department of Microbiology,

STET Women's College (Autonomous),

Sundarakkottai, Mannargudi, Thiruvarur, (Dt), Tamil Nadu, India.

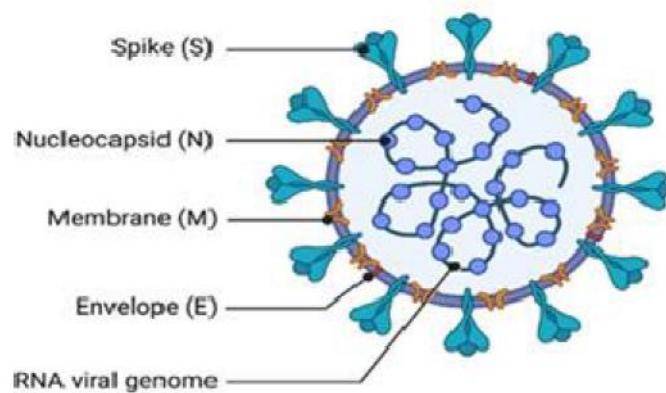
Phone number: 9942083056, 9080881425

Email Id: umasamyamf@gmail.com

Introduction

Coronavirus disease 2019 (COVID-19), the highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a catastrophic effect on the world's demographics resulting in more than 6 million deaths worldwide, emerging as the most consequential global health crisis since the era of the influenza pandemic of 1918. After the first cases of this predominantly respiratory viral illness were first reported in Wuhan, Hubei Province, China, in late December 2019, SARS-CoV-2 rapidly disseminated across the world in a short span of time, compelling the World Health Organization (WHO) to declare it as a global pandemic on March 11, 2020. Since being declared a global pandemic, COVID-19 has ravaged many countries worldwide and has overwhelmed many healthcare systems. The pandemic has also resulted in the loss of livelihoods due to prolonged shutdowns, which have had a rippling effect on the global economy. Even though substantial progress in clinical research has led to a better understanding of SARS-CoV-2 and the management of COVID-19, limiting the continuing spread of this virus and its variants has become an issue of increasing concern, as SARS-CoV-2 continues to wreak havoc across the world, with many countries enduring a second or third wave of outbreaks of this viral illness attributed mainly due to the emergence of mutant variants of the virus.

Coronavirus Structure



Etiology

Coronaviruses (CoVs) are positive-stranded RNA(+ssRNA) viruses with a crown-like appearance under an electron microscope (*coronam* is the Latin term for crown) due to the presence of spike glycoproteins on the envelope. The subfamily *Orthocoronavirinae* of the *Coronaviridae* family (order *Nidovirales*) classifies into four genera of CoVs:

- Alphacoronavirus (alphaCoV)
- Betacoronavirus (betaCoV)
- Deltacoronavirus (deltaCoV)
- Gammacoronavirus (gammaCoV)

Common human CoVs: HCoV-OC43, and HCoV-HKU1 (betaCoVs of the A lineage); HCoV-229E, and HCoV-NL63 (alphaCoVs). These viruses can cause common colds and self-limiting upper respiratory tract infections in immunocompetent individuals. However, in immunocompromised subjects and the elderly, lower respiratory tract infections can occur due to these viruses.

Other human CoVs: SARS-CoV and MERS-CoV (betaCoVs of the B and C lineage, respectively). These viruses are considered to be more virulent and capable of causing epidemics manifesting with respiratory and extra-respiratory manifestations of variable clinical severity.

SARS-CoV-2 Variants of Concern (VOCs)

Alpha (B.1.1.7 lineage)

In late December 2020, a new SARS-CoV-2 variant of concern, **B.1.1.7 lineage**, also referred to as **Alpha variant** or **GRY**(formerly GR/501Y.V1), was reported in the UK based on whole-genome sequencing of samples from patients who tested positive for SARS-CoV-2.

In addition to being detected by genomic sequencing, **the B.1.1.7** variant was identified in a frequently used commercial assay characterized by the absence of the S gene (S-gene target failure, SGTF) PCR samples. The B.1.1.7 variant includes 17 mutations in the viral genome. Of these, eight mutations (69-70 deletion, 144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H) are in the spike (S) protein. N501Y shows an increased affinity of the spike protein to ACE 2 receptors, enhancing the viral attachment and subsequent entry into host cells.

Beta (B.1.351 lineage)

Another variant of SARS-CoV-2, **B.1.351** also referred to as **Beta variant** or **GH501Y.V2** with multiple spike mutations, resulted in the second wave of COVID-19 infections, was first detected in South Africa in October 2020.

The B.1.351 variant includes nine mutations (L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, and A701V) in the spike protein, of which three mutations (K417N, E484K, and N501Y) are located in the RBD and increase the binding affinity for the ACE receptors. SARS-CoV-2 501Y.V2(B.1.351 lineage) was reported in the US at the end of January 2021.

This variant is reported to have an increased risk of transmission and reduced neutralization by monoclonal antibody therapy, convalescent sera, and post-vaccination sera.

Gamma (P.1 lineage)

The third variant of concern, the **P.1 variant** also known as **Gamma variant** or **GR/501Y.V3**, was identified in December 2020 in Brazil and was first detected in the US in January 2021.

The B.1.1.28 variant harbors ten mutations in the spike protein (L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I V1176, K417T, E484K, and N501Y). Three mutations (L18F, K417N, E484K) are located in the RBD, similar to the B.1.351 variant.

Notably, this variant may have reduced neutralization by monoclonal antibody therapies, convalescent sera, and post-vaccination sera.

Delta (B.1.617.2 lineage)

The fourth variant of concern, B.1.617.2 also referred to as the **Delta variant** was initially identified in December 2020 in India and was responsible for the deadly second wave of COVID-19 infections in April 2021 in India. In the United States, this variant was first detected in March 2021

The Delta variant was initially considered a variant of interest. However, this variant rapidly spread around the world prompting the WHO to classify it as a VOC in May 2021

The B.1.617.2 variant harbors ten mutations (T19R, (G142D*), 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N) in the spike protein.

Omicron (B.1.1.529 lineage)

The fifth variant of concern **B.1.1.529**, also designated as the **Omicron variant** by the WHO was first identified in South Africa on 23 November 2021 after an uptick in the number of cases of COVID-19 .

Omicron was quickly recognized as a VOC due to more than 30 changes to the spike protein of the virus along with the sharp rise in the number of cases observed in South Africa. The reported mutations include T91 in the envelope, P13L, E31del, R32del, S33del, R203K, G204R in the nucleocapsid protein, D3G, Q19E, A63T in the matrix, N211del/L212I, Y145del, Y144del, Y143del, G142D, T95I, V70del, H69del, A67V in the N-terminal domain of the spike, Y505H, N501Y, Q498R, G496S, Q493R, E484A, T478K, S477N, G446S, N440K, K417N, S375F, S373P, S371L, G339D in the receptor-binding domain of the spike, D796Y in the fusion peptide of the spike, L981F, N969K, Q954H in the heptad repeat 1 of the spike as well as multiple other mutations in the non-structural proteins and spike protein.

Epidemiology

According to the World Health Organization (WHO), the emergence of viral diseases represents a serious public health risk. In the past two decades, several epidemics caused by viruses such as the severe acute respiratory syndrome coronavirus (SARS-CoV) from 2002 to 2003, and H1N1 influenza in 2009, and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 have been described which have had a significant impact on global health. Since being declared a global pandemic by the WHO, SARS-CoV-2, the virus responsible for COVID-19 has spread to 223 countries with more than

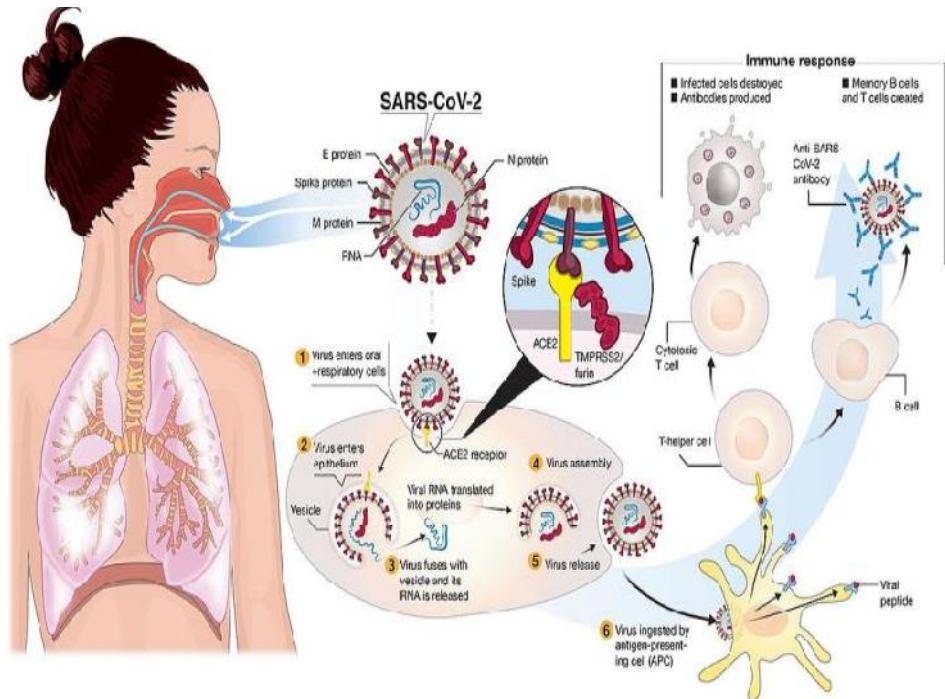
593 million cases, and more than 6 million deaths reported globally. A recent epidemiological update by WHO reported that more than 200 countries around the world have reported SARS-CoV-2 variants of concern of which the Omicron VOC has been reported as the most dominant current circulating VOC since first being reported in November 2021. The U.S. has experienced the highest number of SARS-CoV-2 infections and COVID-19 related deaths followed by India and Brazil. In fact, COVID-19 was the third leading cause of death in the U.S. in 2020 after heart disease and cancer, with approximately 375,000 death reported. The WHO's current estimate of the global case fatality rate for COVID-19 is 2.2%.

Pathophysiology

Pathogenesis of SARS-CoV-2

Structurally and phylogenetically, SARS-CoV-2 is similar to SARS-CoV and MERS-CoV and is composed of four main structural proteins: spike (S), envelope (E) glycoprotein, nucleocapsid (N), membrane (M) protein, along with 16 nonstructural proteins, and 5-8 accessory proteins. The surface spike (S) glycoprotein, which resembles a crown, is located on the outer surface of the virion and undergoes cleavage into an amino (N)-terminal S1 subunit, which facilitates the incorporation of the virus into the host cell and a carboxyl (C)-terminal S2 subunit containing a fusion peptide, a transmembrane domain, and cytoplasmic domain is responsible for virus-cell membrane fusion. The S1 subunit is further divided into a receptor-binding domain (RBD) and N-terminal domain (NTD), which facilitates viral entry into the host cell and serves as a potential target for neutralization in response to antisera or vaccines. The RBD is a fundamental peptide domain in the pathogenesis of infection as it represents a binding site for the human angiotensin-converting enzyme 2 (ACE2) receptors. Inhibition of the renin-angiotensin-aldosterone system(RAAS), as previously hypothesized, does not increase the risk of hospitalization for COVID-19 and severe disease.

SARS-CoV-2 gains entry into the hosts' cells by binding the SARS-CoV-2 spike or S protein (S1) to the ACE2 receptors abundantly on respiratory epithelium such as type II alveolar epithelial cells. Besides the respiratory epithelium, ACE2 receptors are also expressed by other organs such as the upper esophagus, enterocytes from the ileum, myocardial cells, proximal tubular cells of the kidney, and urothelial cells of the bladder. The viral attachment process is followed by priming the spike protein S2 subunit by the host transmembrane serine protease 2 (TMPRSS2) that facilitates cell entry and subsequent viral replication endocytosis with the assembly of virions.



Effect of SARS-CoV-2 on Extrapulmonary Organ Systems

Although the respiratory system is the principal target for SARS-CoV-2 as described above, it can affect other major organ systems such as the gastrointestinal tract (GI), hepatobiliary, cardiovascular, renal, and central nervous system.

Cardiovascular system (CVS): Although the exact mechanism of cardiac involvement in COVID-19 is unknown, it is likely multifactorial. ACE2 receptors are also exhibited by myocardial cells implicating direct cytotoxicity by the SARS-CoV-2 on the myocardium leading to myocarditis. Proinflammatory cytokines such as IL-6 can also lead to vascular inflammation, myocarditis, and cardiac arrhythmias.

Hematological: SARS-CoV-2 has a significant effect on the hematological and hemostatic systems. The mechanism of leukopenia, one of the most common laboratory abnormalities encountered in COVID-19, is unknown. Several hypotheses have been postulated that include ACE 2 mediated lymphocyte destruction by direct invasion by the virus, lymphocyte apoptosis due to proinflammatory cytokines, and possible invasion of the virus of the lymphatic organs.

Central Nervous System (CNS): There is emerging evidence of ACE2 receptors in human and mouse brains, implicating the potential infection of the brain by SARS-CoV-2. The possible routes by which SARS-CoV-2 can invade the central nervous system are transsynaptic transfer across infected neurons via the olfactory nerve, vascular endothelial cell infection, or migration of leukocytes across the blood-brain barrier.

Gastrointestinal (GI) Tract: The pathogenesis of GI manifestations of COVID-19 is unknown and is likely considered to be multifactorial due to several potential mechanisms that include the direct ACE 2-mediated viral cytotoxicity of the intestinal mucosa, cytokine-induced inflammation, gut dysbiosis, and vascular abnormalities.

Hepatobiliary: Although the pathogenesis of liver injury in COVID-19 patients is unknown, hepatic injury in COVID-19 is likely multifactorial and is explained by many mechanisms alone or in combination that includes ACE-2-mediated viral replication in the liver, direct virus-mediated damage, hypoxic or ischemic injury, immune-mediated inflammatory response, drug-induced liver injury (DILI), or worsening of preexisting liver disease.

Renal: The pathogenesis of COVID-19 associated kidney injury is unknown and is likely multifactorial explained by a single or a combination of many factors such as direct cytotoxic injury from the virus, imbalance in the RAAS, associated cytokine-induced hyperinflammatory state, microvascular injury, and the prothrombotic state associated with COVID-19.

Histopathology

Lungs: A multicenter analysis of lung tissue obtained during autopsies of patients who tested positive for COVID-19 demonstrated typical diffuse alveolar damage features in 87% of cases. Additionally, there was a frequent presence of type II pneumocyte hyperplasia, airway inflammation, and hyaline membranes in alveolar zones. Forty-two percent of patients were noted to have large vessel thrombi, platelet (CD61 positive), and/or fibrin microthrombi were present in 84% of cases.

Brain: A single-center histopathological study of brain specimens obtained from 18 patients who succumbed to COVID-19 demonstrated acute hypoxic injury in all patients' cerebrum and cerebellum. Notably, no features of encephalitis or other specific brain changes were seen. Additionally, immunohistochemical analysis of brain tissue did not show cytoplasmic viral staining.

Heart: Analysis of cardiac tissue from 39 autopsy cases of patients who tested positive for SARS-CoV-2 demonstrated the presence of SARS-CoV-2 viral genome within the myocardium.

Kidney: Histopathology analysis of kidney specimens obtained from autopsies of 26 patients with confirmed COVID-19 demonstrated signs of diffuse proximal tubular injury with loss of brush border, non-isometric vacuolar degeneration, and necrosis. Additionally, electron microscopy showed clusters of coronavirus-like particles with spikes in the tubular epithelium and podocytes.

GI Tract: Endoscopic specimens demonstrated positive staining of the viral nucleocapsid protein in the gastric, duodenal, and rectal epithelium cytoplasm. Numerous infiltrating plasma cells and lymphocytes with interstitial edema were seen in the lamina propria of the stomach, duodenum, and rectum.

Liver: A prospective single-center clinicopathologic case series study involving the postmortem histopathological exam of major organs of 11 deceased patients with COVID-19 reported hepatic steatosis findings in all patients. The liver specimens of 73% of patients demonstrated chronic congestion. Different forms of hepatocyte necrosis were noted in 4 patients, and 70% showed nodular proliferation.

Treatment and management

Initially, early in the pandemic, the understanding of COVID-19 and its therapeutic management was limited, creating an urgency to mitigate this new viral illness with experimental therapies and drug repurposing. Since then, due to the intense efforts of clinical researchers globally, significant progress has been made, which has led to a better understanding of not only COVID-19 and its management but also has resulted in the development of novel therapeutics and vaccine development at an unprecedented speed.

Pharmacologic Therapies In The Management Of Adults With COVID-19

Currently, a variety of therapeutic options are available that include antiviral drugs (e.g., molnupiravir, paxlovid, remdesivir), anti-SARS-CoV-2 monoclonal antibodies (e.g., bamlanivimab/etesevimab, casirivimab/imdevimab, sotrovimab, bebtelovimab), anti-inflammatory drugs (e.g., dexamethasone), immunomodulators agents (e.g., baricitinib, tocilizumab) are available under FDA issued Emergency Use Authorization (EUA) or being evaluated in the management of COVID-19.

The clinical utility of these treatments is specific and is based on the severity of illness or certain risk factors. The clinical course of the COVID-19 illness occurs in 2 phases, an early phase when SARS-CoV-2 replication is greatest before or soon after the onset of symptoms. Antiviral medications and antibody-based treatments are likely to be more effective during this stage of viral replication. The later phase of the illness is driven by a hyperinflammatory state induced by the release of cytokines and the coagulation system's activation that causes a prothrombotic state. Anti-inflammatory drugs such as corticosteroids, immunomodulating therapies, or a combination of these therapies may help combat this hyperinflammatory state more than antiviral therapies. Below is a summary of the latest potential therapeutic options proposed, authorized, or approved for clinical use in the management of COVID-19.

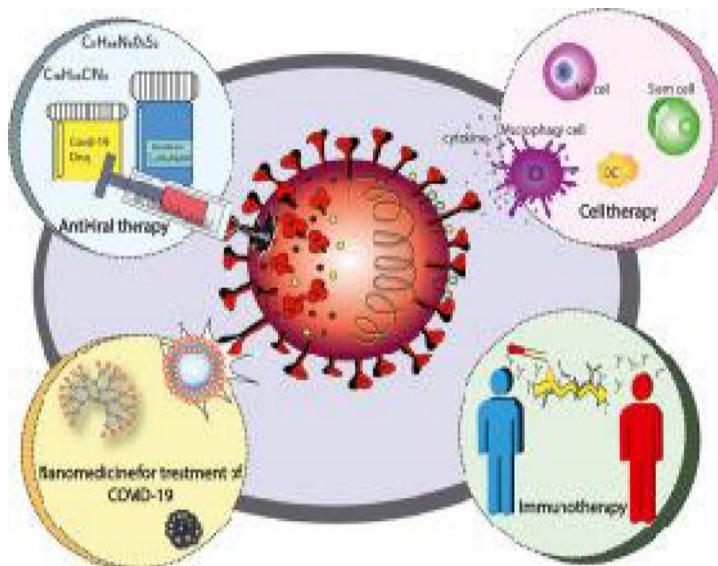
Antiviral Therapies

Molnupiravir (named after the Norse god Thor's hammer Mjölnir) is a directly acting broad-spectrum oral antiviral agent acting on the RdRp enzyme was initially developed as a possible antiviral treatment for influenza, alphaviruses including Eastern, Western, and Venezuelan equine encephalitic viruses. Based on a meta-analysis of available phase 1-3 studies, molnupiravir was noted to demonstrate a significant reduction in hospitalization and death in mild COVID-19 disease

Paxlovid (ritonavir in combination with nirmatrelvir) is an oral combination pill of two antiviral agents which on an interim analysis of phase 2-3 data (reported via press release) which included 1219 patients, found that the risk of COVID-19 related hospital admission or all-cause mortality was 89% lower in the paxlovid group when compared to placebo when started within three days of symptom onset.

Remdesivir is a broad-spectrum antiviral agent that previously demonstrated antiviral activity against SARS-CoV-2 *in vitro*. Based on results from three randomized, controlled clinical trials that showed that remdesivir was superior to placebo in shortening the time to recovery in adults who were hospitalized with mild-to-severe COVID-19, the U.S. Food and Drug Administration (FDA) approved remdesivir for clinical use in adults and pediatric patients (over age 12 years and weighing at least 40 kilograms or more) to treat hospitalized patients with COVID-19.

Hydroxychloroquine and chloroquine were proposed as antiviral treatments for COVID-19 initially during the pandemic. However, data from randomized control trials evaluating the use of hydroxychloroquine with or without azithromycin in hospitalized patients did not improve the clinical status or overall mortality compared to placebo. Data from randomized control trials of hydroxychloroquine used as postexposure prophylaxis did not prevent SARS-CoV-2 infection or symptomatic COVID-19 illness.



Oxygenation and Ventilation Management In COVID-19

Conventional Oxygen Therapy

COVID-19 patients with associated respiratory insufficiency should be monitored closely with continuous pulse oximetry. Supplemental oxygen supplementation via nasal cannula or Venturi mask must be administered to maintain oxygen saturation (SpO_2) between 92 to 96% (< 88-90% if COPD). If there is improvement in clinical and oxygen saturation, supplemental oxygen should be continued with periodic reassessment. If there is no clinical improvement or worsening of symptoms and/or oxygen saturation, noninvasive treatments such as High-Flow Nasal Cannula (HFNC) or Noninvasive Positive Pressure Ventilation (NIPPV) are recommended.

Management of Acute Hypoxemic Respiratory Failure in COVID-19

Acute hypoxemic respiratory failure is the most common complication in adult patients with COVID-19, and conventional oxygen therapy is not helpful to address the oxygen demand in these patients. These patients should be managed with enhanced respiratory support modalities such as high-flow nasal cannula (HFNC), noninvasive positive pressure ventilation (NIPPV), endotracheal intubation, and invasive mechanical ventilation (IMV) or extracorporeal membrane oxygenation (ECMO)

High-Flow Nasal Cannula (HFNC) and Noninvasive Positive Pressure Ventilation (NIPPV)

HFNC and NIPPV are noninvasive enhanced respiratory support modalities available in managing COVID-19-associated acute hypoxemic respiratory failure and are instrumental in avoiding invasive mechanical ventilation in carefully selected patients. A meta-analysis study evaluating the effectiveness of HFNC compared to conventional oxygen therapy and NIPPV before mechanical ventilation reported that HFNC, when used before mechanical ventilation, could improve the prognosis of patients compared to conventional oxygen therapy and NIPPV.

Vaccination to prevent SARS-CoV-2 infection

Besides the importance of imposing public health and infection control measures to prevent or decrease the transmission of SARS-CoV-2, the most crucial step to contain this global pandemic is by vaccination to prevent SARS-CoV-2 infection in communities across the world. Extraordinary efforts by clinical researchers worldwide during this pandemic have resulted in the development of novel vaccines against SARS-CoV-2 at an unprecedented speed to contain this viral illness that has devastated communities worldwide. Vaccination triggers the immune system leading to the production of neutralizing antibodies against SARS-CoV-2. As per the WHO Coronavirus (COVID-19) Dashboard, more than 12 billion doses of vaccine doses have been administered so far.

BNT162b2 vaccine: Results of an ongoing multinational, placebo-controlled, observer-blinded, pivotal efficacy trial reported that individuals 16 years of age or older receiving two-dose regimen the trial vaccine BNT162b2 (mRNA-based, BioNTech/Pfizer) when given 21 days apart conferred 95% protection against COVID-19 with a safety profile similar to other viral vaccines. After granting an initial EUA, The US FDA approved the clinical use of BNT162b2 vaccine to prevent COVID-19 in August 2021.

mRNA-1273 vaccine: Results from another multicenter, Phase 3, randomized, observer-blinded, placebo-controlled trial demonstrated that individuals who were randomized to receive two doses of mRNA-1273 (mRNA based, Moderna) vaccine given 28 days apart showed 94.1% efficacy at preventing COVID-19 illness and no safety concerns were noted besides transient local and systemic reactions. After granting an initial EUA, The US FDA approved the clinical use of mRNA-1273 vaccine to prevent COVID-19 in January 2022.

Ad26.COV2.S vaccine: A third vaccine Ad26.COV2.S vaccine for the prevention of COVID-19 received EUA by the FDA on February 27, 2021, based on the results of an international multicenter, randomized, placebo-controlled multicenter, phase 3 trial showed that a single dose of Ad26.COV2.S vaccine conferred 73.1% efficacy in preventing COVID-19 in adult participants who were randomized to receive the vaccine.

Covishield: It is a recombinant, replication-deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 Spike (S) glycoprotein. Following administration, the genetic material of part of Who made COVISHIELD vaccine in India?

Both COVISHIELD (manufactured by **Serum Institute of India Pvt Ltd**) and COVID-19 Vaccine AstraZeneca (manufactured by AstraZeneca) are ChAdOx1 nCoV- 19 Corona Virus Vaccines (Recombinant).

Corona virus is expressed which stimulates an immune response.

COVISHIELDTM contains the following excipients:

L-Histidine
L-Histidine hydrochloride monohydrate
Magnesium chloride hexahydrate
Polysorbate 80
Ethanol
Sucrose
Sodium chloride
Disodium edetate dihydrate (EDTA)

Covaxin:

Bharat Biotech has successfully developed COVAXINTM, India's 1st vaccine candidate for COVID-19, in collaboration with the Indian Council of Medical Research (ICMR) - National Institute of Virology (NIV). Vaccine efficacy against COVID-19 of any severity, 14 or more days post dose 2, was **78%**. Vaccine efficacy against severe disease is 93%. In adults aged less than 60 years, efficacy was 79%; and in those aged 60 years and over it was 68%. As of June 2022, more than 77 million doses of COVAXIN have been distributed and administered in India.

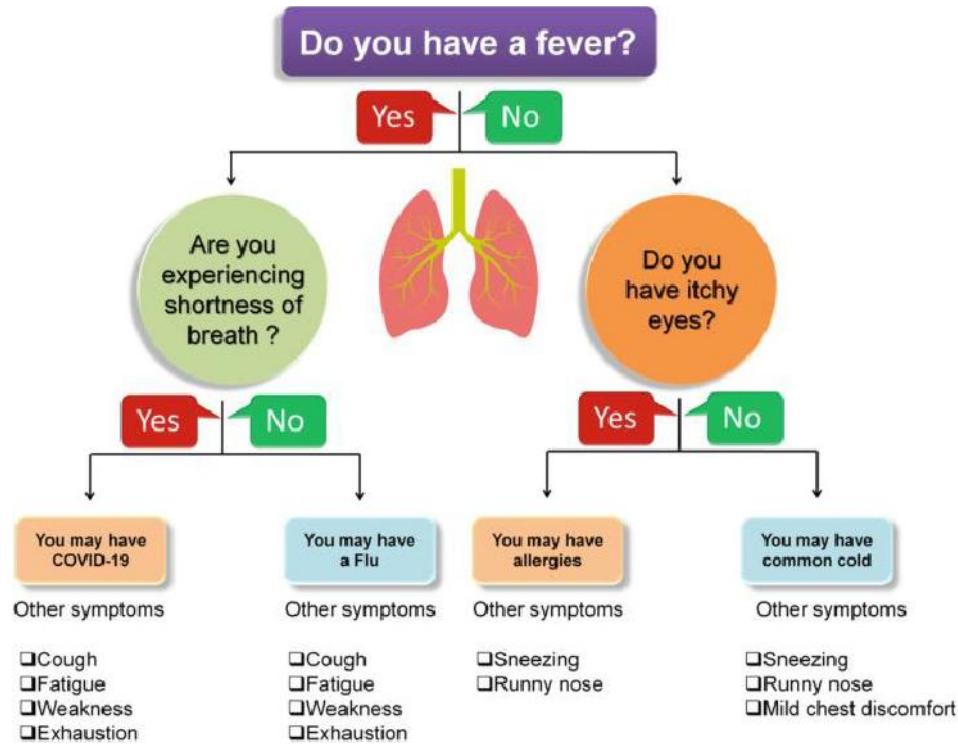


Differential diagnosis

The symptoms of the early stages of the disease are nonspecific. Differential diagnosis should include the possibility of a wide range of infectious and non-infectious (e.g., vasculitis, dermatomyositis) respiratory disorders.

- Adenovirus
- Influenza
- Human metapneumovirus (HmPV)
- Parainfluenza
- Respiratory syncytial virus (RSV)
- Rhinovirus (common cold)

For suspected cases, rapid antigen detection and other investigations should be adopted for evaluating common respiratory pathogens and non-infectious conditions.



Complications

COVID-19 can be regarded as a systemic viral illness based on its involvement in multiple major organ systems.

- Patients with advanced age and comorbid conditions such as obesity, diabetes mellitus, chronic lung disease, cardiovascular disease, chronic kidney disease, chronic liver disease, and neoplastic conditions are at risk of developing severe COVID-19 and its associated complications. The most common complication of severe COVID-19 illness is progressive or sudden clinical deterioration leading to acute respiratory failure and ARDS and/or multiorgan failure leading to death.

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- Patients with COVID-19 illness are also at increased risk of developing prothrombotic complications such as PE, DVT, MI, ischemic strokes, and arterial thrombosis.
- Cardiovascular system involvement results in malignant arrhythmias, cardiomyopathy, and cardiogenic shock.
- GI complications such as bowel ischemia, transaminitis, gastrointestinal bleeding, pancreatitis, Ogilvie syndrome, mesenteric ischemia, and severe ileus are often noted in critically ill patients with COVID-19
- Acute renal failure is the most common extrapulmonary manifestation of COVID-19 and is associated with an increased risk of mortality.

Symptoms:

People with COVID-19 have had a wide range of symptoms reported – ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. Anyone can have mild to severe symptoms.

Possible symptoms include:

- Fever or chills
- Cough
- Shortness of breath or difficulty breathing
- Fatigue
- Muscle or body aches
- Headache
- New loss of taste or smell
- Sore throat
- Congestion or runny nose
- Nausea or vomiting
- Diarrhea

This list does not include all possible symptoms. Symptoms may change with new COVID-19 variants and can vary depending on vaccination status. CDC will continue to update this list as we learn more about COVID-19. Older adults and people who have underlying medical conditions like heart or lung disease or diabetes are at higher risk for getting very sick from COVID-19.

Treatment:

Treatment for COVID-19 depends on the severity of the infection and risk factors affecting individuals. For milder illness, resting at home and taking medicine to reduce fever is often sufficient. A doctor may prescribe antiviral pills if a patient is at high risk of severe infection or has other indications for this therapy. More severe cases may require hospitalization.

Antiviral Medication

Antiviral medications are available to treat several viral infections, such as influenza. Antiviral drugs generally don't kill a virus but instead limit the production of new viruses inside host cells. Effective antiviral treatments can shorten the duration of the illness and lessen complications for some people.

COVID Pills: Paxlovid and Molnupiravir

Two pills, taken by mouth, can treat COVID-19 in some people. One pill, molnupiravir, is produced by Merck. The other, Paxlovid (nirmatrelvir and ritonavir tablets, co-packaged for oral use), is made by Pfizer. Both medications were granted an emergency use authorization (EUA) by the U.S. Food and Drug Administration (FDA) in December 2021.

According to FDA criteria, people who can get Paxlovid are those who meet all of these criteria:

- Have tested positive for COVID-19
- Are at least 12 years old
- Weigh at least 88 lbs
- Have certain health conditions, such as cancers, diabetes, obesity or others associated with more severe cases of COVID-19

To be eligible for these pills, people must be at high risk for progression to severe COVID-19, including hospitalization or death. Paxlovid and molnupiravir are available by prescription only and should be started as soon as possible after diagnosis of COVID-19 — no later than five days after symptoms began.

If you are prescribed Paxlovid for treatment of COVID-19, be sure to tell all of your doctors and care team members about other medicines you are taking and ask about drug interactions with Paxlovid. Some medicines, including certain blood thinners and immunosuppressants, are not safe to take with Paxlovid. Your usual medications or doses may need to be temporarily adjusted.

Monoclonal Antibodies

Manufactured in a laboratory, monoclonal antibodies are proteins that in some cases, can help your body fight infectious disease. Monoclonal antibody treatment is given by infusing the material into the bloodstream.

In January 2022, the CDC discontinued treatment with some types of monoclonal antibodies because those therapies did not work on the recent coronavirus variants (mutated viruses).

Current recommendations issued by the National Institutes of Health say that a monoclonal antibody called bebtelovimab can be given to patients age 12 and older, only when Paxlovid and remdesivir are not available or not clinically appropriate for the patient.

Immunomodulators

In June 2021, the FDA granted an EUA for tocilizumab for treatment of adults and children hospitalized with severe COVID-19. This biologic agent can reduce inflammation and is FDA approved to treat autoimmune illnesses such as rheumatoid arthritis. It is given by medical practitioners as an injection or infusion.

Another medication, baricitinib, is being studied to determine if it can benefit people with COVID-19 in a similar way.

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A Review on Strategies Adopted by bacterial Community to Remediate Lead Contamination in the Environment

Kasthuri.S¹, Shanmuga Priya.R^{2*}

¹ Research Scholar, Department of Microbiology, PSG College of Arts & Science, Coimbatore, Tamil Nadu. *kasthurisivakumar0607@gmail.com*.

² Associate Professor, Department of Microbiology, PSG College of Arts & Science, Coimbatore, Tamil Nadu. *priyajasper@gmail.com*.

* Corresponding author: *priyajasper@gmail.com*

Abstract

Lead is a toxic, non-essential and carcinogenic heavy metal that was found in the environment. Untreated industrial effluent contains various forms of lead, such as lead salts, sulphides and oxides which are directly discharged into the environment, and consequently affect our ecosystem. Coal burning, roasting of minerals and casting industries are significant anthropogenic sources of lead. As a result of extensive anthropogenic activities, lead gets increased significantly and accumulates over time in sediments, soil, plants and animals. The food chain and food web are affected by lead pollution. In plants lead causes impeding plant development, breakage of roots and defective photosynthesis process. In humans lead causes serious health risks. Several physical and chemical remediation methods are employed to reduce lead concentrations in the environment, including excavation, soil replacement, surface capping, soil encapsulation, soil washing, and stabilizing agents. Alternative methods are needed for lead remediation because conventional methods are less effective and more expensive. The use of bioremediation as an alternative method for removing lead contamination and cleaning up our natural biota is a sustainable approach to lead removal. Bacteria can remediate lead by using various mechanisms such as biosorption, bioaccumulation, exopolysaccharides production, volatilisation and precipitation thus restoring our ecosystem clean and safe.

Key words: Heavy metals, Lead, Bioremediation, Toxicity, Bacteria

Introduction

The group of metallic compounds with high atomic weight and a high density of more than $5\text{g}/\text{cm}^3$ are explicated as heavy metals. Soil erosion, metal corrosion, atmospheric deposition, leaching of heavy metals and natural weathering of the earth's crust are the significant natural sources for lot of heavy metals. Industries which use metals as their sole primary materials such as mines, smelters and foundries considerably liberate heavy metals and pollute our natural ecosystem. The most frequently existing heavy metals in the environment are arsenic, cadmium, chromium, copper, lead, nickel and zinc. Even minute concentration of hazardous heavy metal in the environment has an adverse impact on the entire tropical level (Kumar and Singh, 2023). Lead is the most common heavy metal used from a very early time well before the time of the Roman kingdom. The first metal discovered by humans was lead which is a blue-grey metal present in the environment. In the periodic table, lead is located in group 14, period 6 and with an atomic number of 82 (Wani *et al.*, 2015). In nature, lead can exist in various forms which are toxic to the environment. Lead acetate, lead chloride, lead chromate, lead nitrate and lead oxide are the lead compounds discharged by various anthropogenic sources and via the food chain and food web these forms are ingested by all biological systems (Patra *et al.*, 2011). Various, physical and chemical restoration methods are employed to remediate lead contamination in the atmosphere. Due to the unsustainable property of conventional methods, bioremediation comes accounted to detoxify environmental pollutants (Kumar and Bharadvaja 2020). Employing microorganisms to remediate heavy metal pollution is described as bioremediation (Pande *et al.*, 2019). Attributable to the conversion of toxic metals to nontoxic forms, bioremediation is a widely accepted and employed tactic to remediate metal pollution (Girma, 2015).

Source of lead:

Lead has special physical and chemical characteristics like softness, malleability, ductility, poor conductivity and resistance to corrosion that make it suited for a wide range of application; lead has been used by humans for many years and is now a frequent environmental contaminant. Lead is liberated into the environment by natural and anthropogenic sources. In nature, lead was present in ores along with other metals. Coal burning, metal plating, wastes from battery industries, exhaust from automobiles, lead mining, smelting, waste incineration, additives in gasoline, use of fertilizer, pesticides and metal processing industry releases huge levels of lead into the environment (Cheng and Hu, 2010), (Kumar and Bharadvaja 2020).

Toxicity of lead:

Lead is considered a multi-target environmental pollutant and due to its non-degradable property it gets accumulated in the environment and produces a large number of acute and chronic diseases in the entire living organism. From various natural and anthropogenic sources, lead gets incorporated into the environment and causes inhalation toxicity in the gastrointestinal tract, respiratory tract, skin and mucosa of humans, thereby gaining entry into the bloodstream. After adsorption, lead gets bounded to various body tissues like bone, hair, teeth, brain, kidney and bone marrow. Other than body tissues lead also gets adsorbed into human body through plasma protein and erythrocytes. Left out remains of lead gets excreted via urine, sweat, and faeces. Lead further proves to be hepatotoxic, renal Toxic, neurotoxic, reproductive toxic and bone toxic heavy metal (Sevak *et al.*, 2021); Table 1.

S.No	Human organs and system	Toxic bio-hazards of lead
1	Liver	Chronic lead exposure causes liver enlargement, liver cirrhosis and necrosis
2	Kidney	Causes reduction in the glomerular filtration rate, renal failure and renal hypertension.
3	Brain	Causes various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease. Accumulation in the cerebral cortex, hippocampal gyrus, and cerebellum of the brain, causes astrocyte and microglia proliferation, synaptic formation disorders, cell nuclear membrane contraction, and mitochondrial swelling.
4	Bones	Deposition in bones causes reduction of bone density and trabecular bone quality.
5	Immune system	Affects the synthesis and expression of immune cells T lymphocyte DNA and IgE antibody.
6	Hematopoietic system	Interferes with heme synthesis and affects the Red Blood Cells causing shrinkage and rupture.

7	Reproductive system	Causes decreased sperm motility, chromosomal damage, infertility, sperm count reduction, abnormal prostatic function and serum testosterone changes in men. Infertility, miscarriage, premature membrane rupture, preeclampsia, pregnancy hypertension and premature delivery in women can also be caused by toxic effects of lead.
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Table 1: Toxicity of lead (Sevak *et al.*,2021), (Winder, 1993)

Removal of lead through conventional methods:

As lead is omnipresent in land, air and water resources, through the food chain and food web, it gets deposited in living beings. Due to the negative effect of lead, it is essential to implement a sustainable remediation strategy to safeguard our natural environment (Sevak *et al.*, 2021). Diverse restoration techniques are existing for the removal of lead, among which the significant methods are denoted as, physical, chemical and biological remediation. Common physical and chemical methods employed to remove lead from the environment are sedimentation, filtration, membrane separation, ion exchange, chemical precipitation, solvent extraction, coagulation and granulated activated carbon adsorption (Abdel-Raouf and Abdul-Raheim, 2017). Because of the limitations of physical and chemical procedures, such as economically expensive processes, the intricacy of field application, the development of secondary chemical pollutants, and the unsustainable nature of the processes, alternate solutions for removing lead contamination are required (Sevak *et al.*, 2021).

Removal of lead through various bioremediation strategies:

Bioremediation is a sustainable and eco-friendly process to remediate lead contamination in the environment. Due to the disadvantages of conventional methods, bioremediation stands a promising strategy to remediate problematic lead from our environment. Bioremediation can be attained by biological agents for example; bacteria, fungus, algae, plants or any compounds produced by living organisms like enzymes or exopolysaccharides. Among various biological agents bacteria are commonly used for lead remediation, due to their diversity and ability to develop several mechanisms to reduce or tolerate heavy metals. *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Mesorhizobium*, *Microbacterium*,

Pseudomonas, *Rhizobium*, *Rhodococcus*, and *Variovorax* are found to be common bacterial species for lead remediation (Gonzalez Henao and Ghneim-Herrera, 2021).

Bacteria utilize a variety of methods to address the lead pollution in our natural ecosystem, including biosorption, exopolysaccharide production, bioprecipitation, and bioaccumulation mechanism. Biosorption is a significant remediation mechanism used by various bacteria for lead remediation because of its small size and lesser generation time. Lead is adsorbed to the cell surface of bacteria by physical (van der waals force) or chemical (surface functional groups) interaction which mediates bacteria-metal complex formation (Ayangbenro *et al.*, 2019). *Oceanobacillus profundus* is a bacterium isolated from mine industry and able to remediate 97% of lead by biosorption process mechanism (Mwandira *et al.*, 2020). In bioprecipitation process toxic forms of lead gets converted into nontoxic or less toxic forms of lead. Usually phosphatase enzyme producing bacteria was found to produce lead phosphate which is less toxic in nature. Consequently the produced lead phosphate compound was deposited on the surface of the bacterial cell wall. The lead resistant bacterium *Providencia alcalifaciens* was isolated from car battery waste dumping site and this bacterium was found to convert lead into lead orthophosphate mineral which is a less toxic form of lead (Naik *et al.*, 2013). Exopolysaccharide is generally present on the outer surface of bacterial cells as a capsule and is composed of combinations of protein, polysaccharides, humic acid, uronic acid, lipids, nucleic acid, and organic, inorganic components. Exopolysaccharide is a defence system produced by bacteria at various stress conditions. Exopolysaccharide significantly plays a curial role in lead bioremediation. *Pseudomonas sp.* W6 isolated from hot spring produce exopolysaccharide which is anionic in nature and can bind with cationic lead (Kalita and Joshi, 2017). Volatilisation is a process which involves in the transformation of ionic species of metals into volatile compound, due to which the concentration of lead in the environment gets decreased (Meyer *et al.*, 2008). Detoxification of lead by volatilisation was investigated by *Klebsiella aerogenes* in phosphate limited growth medium (Aiking *et al.*, 1985). Bioaccumulation is one of the remediation methods used by bacteria to diminish the toxicity of lead by plummeting the concentration of freely available lead in the environment. The process of bioaccumulation can happen either actively which requires transporters to carry the lead into the cell, or passively, involving diffusion from higher concentrations to lower concentrations. The bacterial strain called *Klebsiella pneumoniae* MB361, has the ability to remove lead at the percentage of 85.30% by bioaccumulation

process (Aslam *et al.*, 2020). Usually heavy metal contaminated sites possess specific microbial communities which are resistant to heavy metals as these bacteria are effective candidates for metal removal. Lead resistance bacteria isolated from the lead contaminated site are found to possess antioxidant, antibacterial, antifungal, drug resistant activity and metal detoxification mechanisms due to which bacteria are acting as excellent candidate for metal remediation. Lead resistant bacteria which are present at metal contaminated sites can be biostimulated by the addition of nutrients and can be used to remediate contaminated industrial waste. In order to effectively remediate lead on contaminated sites, indigenous bacteria could be used, due to the fact that they are possessing excellent adaptable nature for the lead polluted environments. Thus the biostimulation of indigenous bacteria for bioremediation is one of the developing sustainable technologies. (Li *et al.*, 2020).

Conclusion

Lead is a metal which is hazardous and carcinogenic in nature which acts as a significant contaminant in the environment. Due to industrialization, huge amount of lead containing waste materials are discharged into the environment which shows the emergency of developing a sustainable lead remediation approach. Due to the disadvantages of conventional methods bacterial bioremediation methods, such as biosorption, exopolysaccharide production, bioprecipitation, bioaccumulation and biostimulation are considered as a best alternative method for lead remediation and they have tremendous usage in cleaning our environment. Considering the applications of the biological way of handling lead contamination, most of the researchers have concentrated on harnessing the power of microorganisms in remediation of lead contamination. Thus, bioremediation is considered to be a universally accepted and developing sector of research.

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Food Preservation

V.Gayathri* and B.Varshini

*Associate Professor, Department of Microbiology,
Ethiraj College for women, Chennai, Tamil Nadu, India
E-mail: gayathri16@ethirajcollege.edu.in

Introduction

Food preservation refers to the process of handling and processing food to prevent or delay food decomposition, loss of quality or nutritional content and enable prolonged food storage.

Principles of Food Preservation:

Food can be preserved using a number of chemical and physical methods. However, choosing an appropriate method is important in preserving food quality and nutritional value. The following principles are involved in preservation using these methods.

-) Asepsis
-) Removal of microorganisms
-) Maintenance of anaerobic condition
-) Use of high temperature
-) Improving texture & consistency
-) Minimized nutrients loss
-) Extending the self-life
-) Drying and smoking

Food self-decomposition can be avoided by:

- Destroying the self-enzyme of the food that causes self-decomposition. For example, washing fruits in hot water can damage their ripening enzymes.
- Preventing purely chemical reactions in food from causing damage to the food. Anti-oxidant addition, for example, can prevent lipid oxidative rancidity.
- Preventing mechanical food damage caused by insects, birds, food handling devices, and so on.

Microorganisms are inhibited or killed in the following ways:

Various methods for preserving food, either by killing microorganisms or slowing their growth and activity, include:

- High temperature preservation (heat)
- Low temperature preservation
- Food preservation by drying
- Food preservation by radiation (irradiation)
- Chemical agents or additives used in food preservation

Preservation of food by high temperature (HEAT):

The most common method used for preserving the food is by heating at high temperature. Heat kills the spores and microorganisms that spoil the food by denaturation of protein and enzymes. A few methods that heat might kill microbes in food are as follows:

Pasteurization:

Pasteurization is a method of selective heating that kills some but not all microorganisms found in milk. Pasteurization is now used to preserve other beverages such as beer and fruit juice. Pasteurization involves heating milk and beverages to a certain temperature that kills spoilage or pathogenic organisms without causing changes to the product.

Pasteurization time-temperature relationships are chosen based on three bacteria that are transmitted through milk (*Mycobacterium bovis*, *Coxiella burnetii* and *Brucella abortus*).

- Low temperature holding (LTH) or Vat pasteurisation: Milk is heated at 62.8 °C for 30 minutes in this method.
- High temperature short time (HTST) method: Milk is heated at 71.7 °C for 15 seconds in this method.
- Ultra-pasteurization: In this method, milk is heated at about 137.8°C for 2 seconds.

Blanching:

Blanching is the process of washing vegetables or fruits in warm water before storage.

Blanching has the following benefits:

- It minimizes the quantity of microbes on the food's surface and inactivates food enzymes that lead to self-decomposition
- It enhances the ability to fixate on the bright green colour of fruits and vegetables.

Preservation of food by low temperature:

Low temperature is a common physical method of food preservation. Food is preserved by slowing the growth and activity of spoilage organisms in food.

- By slowing the rate of chemical reactions or other enzymatic activity that results in food self-decomposition.
- Low temperatures can sometimes kill spoilage organisms in food. For example: deep freezing.

Chilling or cold storage:

- In chilling or cold storage, storage temperature is usually between (0-10)°C.
- At this temperature, growth and activity of most bacteria is slowed down except psychrophiles.
- Selection of appropriate temperature is very important because quality of some food is damaged at lower temperature.

This method is most commonly used for short term storage of highly perishable foods like meat, milk and fish etc.

Freezing or frozen storage:

In freezing storage, the temperature is between (-10 to -30)°C. At this temperature, growth of even psychrophilic bacteria is completely inhibited when food is frozen. The water in food forms ice-crystals. The microbial cell's cytoplasmic water then gradually diffuses outside and into the nearby ice crystal. A microbial cell's remaining cytoplasm becomes more and more packed together.

Drying for food preservation:

Many spoilage microorganisms are highly susceptible to drying. Drying is accomplished by either evaporating water from the food or lowering the Aw value of the food through the addition of salt and sugar. Several parameters, such as drying time, temperature of the air should be controlled when drying food by evaporation.

Smoking:

This method involves heating the food using smoke from various types of woods to preserve the food.

The smoke produces heat, which kills the microorganisms on surface. They also kill the vegetative cells as well as spores.

This method also enhances the taste and flavor of food. Example: Smoked fish.

Canning:

The process of sealing foodstuffs in air tight containers and sterilizing them by heat for long term storage is known as canning. Canning is the preservation method that involves preservation of foods in sealed containers such as metal, glass jars, plastic cans, thermostable plastic, or a multilayered flexible pouch. The main objective of heat application is to destroy pathogenic and spoilage microorganisms. Canning is done in many industries for processed foods packing.

Causes of spoilage

Improper handling:

Foods that are not fresh, cool and in prime condition when canned, spoilage organisms begin to grow and multiply rapidly. Food which are not cleaned properly will have more organisms and thus will be harder to kill them.

Broken seals or leaking cans:

It occurs because of manufacturing defects, punctures or rough handling. Microbes are introduced into can by either through holes or improper seals. Leakage may also be responsible for release of vacuum, which can favor the growth of microorganisms.

Types of spoilage in canned foods:

- | Microbial spoilage
- | Chemical spoilage
- | Enzymatic spoilage
- | Flat sour spoilage: Production of some acids, like lactic acids by microorganisms present in the food may cause this type of spoilage.
- | Sulfide stinker spoilage: The amino acids present in the food reacts with sugars and iron present in the coating material and forms a stinking smell due to formation of SO₂ gas

Food contamination:

Food contamination is caused by microbes and enzymes in food which enters food during manufacture, processing, preparation and packing of food.

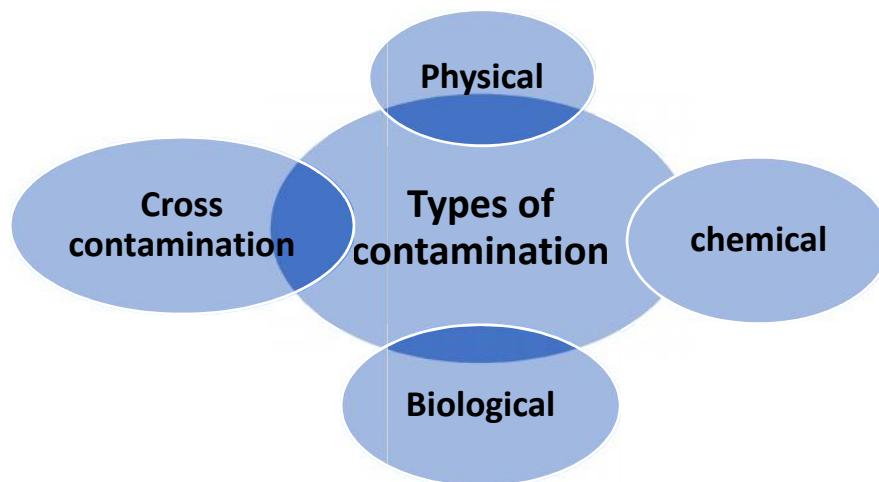
The common reasons for food contamination are:

-) Improper storing, handling and preparation of food
-) Usage of unclean or unsterilized utensils
-) Contamination by insects, and pests.

Examples of food contamination:

Food can get contaminated by-

- * Water used for cooking/washing
- * Soil in which food is grown
- * Container used for storage, preparation and service
- * Personnel handling food at various stages
- * GM varieties of maize
- * Pesticide residues



Types of food contamination:

Physical: Physical contamination refers to food that has been contaminated by a foreign objects. The objects may also carry microorganisms posing risk to the consumer. It is seen via naked eyes and it can be removed manually. If those contaminants are not removed they cause a risk of choking . Common contaminants include:

-) Tie your hair when handling food
-) Clean away cracked or broken crockery and utensils to avoid contamination
-) Wear minimum jewelry when preparing food.

Some of the physical contaminants includes hair, glass, paper, plastic, scabs, rodent droppings, flies, bones from meat/ fish.

Chemical:

Chemical contamination refers to food that has been contaminated by some type of chemical substance. Moreover, some foods naturally contain chemicals, such as the poisons found in some fish, and in some instances, a small amount of chemical contamination may not actually cause illness. To prevent chemical contamination, the food handler must always be aware of the presence of chemicals in food and take all necessary precautions.

Some typical chemical pollutants are:

- * cleaning products (e.g. detergent, sanitizer)
- * pesticides/herbicides
- * toxic chemicals in metals and plastic
- * preservatives

* naturally occurring toxins Naturally occurring toxins are toxic compounds that are produced by living organisms, some of which are staples of the human diet (e.g. shellfish, potatoes, fish). These toxins are not harmful to the organisms themselves but can be harmful to us if we eat them. Health impacts: various chemical contamination causes illness as they are harmful to consumer . There may be some allergic reactions when such chemicals are consumed.

Biological:

Health effects – A bacterium or its toxins may directly affect one's health. (For instance, aflotoxin generated by *Aspergillus sp.*) The symptoms appear within hours of ingesting the toxin. In general, the symptoms include nausea, vomiting, and diarrhoea. Microbes can develop faster when conditions like ambient temperature, oxygen, food, moisture, and acidity are favourable.

Cross contamination:

Cross-contamination takes place when pathogens are transported from any object that you use in the kitchen. Dirty kitchen clothes, unclean utensils, pests, raw food storage can lead to cross-contamination. Ways to avoid cross-contamination: Utensils- Use separate utensils to prepare different types of foods. Avoid using the same chopping board and knife for ready to eat foods Storing Food- Make sure raw foods don't come in contact with ready to eat foods. Cover and store raw foods below cooked foods to prevent cross-contamination. (mostly occurs in refrigerators- when we store food) Most food-borne illnesses in Canada are caused by bacteria or viruses, with the most common being: Norovirus, Listeria, Salmonella, *E. coli*, Campylobacter, Food-borne illness occurs when disease-causing microorganisms, also called pathogens, get into food and multiply to unsafe levels before being consumed. This can happen remarkably quickly; in conditions ideal for bacterial growth, one single-cell bacteria can become two million in just seven hours. Ways to avoid contamination of food:

*To slow down the growth of bacteria and prevent food safety risks, you need to follow best practices of food safety designed to control bacterial growth through proper food handling techniques, rigorous cleaning, sanitizing procedures and time- temperature control of food.

*purchase, store, thaw, prepare, cook and serve high-risk foods properly

*regularly clean and sanitize all food contact surfaces and equipment

*maintain good overall hygiene and sanitation of the premises

*maintain personal hygiene.

Health risk:

- *Carcinogenic
- *Mutagenic
- *Teratogenic (interfere with normal embryonic development)
- *Birth defects
- *Reproductive problem, headache, nausea, dizziness damage
- *Other fatal effect

Emerging techniques in food preservation:

The technology is favoured because it has a variety of characteristics, including gradual release action, target specificity, precise action on active areas, and high surface area (Joshi et al. 2019). Nanotechnology's success can be attributed to its promising outcomes, lack of pollutant emission, energy efficiency, and little space needs. In addition to these success elements, nanotechnology has demonstrated a wide range of applications for risk assessment in the domains of agriculture, food, and the environment.

Novel non thermal food processing methods are in which microbial inactivation takes place by without applying heat directly. Such technologies are pulse electric fields (PEF), high-pressure processing (HPP), ozone treatment pulsed light, non-thermal plasma/cold plasma (NTP) and ultrasound technology. Combining two or more methods can also enhance the efficacy of microbial inactivation in foods. The technologies can be grouped into two major groups:

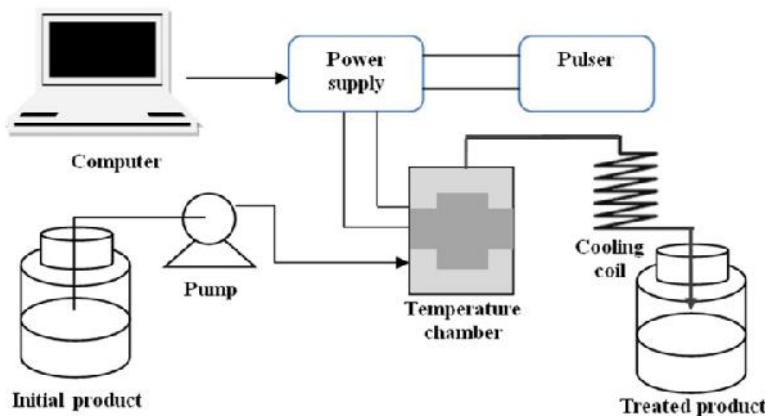
1. Physical processes- pulse electric field, high pressure processing, ultraviolet radiation, pulsed light, ultrasound and ionizing radiation
2. Chemical processes- Ozone treatment, and cold plasma.

Physical processes:

Pulsed electric fields:

Productivity Boosters High intensity electric fields operating between 20 and 80 kv/cm are used in pulsed electric field (PEF) processing (1- 100 milliseconds). Electroporation, in which the depolarization of the cell membrane causes holes in the lipid bilayer to open, is the main antibacterial method of action. The process parameters, such as the electric field strength, the pulse width, frequency, and length, the overall treatment time, and the input energy, all affect how effective the procedure is. The use of PEF in liquid food

such as juices and liquid eggs to kill the food borne pathogens .The PEF has been evaluated for improved shelf life and preservation of semiliquid food products, and improved extraction of bioactive and neutraceutical cellular components such as antioxidants , essential oils and algal protein.



Structure and Function of Food Engineering. (2012). Croatia: IntechOpen.

High pressure processing:

HPP treatment typically involves applying pressures of up to 600 MPa . The pressure range of 100-800 MPa and temperature as low as 20 °C applied together, with the time of exposure of the foodstuff usually seconds to minutes, is important in an HPP process which is reported in the work of Heinz and Buckow and Mjica-Paz et al. Unnecessary damage of the food can be avoided along with destroying microbes by this method. The Ultra-pressure applied in this method are thought to alter the anatomy of bacterial cells as well as thwart enzymatic functions, resulting in the weakening and eventual death of the foodborne pathogen. HPP technology can be used specifically in multi pulse HPP technology, which involves the use of repeated short high-pressure treatments for several cycles. HPP conditions of 593.96 MPa for 233 s decreased significantly S. aureus and B. cereus in human milk, thus providing a reliable alternative to pasteurisation in human milk banks.[6]

In addition to operating at normal temperatures, HPP can extend the shelf-life of foods while preventing negative changes in their nutritional content . HPP has also been used to process liquid foods such as juices . This method is known to be used in the treatment of foods with a water activity greater than 0.8 . Fruits, meats, vegetables, milk and its products , juices,

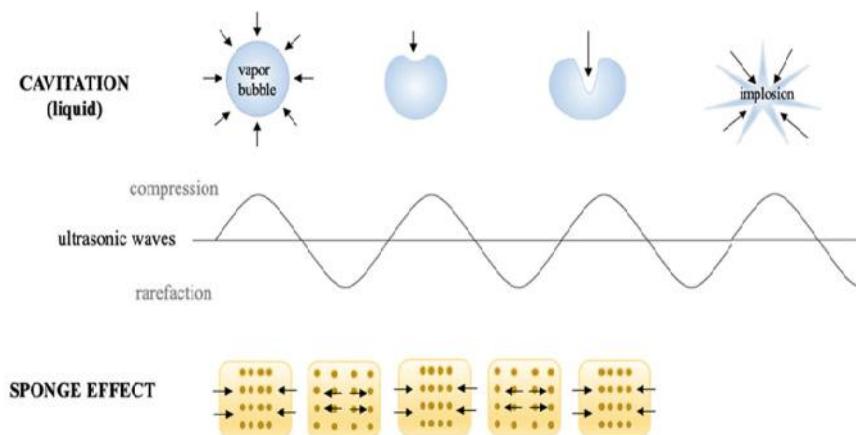
beverages, seafood, and fish are among the major groups processed using HPP.[9]

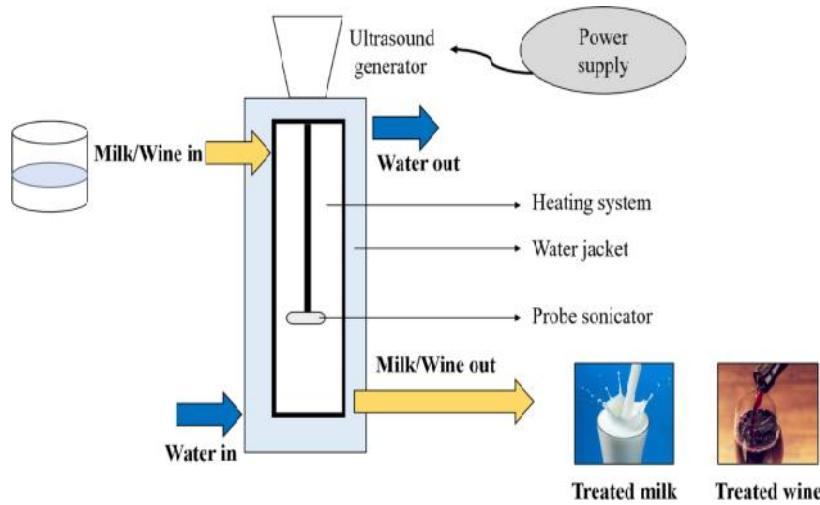
HPP may aid in the retention of good fats, the reduction of salt consumption, and the reduction of allergen development and toxin generation capacity of foods. However, once the operational pressure has been reached, there is no additional energy required to maintain the pressure. In comparison to traditional heat-using technologies, HPP does not require additional energy to cool the food product after the estimated treatment period .

Ultrasound method:

The ultrasound method is used to pasteurise milk. It demonstrates a high degree of homogenization and improved stability after processing.Ultrasound uses pressure waves with frequencies ranging from 20 to 100 kHz. Ultrasound at high frequencies (100 kHz to 1 MHz) and diagnostic ultrasound (1–10 MHz). There is formation of bubbles due to activity of mechanical waves that arise as a result of intense energy solicitations.

The breakdown of the bubbles produces adverse confined pressure (approximately 1000 atm) and temperature (approximately 5000 K) conditions, which cause conformational changes in the target microorganisms .During sonication process there is formation of free radicals. These free radicals (hydroxyl and hydrogen) kill microbes by destroying their cell walls, cell membranes, liposomes, and genetic material. This causes cell disintegration and death.[10]





Ultraviolet (UV) radiation:

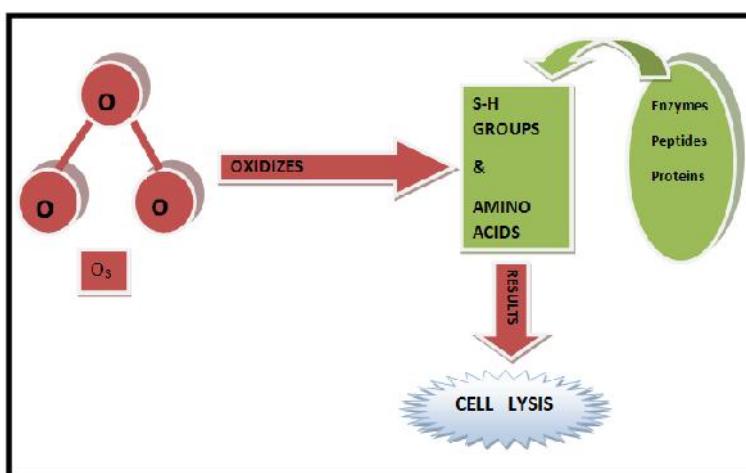
This method is used to decontaminate the food and to increase the shelf life of the food products. The germicidal properties of UV is observed at the range of 200-280nm. Generally, UV light falls in the range of 100 to about 400 nm and the range can be further categorized into UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm) and UV- Vacuum (100–200 nm). UV rays kills the microorganism by forming thymine dimmers in their DNA thereby causing the death of the organism. Bacteria, yeast, fungus, protozoa, and algae, for example, require UV-inactivation doses of 1-10, 2-8, 20-200, 100-150, and 300-400 mJ/cm², respectively, indicating that algae is the most resistant because it requires the highest dose when compared to other pathogenic microbes.

A study found that UV-C treatment of raw milk reduced the total mesophilic aerobic bacteria and yeast-mold count by 2 and 3 logs, respectively. Moreover, UV-treatment resulted in 2-3 log reductions of inoculated *Salmonella*, *L. monocytogenes*, *S. aureas*, and *E. coli*. However, in order to achieve a more effective reduction in bacterial load, this study suggested that UV light be used in conjunction with other technologies rather than as a stand-alone strategy . A UV-C treatment of 127.2 mJ/cm² for 30 seconds was also found to be effective in reducing the bacterial load of raw salmon in another study.[11]

Chemical processes:

Ozone treatment:

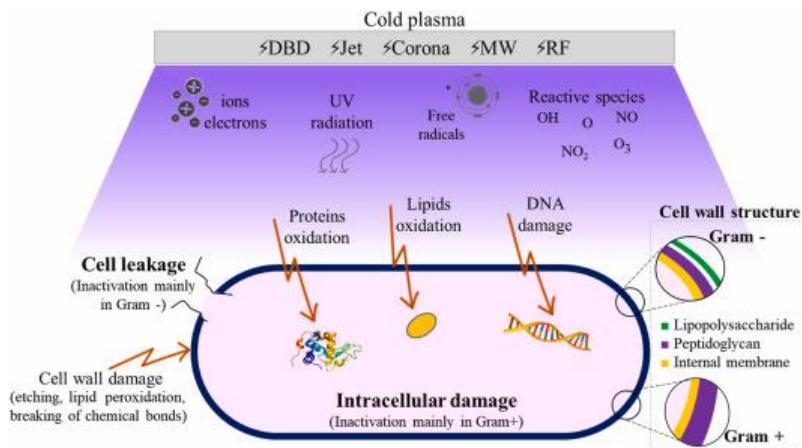
Ozone is a powerful oxidizing agent against wide range of microorganisms. In recent years this property of ozone is applied in food industries to protect the food from decaying and to keep them away from microorganisms. The ozone gas reduces the biochemical reactions that are happening in the food thereby increasing the shelf life of the product. Ozone is a light blue gas and partially soluble in water. It has a high oxidation-reduction potential (2.07 V), making it an excellent oxidant for food applications [18]. The ozone gas attacks the cell wall and cell membrane in bacteria leading to its cell death. Ozone also forms free radicals thereby causing destruction in the cellular activity. Cell death, genetic material degradation, and cellular component leakage are all results of oxidation processes. DNA and RNA can both bind to ozone. It has been demonstrated that ozone can eliminate viruses like hepatitis, influenza, and infectious bovine rhinotracheitis virus [17].



Cold plasma treatment:

Cold plasma (CP) is a new technology for processing food at low temperatures and in a short amount of time. Plasma is composed of free electrons, ions and free radicals. It is created by ionizing gas. Plasma system has sufficient density of reactive products to sanitize foods without damaging the product. Cold plasma inactivates foodborne pathogens by causing DNA damage. Due to partial denaturation of protein structures and cell leakage, the CP process has a high efficiency at low pH. Furthermore, the hydrogen peroxide formed by water vapour in the gas phase is a strong oxidizer that oxidises the outer cell structure, resulting in cell death. The reactive oxygen

species inactivate microbial cell wall causes protein denaturation, enzyme inactivation, oxidative stress. In Gram-negative (GN) and Gram-positive (GP) bacteria, two distinct cell damage mechanisms were observed. ROS and UV radiation primarily attack the cell envelope, causing irreversible wall damage, oxidation, and the release of intracellular compounds such as protein, DNA, and lipids. Due to differences in peptidoglycan cell-wall thickness, some studies have shown that CP is more effective against GN bacteria than against GP bacteria. *Pseudomonas fluorescens* was discovered to be sensitive to plasma-activated water treatment, and after 3 minutes of CP treatment, it was reduced to below detection limits[20]



Mechanism of Gram-negative and Gram-positive bacteria inactivation using CP.

Nanotechnology:

The introduction of nanotechnology in research and a combination of several advanced technologies. So, constructive actions must be taken to focus on these technologies' improvements in order to live sustainably and cheaply. Nanotechnology's success can be attributed to its promising outcomes, lack of pollutant emission, energy efficiency, and little space needs. In addition to these success elements, nanotechnology has demonstrated a wide range of applications for risk assessment in the domains of agriculture, food, and the environment.



AGRICULTURE



FOOD PROCESSING



FOOD PACKAGING

Nanosensors

- Precision agriculture

Nanomaterials

- nTiO_2
- Gold/Silver Nanoparticles
- Chitosan
- Nano formulated agro-chemicals

Sensorial attributes

- Nanocapsules for flavor enhancement

Nutrient delivery

- Targeted nutrient delivery to plants
- Detection of enzyme-substrate interactions

Smart packaging

- Detection
- Sensors

Active packaging

- Antimicrobials
- TiO_2 , SiO_2 and Chitosan based additives

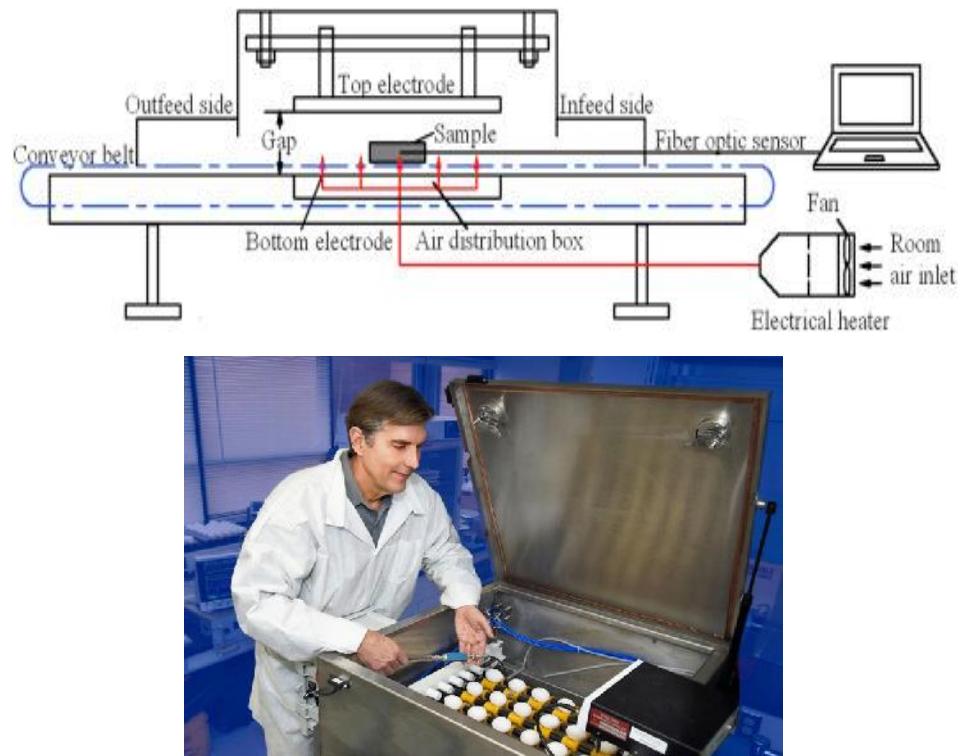
Nanotechnology in food processing and preservation:

Bacter *Pichia fermentans JA 2* *Pichia fermentans JA 2* Silver and zinc oxide NPs UV-vis, XRD, and FE-SEM- EDX analysis ZnO NPs inhibited only *Pseudomonas aeruginosa* [15].

Serratia sp. BHU S4 Silver NPs TEM (10– 20 nm), X RD, EDXA, FTIR As fungicide against phytopathogen *Bipolaris oryzae* causing spot blotch disease in wheat reduction and stabilization [16]

Radio frequency pasteurization/ salmonella killer :

The RF pasteurization process effectively inactivates Salmonella while preserving the shell quality. The shell eggs are in direct contact with curved electrodes, which pulse RF energy through the albumin. This Resistance Capacitance circuit of the coupled albumin and yolk creates heat inside the eggs, while the dielectric nature of the eggs shell creates series capacitance. The external RF field leads to generation of uniform ohmic heat from the electric fields maintained inside the egg, arising from the dissipation of the applied power. The net effect is that the RF process heats the egg from the inside out, in contrast to conventional hot water submersion ,which heats the egg from the outside. This method kills Salmonella but the albumin is unaffected. The process is further enhanced by egg rotation and the application of a water stream across the shell to enhance the electric contact.[21]

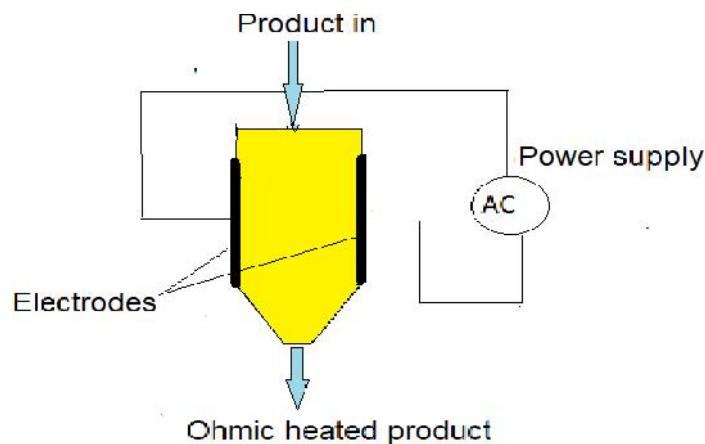


Plasma activated water:

Cold plasma treatment capturing water either in Plasma activated water or in discrete fine drops (Plasma activated mist). Plasma is generated and injected into a mass of water and the water droplets are passed through a dielectric barrier discharge or Plasma jet. The short lived products are typically lost and the long lived reaction products are suspended in water which can be taken for use. It is used chemical sanitizers but with a chlorine free composition. While the cold plasma reactivity is lost by the capture process, much flexibility can be gained by the creation of an aqueous sanitizing solution. The applications for ice packed fresh and fresh cut produce and sea food shipping are under research.

Ohmic heating of foods:

Ohmic heating is a sophisticated thermal processing technique in which the food item is heated by electrical current flowing through it, acting as an electrical resistor. Heating happens quickly and evenly because electrical energy is converted to heat. Electrical charges can accumulate and create holes across microbial cells at low frequencies (50–60 Hz). There are many uses for ohmic heating in the food industry, including electrical resistance heating, Joule heating, and electro-heating. Ohmic heating can be used to heat heat-sensitive liquids as well as liquid foods with large particles, such as soups, stews, and fruit slices in syrups and sauces. This technique is useful for handling proteinaceous foods since they get denatured and coagulate when heated. [22]



Biopreservatives:

Bio-preservatives are naturally occurring compounds derived from plants, animals, and microorganisms that extend the shelf life of food [13]. These compounds eliminate pathogenic organisms in food and improve food quality. Many of these compounds not only act as antimicrobials, but also as antioxidants, break down cell membranes, and disrupt biosynthetic microbe pathways [14]. Essential oil are made up of many different components, and it is likely that their mode of action involves multiple targets in the bacterial cell. Their hydrophobicity allows them to partition in the lipids of the cell membrane and mitochondria, making them permeable and allowing cell contents to leak.

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Review of organic biofertilizers in agricultural environment

Subashini S¹, Abdul Farook S² and Thaiyalnayagi S³

¹Research Scholar, Department Of Microbiology, Cauvery College For Women (Autonomous), Trichy.

²Research Scholar, Department Of Microbiology, Cauvery College For Women (Autonomous), Trichy.

³Assistant Professor , Department of Microbiology, Sri Bharathi Arts and Science College For Women, Pudukkottai.

Email: subamicro4897@gmail.com, Contact: 8667595203.

Abstract

Biofertilizer are natural fertilizer. Which are microbial inoculants of bacteria, fungi, algae alone or combination and they argument the availability of nutrients of the plants. The role of biofertilizers in agriculture assumes special significance particularly in the present context of increased cost of chemical fertilizers and their hazardous chemical fertilizers and their hazardous effects on soil health. The need for the use of biofertilizer thus arises primary for two reasons first because increase in the use of biofertilizer to increased crop productivity. Second, because increased usage chemical fertilizers leads to damage in soil texture and arises other environmental problems use of biofertilizers of integrated nutrients management as they are cost effective and renewable source of plants nutrients to supplement the chemical fertilizers for sustainable agriculture several microorganisms and their association with crop plants of being exploited in the production of biofertilizers. Biofertilizers requires to the fertility to the soil prolonged use of chemical fertilizers. Degraded the soil and affects crop yield. Biofertilizers on the other hand enhance the water holding capacity of the soil and add essential nutrients such as nitrogen, vitamins and proteins to the soil. They are the natural form of fertilizers and hence widely used in agriculture.

Key words: Biofertilizers, Types, Classification, Importance, Advantages

Introduction

Bio-fertilizer is a material which contains living microorganisms. When applied to plant surfaces, they promotes plant growth by increasing the supply of primary nutrients to the host plant. Bio-fertilizers add nutrients through natural processes like nitrogen fixation, phosphorus solubilisation and stimulating plant growth along with the synthesis of growth-promoting substances. Some PGPR promote the growth by acting as bio-fertilizer. Microorganisms mainly nitrogen fixers, phosphate solubilizers and mycorrhizae are the main sources of bio-fertilizers.



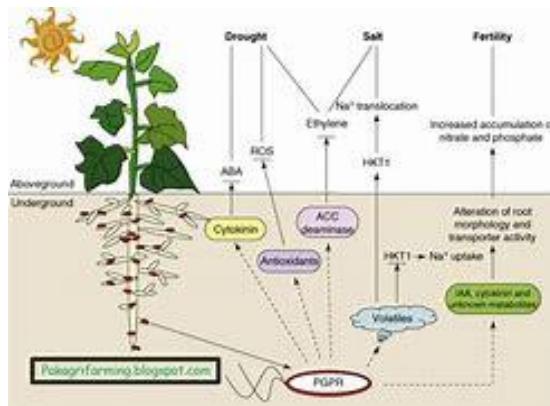
The microorganisms used for the biofertilizer are bacteria like *Bacillus*, *Pseudomonas*, *Lactobacillus*, photosynthetic bacteria, nitrogen fixing bacteria and fungi like *Trichoderma* and yeast. Bio-fertilizers have shown great potential as a renewable and environmental friendly source of plant nutrients. Foregoing researches have reported positive, rarely negative and the lack of any significant influence of biofertilizers on soil physical, chemical and biological properties. In view of the growing demand for safer and healthier food and concerns for environmental sustainability, organic farming has emerged as an important priority area globally.

History of biofertilizers

Bio-fertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi. Historically, the bio-fertilizers were initially identified by a Dutch scientist in 1888, thereafter bio-fertilizers use started with the launch of Nitragin by Nobe and Hiltner with a laboratory culture of *Rhizobia* in 1895 (Ghosh, 2004). In India the first commercial production of

bio-fertilizer started in 1956 under the supervision of N.V Joshi. The NIKU Bio Research Laboratory was established in the year 1997 at Pune. The initial name signifies N=Natural, I=Input, K=Complete and U=Utilization.

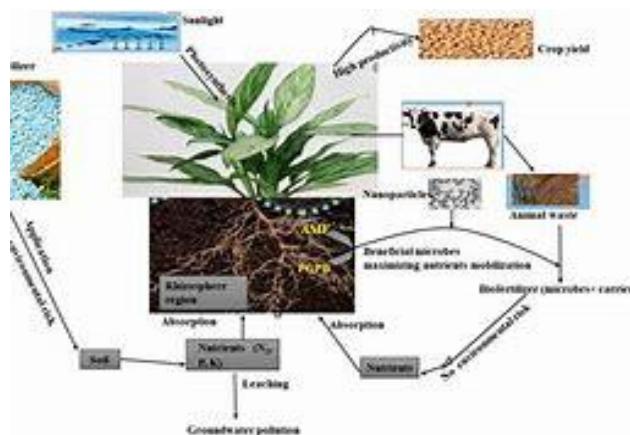
Modern agriculture emphasizes in using hybrid seeds and high yielding varieties that are highly responsive to large doses of chemical fertilizers and irrigation. Indiscriminative use of synthetic fertilizers has lead to pollution and contamination of soil and water basins. The soil driven of essential plant nutrients and organic matter. It has lead to depletion of beneficial microorganisms and insect indirectly reducing soil fertility and making crops more prone to disease. It is estimated that by 2020, to achieve the target production of 321 millions tons of food grain, the requirement of nutrient will be 28.8 million tons, while their availability will be only 21.6 millions tons being a deficit of about 7.2 millions tons thus depleting feed stock/fossils fuels (energy crisis) and increasing cost of fertilizers which would be unfavourable to small and marginal farmers , thus intensifying the depleting leavel of soils fertility due to widening gap between nutrient removal and supplies.



Chemical fertilizers which are now being used extensively since the green revolution have depleted soil health by making the soil ecology non-indispensable for soil micro flora and micro-fauna which are largely responsible for maintaining soil fertility and providing some essential and indispensable nutrients to plants. Biofertilizers are the product containing one or more species of microorganisms which have the ability to mobilize chemical fertilizers nutritionally important elements elements from non usable to usable from through biological process such as nitrogen fixatioion phosphatase solubilisation excretion of plant growth promoting substances or cellulose and biodegradation in soil, compost and other environments. In other

words biofertilizers are natural fertilizers which are living microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrient to the plants. The role of biofertilizers in agriculture assumes special significance particularly in the present context of increased cost of chemical fertilizer and their hazardous effects on soil health.

Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environments pollution.associated with more use of agrochemicals. Though the use of chemical inputs in agriculture is inevitable to meet growing demand for food in world there are opportunities in selected crops and inche areas where organic production can be encouraged to tape the domestic export market (Mishra et al., 2013). Biofertilizers is a substances which contains living organisms. which when applied to seed plant surfaces or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant areas with the objectives of increasing number of such microorganisms and accelerate those microbial processes, which agument the availability of nutrients that can be easily assimilated by plants.



Bio fertilizers play a very significant role in improving soil fertility by adding nutrient through the natural process of nitrogen fixation solubilizing phosphorus, and stimulating plant growth through the synthesis of growthpromoting substance in the soil. They are in the fact being promoted to hervest the naturally available,biological system of nutrient mobilization.

In India due to growing human population it is necessary to increase crop production and land productivity to fulfil their food requirements. It is estimated that up to 2020, total production required by country will become 321 millions tones of food grains and for their food proper growth nutrient requirement will became 28.8 millions tones but only 21.6 millions tones nutrient will be available from a deficit about 7.2 million tones. To increase crop production use chemical fertilizers and plant production materials is also increased. Increasing use of chemical fertilizers in agriculture make country self sufficient in food production but it pollute environment and cause slow deterioration of living being (Sexana and Joshi, 2020).

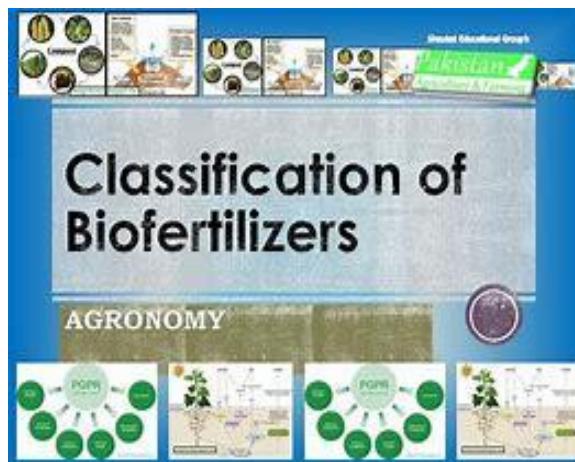
Plant require essential nutrient like nitrogen (N), Phosphorus (P), Potassium (K) and several other minerals for their growth and receive them from soil. (Arya, 2000). Nitrogen and phosphate relationship is very important and show a direct impact on productivity of soil. (Hutchinson and Richards 1921). The fertilizers containing NPK are applied in soil to improve the crop productivity but it is not utilized by crop completely. The remaining fertilizers pollutes the soil and also pollute the ground as well as surface through percolation and surface run off during monsoon period. There are some other nutrient also contaminate the water bodies and leads to Eutrophication. India is one of the agriculture dominated country, hence most of the population dependent on agriculture. As India also have a large number of peoples and stand second position in world in population with 1.25 billions as per census 2011. In India agriculture is the most important part of economic growth.

They are urgent need to increase the crop production hence farmers are using large amount of chemical fertilizers without following standard guidelines. This discriminative use of synthetic fertilizers affect the soil properties and also caused water pollution through runoff in rainy season. The liberal use of chemical fertilizers especially in paddy fields may effects the growth inhibiting microorganisms (Bishara, 1978, Konar and Sarkar, 1983). These fertilizers, reach into water bodies, also affect fishes (Palanichamy, 1985) and other organisms. The extensive applications of synthetic fertilizers from last several years have not only caused soil degradation but also polluted the soil and water with health implications in populations. In agriculture, chemical fertilizers are used extensively but they are costly and also have various adverse effect on the soils i.e. deplete water holding capacity soil fertility and disparity in soil nutrients. Due to insufficient uptake of these fertilizers by plants results in the leaching away from soil. Hence, it is necessary to develop some low cost fertilizers which work without disturbing nature. Biofertilizers are biological cell that have potential to fix atmospheric nitrogen

in to nitrates. Biofertilizers is a mixture which contains living cell specially microorganisms that help the root system with good seed germination by providing the nutrients to them. These living cells can also solubilise insoluble salts like phosphate and can produce fertilizing substance in soil. (Mazid et.al. 2014).

Classification of biofertilizers

Several microorganisms and their association with crop plants are being exploited in the production f biofertilizers they can be grouped in different ways based on their nature and function.



Rhizobium : Rhizobium is a soil habitat bacterium , which colonize legume roots and fixes atmospheric nitrogen symbiotically. The morphology and physiology of Rhizzobium vary from free living condition to bacteriod of nodules they are most efficient biofertilizers as oer the quantity of nitrogen fixed concerned. They have seven genera and are highly specific to form nodule in legumes referred as cross inoculation group.

Azotobacter of the several species of Azotobacterr A.chroochonum happen to be the dominant inhabitant in arable soils capable of fixing N₂ (2-15 mg N₂ fixed/g of carbon source) in culture media . te bacterium produces abundant slime which helps in soil aggregation . the numbers of a.chrooconum in india soils rarely exceeds 105/g soil due to lack of organic matter and the presence of antsgonistic microorganisms in soils

Azospirillum: Azospirillum lipoferum and A.brasilens (Spirillum lipoferumin earlier literature) are primary inhabitants of soils, the rhizoshpere and intracellular spaces of root cortex of germinaceous plants. They develop

associate symbiotic relationship with germinaceous plants. Apart from nitrogen fixation, growth promoting substances production (IAA), disease resistance and drought tolerance are some of the additional benefits of inoculation with azospirillum

Cyanobacteria: both free living as well as symbiotic cyanobacteria (blue green algae) have been harvested in rice cultivation in india. Once so much publicized as a biofertilizer for rice crop , it hasnot presently attracted the attention of rice growers all over. India the benefit due to algalisation could be to the extend of 20-30kgmNm/ha under ideal conditions but the labour oriented methodology for the preparation of BGA biofertilizers is in itself a limitations.

Azolla: Azolla is a free floating water term that floats in water and fixes atmospheric nitrogen in association with nitrogen fixation blue green algae. Anabeana azolla either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. Azolla is used as biofertilizers for wetland rice and it is known to contribute 40-60 kg/ha per rice crop.

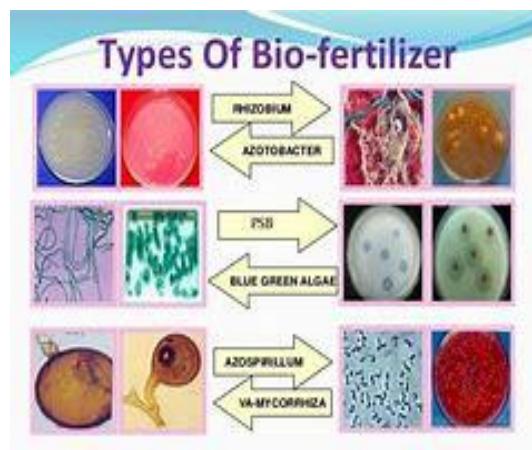
Phosphate solubilizing microorganisms (PSM) several soil bacteria and fungi, notably species of Pseudomonas, Bacillus, Penicillium, Aspergillus etc., secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphate in soil. Increased yields of wheat and potato were demonstrate due to inoculation of peat based culture of Bacillus polymyxa and Pseudomonas striata. AM fungi. The transfer of nutrients mainly phosphate and also zinc and sulphur from the soil milleu to the cells of the root cortex is mediated by intracellular obligate fungal endosymbionts of the green glomus, Gigaspora, Acaulospora, Sclerocysts and Endogone which posess vesicle for storage of nutrients and arbuscles for funneling these nutrients into the root system. By far thecommnest genus appear to be glomus, which has several species distributed in soil. Silicate solubilizing bacteria (SSB). Microorganisms are capable of degrading silicates and aluminium silicates. During the metabolisms of microbes several organic acids are produced and these have a produced and these have a dual role in silicate weathering. They supply H⁺ ions to the medium and promote hydrolysis and the organic acids like citric oxalic acid keto acids and hydroxyl carboxylic acids which form complexes with cations promote their removal and retention in the medium in a dissolved state.

Plant growth promoting rhizobacteria (PGPR). The group bacteria that colonize roots or rhizosphere soil and beneficial to crops are referred to as plant growth promoting rhizobacteria (PGPR). The PGPR inoculants promote

growth through suppression of plant disease (termed Bioprotectants), improved nutrient acquisition (termed Biofertilizers), or phytohormone production (termed bioprotectants), improved nutrient acquisition (termed biofertilizers) or phytohormone production (Biostimulants) species of *Pseudomonas* and *Bacillus* can produce as yet not well characterized phytohormone or growth regulators that cause crops to have greater amounts fine roots which have efficient of increasing the absorptive surface of plant roots for uptake of water and nutrients. These PGPR are referred to asbiostimulants and the phytohormones they produce include indole acetic acid , cytokine, gibberellins and inhibitors of ethylene production.

Types of biofertilizers

N₂ fixing biofertilizers

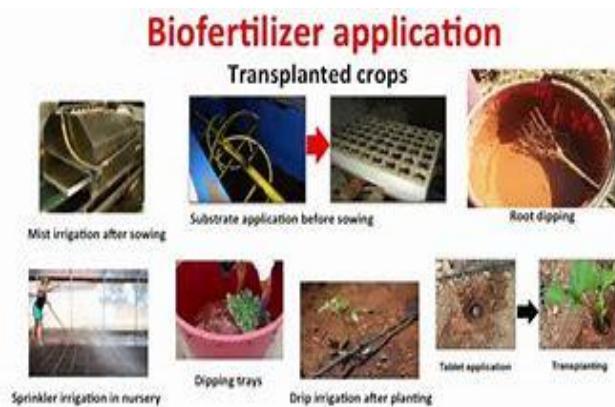


- Free living : Azotobacter, Beijerinckia, Clostridium, Klebsiella, Anabaena, Nostoc
- Symbiotic : Rhizobium, Frankia, Anabaena, Azolla
- Associative symbiotic: Azospirillum, P.solubilising Biofertilizers
- Bacteria:*Bacillus megaterium*, *Bacillus subtilis*, *Bacillus circulans*, *Pseudomonas striata*
- Fungi : Penicillium sp, Aspergillus awamori, P.mobilising bacteria biofertilizers
- Arbuscular mycorrhiza: Glomus sp, Gigaspora sp, Acalospora sp, Scutellospora sp and Sclerocystis sp
- Ectomycorrhiza : Laccaria sp, Pisolithus sp, Boletus sp, Amanita sp.
- Ericoid mycorrhizae: Rhizoctonia
- Orchid mycorrhiza : Rhizoctonia solani

- Biofertilizers for micronutrients
- Silicate and zinc solubilizers: Bacillus sp
- Plant growth promoting Rhizobacteria:Pseudomonas sp, Pseudomonas fluorescence

Advantage of using biofertilizers

- ❖ Some of the advantages associated with biofertilizers include
- ❖ They are ecofriendly as well as cost effective
- ❖ Their used lead to soil enrichment and the quality of the soil improve with time



- ❖ Though they do not show immediate results shown over time are spectacular
- ❖ These fertilizers harness atmospheric nitrogen and make it directly available to the plants.
- ❖ They increase the phosphorus content of the soil by solubilising and releasing unavailable phosphorus
- ❖ Biofertilizers improve root proliferation due to the release of growth promoting hormones
- ❖ Microorganisms convert complex nutrients into simple nutrients for the availability of the plants
- ❖ Biofertilizers contain microorganisms which promote the adequate supply of nutrient to the host plants and ensure their proper development of growth and regulation in their physiology
- ❖ The help in increasing the crop yield by 10-25%
- ❖ Biofertilizers can also protect plants from soil borne disease to a certain degree

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