ISBN: 978-93-91768-12-6

Agricultural Science

Research and Reviews

Volume II

Editors

Dr. Parvinder Khanuja

Dr. Med Ram Verma

Dr. Satyendra Nath

Dr. Mangal S. Kadam

Bhumi Publishing

First Edition: 2021

Agricultural Science: Research and Reviews

Volume II

(ISBN: 978-93-91768-12-6)

Editors

Dr. Parvinder Khanuja

Department of Pharmaceutical Chemistry,

Govt. S. N. P.G. College,

Khandwa (M.P.)

Dr. Med Ram Verma

Principal Scientist (Agricultural Statistics),
Division of Livestock Economics,
Statistics and Information Technology,
ICAR – Indian Veterinary Research Institute,
Izatnagar, Bareilly, Uttar Pradesh

Dr. Satyendra Nath

Department of Environmental Science

And NRM,

College of Forestry, SHUATS, Allahabad

Dr. (Mrs) M. S. Kadam

P. G. Department of Zoology,

Yeshwant Mahavidyalaya,

Nanded (M.S)

2021

First Edition: 2021

ISBN: 978-93-91768-12-6

Published by:

Bhumi Publishing,

Nigave Khalasa, Kolhapur 416207, Maharashtra, India

Website: www.bhumipublishing.com

E-mail: bhumipublishing@gmail.com

Book Available online at:

https://www.bhumipublishing.com/books/

PREFACE

We are delighted to publish our book entitled ***"Agricultural Science: Research and Reviews Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied agricultural science.*

The Indian as well as world population is ever increasing. Hence, it is imperative to boost up agriculture production. This problem can be turned into opportunity by developing skilled manpower to utilize the available resources for food security. Agricultural research can meet this challenge. New technologies have to be evolved and taken from lab to land for sustained yield. The present book on agriculture is to serve as a source of information covering maximum aspects, which can help understand the topics with eagerness to study further research. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editorial Team

Agricultural Science: Research and Reviews Volume II

ISBN: 978-93-91768-12-6

CONTENTS

THE EMERGING IMPORTANCE OF SILVER NANOPARTICLES IN AGRICULTURE

Amit Kumar[1], Ambalika Sharma*[2], Anu[3] and Richa[4]

[1]Department of Intelligent Interior Assistant, Bangalore (Karnataka, India)

[2]Department of Chemistry, Career Point University Hamirpur (H.P.)

[3]Department of Physics, Career Point University Hamirpur (H.P.)

[4]Department of Microbiology, Career Point University Hamirpur (H.P.)

*Corresponding authors E-mail:: ambalikasharma101@gmail.com

Abstract:

Agriculture is the prestigious and main part of human lives. Agriculture is responsible for carrying our basic needs. Without agriculture survival is not possible. Agriculture is the source of income for many people. Nowdays, agriculture is affected by many more reasons. That's why we have attempted here the role of Silver NPs in agriculture.

Introduction:

Agriculture is the foundation of many creating economies, with about 60% of the population depending on agro business. The Indian Department of Agriculture addresses 18% of India's complete creation and utilizes half of the public labor force. Exploration regions, for example, plant diagnostics, soil quality enhancements, nano fertilizers and seed germination are as yet being thought of spots and analysts are drawn to it. To settle this profoundly convincing field, nanotechnologies are a multidisciplinary research technique field [Kale *et al.,* 2021]. Nanotechnology is a promising new field of examination with particles with preferable particles and morphological constructions over many. Nanoparticles have a range of 1-100 nm which expands the surface space of the particles. Because of the expansion in size, the climate builds the natural action of nanoparticles [Anand *et al.*, 2019]. Nanotechnology can possibly change horticultural creation that considers better administration and safeguarding of import and animals information creation. In the field of agriculture, nanotechnology has been utilized to control pesticides utilizing pesticides dependent on nanomaterials, bug sprays, to advance rural creation utilizing bioconjugate nanoparticles (epitome) particularly the sluggish arrival of supplements, water, and hereditarily controlled nanotarticles. Nanotechnology can be utilized for an assortment of rural frameworks with accuracy. NP Agriculture is presently an alluring field for

crop development and harvest decrease by diminishing the utilization of substance composts. Silver nanoparticles (AgNPs) are one of the most broadly utilized nanomaterials in different locales, particularly in the rural area. Plants are a significant piece of human condition and endurance [Yan *et al.*, 2019]. Silver nanoparticles (AgNPs) were acquainted today with further develop seed germination, plant development, and increment quantum proficiency of photosynthesis and as antimicrobial specialists to control plant illnesses [Almutairi *et al.*, 2015]. Worldwide farming creation is declining year on year because of plant infections; accordingly, a large number of dollars have been spent on plant weight the board. Numerous regular and mechanical techniques have been utilized to shield plants from these infections [Gupta *et al.*, 2018]. The most productive utilization of silver has been an amazing mix of antimicrobial, antimicrobial, and antimicrobial properties. Silver nanoparticles can be utilized in food bundling polymers to work on the soundness of human food. It has been shown that if Ag NPs is touchy to drain straight forwardly, accordingly, it lessens the development of microorganisms. These advancements can assist with beating their future farming necessities; lessening air contamination brought about by quality improvement and yield reaping that shields synthetic compounds or plants from ecological pressure [Paramo *et al.*, 2020].

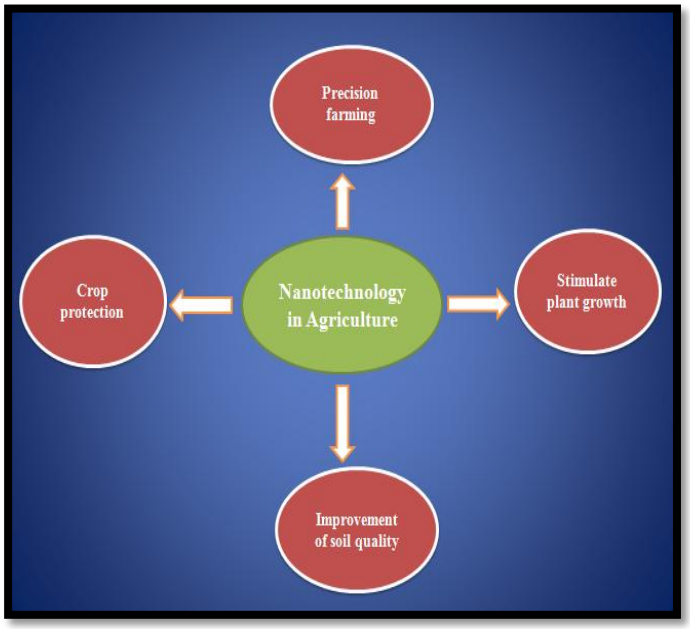


Figure 1: Addressing the job of nanotechnology in agriculture

Elements influencing farming:

There are many elements that influence agribusiness. There are two sorts of tensions pointed toward making genuine harm agribusiness.

A) Biotic Stress: Biotic pressure normally alluded to as injury is brought about by an irresistible sickness that beginnings on gathered foods grown from the ground for the most part brought about by microscopic organisms, growths or yeast [Singla].

B) Abiotic Stress: incorporates possible antagonistic impacts of salt, dry spell, floods, iron harming, high temperatures and low temperatures. There are numerous different factors, for example, openness to UV beams [Vijayalakshmi, 1999]. The stream outline chart given beneath addresses the sorts of tensions and models.

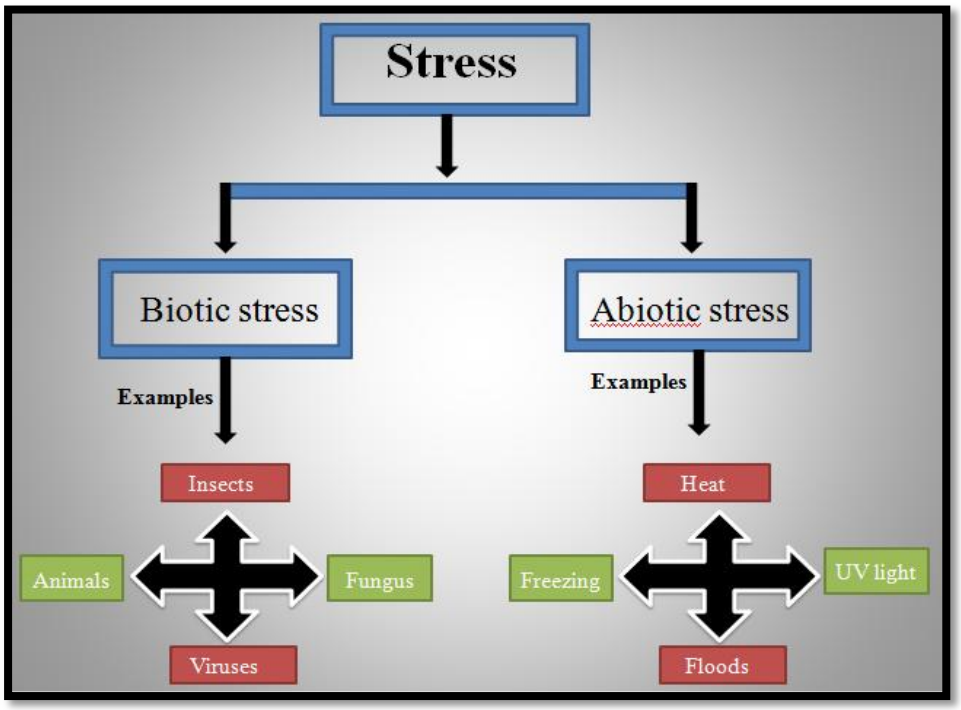


Figure 2: Addresses the sorts of rural tensions

The Role of Silver Nananoparticles (Ag NPs) in Agriculture:

Silver-covered nanoparticles have been utilized as incredible pesticides among various nanoparticles against countless microorganisms, organisms and microbes [Sharma *et al.*, 2018]. Silver nanoparticles can be utilized to straightforwardly dispense with illnesses that cause plant development and creation [Ghazy *et al.*, 2021]. AgNPs advance plant development, which is portrayed by expanded leaf collection and bulb biomass and quick blooming [Salachna *et al.*, 2019]. The disclosure of silver nanoparticles (AgNPs) isn't viable with the treatment of plant illnesses because of their solid bacterial properties. It gives a non-poisonous and harmless to the ecosystem technique [Mishra *et al.*, 2014]. Silver addresses different exercises that hinder miniature creatures [Clement and Jarrett, 1994]. It is a lot more secure to use than synthetic fungicides [Park *et al.*, 2006]. Nano-sized silver particles are developing as innovative advances

make their preparing more conservative [Jo *et al.*, 2009]. Silver is a significant metal with a high enemy of bacterial and low poisonousness work in creature cells and has a long history in customary medication [Mussin *et al.*, 2021]. It is realized that silver assaults different organic cycles in microorganisms, including cell arrangement and capacity [McDonnell and Russell, 1999; Sondi and Salopek-Sondi, 2004; Tak *et al.*, 2015]. Silver additionally hinders the statement of proteins related with ATP creation [Yamanaka *et al.*, 2005]. Until now, restricted investigations have given proof of silver impact on viral control [Singh *et al.*, 2019]. In any case, the productive utilization of silver nanoparticles in the battle against plant contaminations in farming has not been completely concentrated on [Dawadi *et al.*, 2021]. Here are the jobs of Ag NPs recorded underneath:

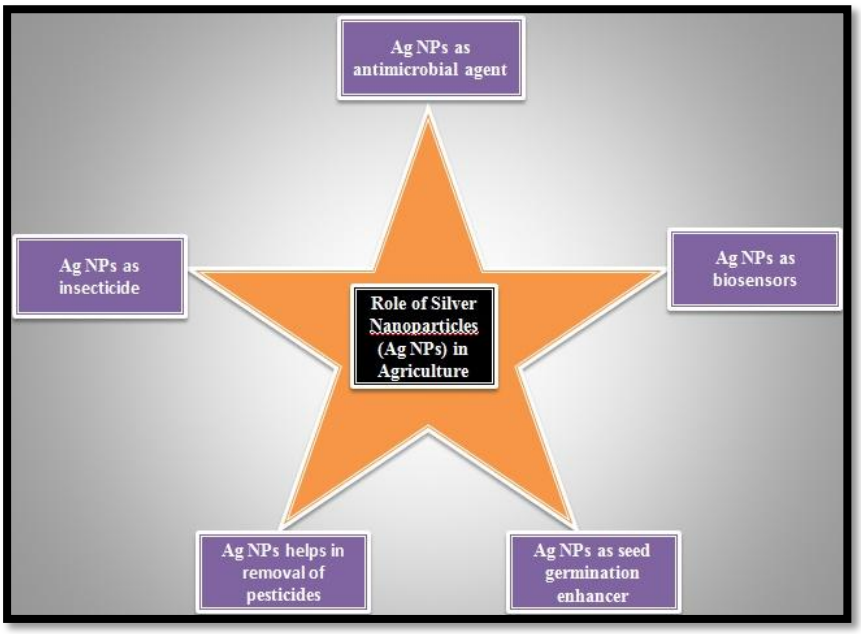


Figure 3: Addresses the job of the Ag NP in agriculture

1) Ag NPs as an antimicrobial specialist in agriculture: Ag NPs, being antimicrobial, are compelling in fighting bacterial diseases [Jo *et al.*, 2009]. Ag NP particles influence colon arrangement and transformation to contagious diseases (*Bipolaris sorokiniana* and *Magnaporthe grisea*) [Ibrahim *et al.*, 2020]. Ag NPs that contain Ag nitrate effectively affect rice and assist with further developing rice creation [Alonso-Díaz *et al.*, 2019; Terra *et al.*, 2019),]. Likewise, fructose-safe Ag NPs have shown hostile to bacterial movement against phytopathogens (*Xanthomonas campestris*, and *Dickeya solani*) [Dzimitrowicz *et al.*, 2018; Chen *et al.*, 2016; Terra *et al.*, 2019]. Graphene-silver oxide nanocomposite (GO-Ag NPs) is utilized to treat contaminated fusarium graminearum leaves [El-Mohamady *et al.*, 2018]. Ag NPs have been

demonstrated to be powerful against herpes infection, which has prompted an increment in farming creation [Noshad *et al.,* 2019].

2) Ag NPs as seed/plant growth enhancer: Silver NPs significantly affect seed development. These tests have been demonstrated by numerous specialists. Treatment of Silver nanoparticle (Ag NPs) in Solanum lycopersicum showed a critical expansion in the pace of development and planting contrasted with untreated seeds. Seed of S. lycopersicum treated with Ag NPs made an increment in seed germination at 3-12 days of germination, while getting treatment a similar impact was noticed for 14–14 days [Acharya *et al.,* 2020]. Ag NP-treated watermelon is shockingly great. The utilization of Ag NPs at first expanded glucose and fructose levels by 96 hours contrasted and non-modern seeds and triploid seeds [Garg *et al.,* 2020].

Figure 4: Addresses the various plant species where these Ag NPs can be used

3) Ag NPs as biosensors: The utilization of nanomaterials in biosensors has shown huge improvement. Biosensors are broadly utilized in food, clinical, wellbeing, natural and different fields to identify pollutions or synthetic substances in different examples. In many investigations the utilization of Ag NP has been demonstrated to be an indication of redox energy for glucose in food and drinks. Silver nanoparticle biosensors have high affectability, speed, burden and minimal expense and have a wide scope of utilizations [Manimegalai *et al.,* 2014].

4) Ag NPs aid the expulsion of pesticides: Pesticides are purposely used to control bugs in horticulture and general wellbeing, some of which are found in drinking water. Since they are broadly utilized, they are available above and beneath groundwater. Silver nanoparticles have been demonstrated to be innocuous and can adequately eliminate pesticides. Silver nanoparticles don't recognize various pesticides and can really be utilized to eliminate pesticides in provincial regions where pesticide tainting is normal.

5) Ag NPs as pesticides: The death pace of oleandra trees has expanded quickly as of late. Aphis nerii is perhaps the most widely recognized creepy crawlies on certain decorative plant. This aphid is thickly populated, found in tropical and subtropical areas of the world. Ag NPs have been demonstrated to be a strong bug contrasted with Aphis nerii. Ag nanoparticles are exceptionally helpful in bug control and can be utilized as significant creepy crawly control programs A. nerii.

Conclusion:

Hence we can reason that Ag NPs assumes an essential part in the field of agribusiness. They are very useful for plant development just as expulsion of risky pesticides. They are not harmful whenever taken in appropriate sum. Specialists have investigated the utilization of many metal NPs in the field of agribusiness.

References:

Acharya P, Jayaprakasha GK, Crosby KM, Jifon JL, Patil BS. (2020): Nanoparticle-mediated seed priming improves germination, growth, yield, and quality of watermelons (Citrullus lanatus) at multi-locations in Texas. Scientific reports. Mar 19; 10 (1):1-6.

Almutairi ZM, Alharbi A. (2015): Effect of silver nanoparticles on seed germination of crop plants. International Journal of Nuclear and Quantum Engineering. Jul 1; 9(6):689-93.

Alonso-Díaz A, Floriach-Clark J, Fuentes J, Capellades M, Coll NS, Laromaine A. (2019 J): Enhancing localized pesticide action through plant foliage by silver-cellulose hybrid patches. ACS biomaterials science & engineering. an 11; 5 (2):413-9.

Anand R, Bhagat M. (2019): Silver nanoparticles (AgNPs): As nanopesticides and nanofertilizers. MOJ Biol. Med. 4:19-20.

Chen J, Sun L, Cheng Y, Lu Z, Shao K, Li T, Hu C, Han H. (2016): Graphene oxide-silver nanocomposite: novel agricultural antifungal agent against Fusarium graminearum for crop disease prevention. ACS applied materials & interfaces. Sep 14; 8 (36):24057-70.

Clement JL, Jarrett PS. (1994): Antibacterial silver. Metal-based drugs. 1 (5-6):467-82.

Dawadi S, Katuwal S, Gupta A, Lamichhane U, Thapa R, Jaisi S, Lamichhane G, Bhattarai DP, Parajuli N. (2021): Current research on silver nanoparticles: Synthesis, characterization, and applications. Journal of Nanomaterials. Feb 8; 2021.

Dzimitrowicz A, Motyka-Pomagruk A, Cyganowski P, Babinska W, Terefinko D, Jamroz P, Lojkowska E, Pohl P, Sledz W. (2018): Antibacterial activity of fructose-stabilized silver nanoparticles produced by direct current atmospheric pressure glow discharge towards quarantine pests. Nanomaterials. Oct; 8 (10):751.

El-Mohamady RS, Ghattas TA, Zawrah MF, Abd El-Hafeiz YG. (2018): Inhibitory effect of silver nanoparticles on bovine herpesvirus-1. International journal of veterinary science and medicine. Dec 1; 6(2):296-300.

Garg D, Sarkar A, Chand P, Bansal P, Gola D, Sharma S, Khantwal S, Mehrotra R, Chauhan N, Bharti RK. (2020): Synthesis of silver nanoparticles utilizing various biological systems: mechanisms and applications—a review. Progress in Biomaterials. Jul 11:1-5.

Ghazy NA, Abd El-Hafez OA, El-Bakery AM, El-Geddawy DI. (2021): Impact of silver nanoparticles and two biological treatments to control soft rot disease in sugar beet (Beta vulgaris L). Egyptian Journal of Biological Pest Control. Dec; 31 (1):1-2.

Gupta N, Upadhyaya CP, Singh A, Abd-Elsalam KA, Prasad R. (2018): Applications of silver nanoparticles in plant protection. InNanobiotechnology Applications in Plant Protection (pp. 247-265). Springer, Cham.

Ibrahim E, Luo J, Ahmed T, Wu W, Yan C, Li B. (2020): Biosynthesis of Silver Nanoparticles Using Onion Endophytic Bacterium and Its Antifungal Activity against Rice Pathogen Magnaporthe oryzae. Journal of Fungi. Dec; 6 (4):294.

Jo YK, Kim BH, Jung G. (2009): Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant disease. Oct; 93(10):1037-43.

Jo YK, Kim BH, Jung G. (2009): Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant disease. Oct; 93 (10):1037-43.

Kale SK, Parishwad GV, Patil AS. Emerging Agriculture Applications of Silver Nanoparticles. ES Food & Agroforestry. 2021 Mar 16; 3:17-22.

Manimegalai G, Shanthakumar S, Sharma C. (2014): Silver nanoparticles: synthesis and application in mineralization of pesticides using membrane support. International Nano Letters. Jun 1; 4 (2):105.

McDonnell G, Russell AD. (1999): Antiseptics and disinfectants: activity, action, and resistance. Clinical microbiology reviews. Jan 1; 12 (1):147-79.

Mishra S, Singh BR, Singh A, Keswani C, Naqvi AH, Singh HB. (2014): Biofabricated silver nanoparticles act as a strong fungicide against Bipolaris sorokiniana causing spot blotch disease in wheat. Plos one. May 19; 9 (5):e97881.

Mussin J, Robles-Botero V, Casañas-Pimentel R, Rojas F, Angiolella L, San Martín-Martínez E, Giusiano G. (2021): Antimicrobial and cytotoxic activity of green synthesis silver nanoparticles targeting skin and soft tissue infectious agents. Scientific reports. Jul 15; 11 (1):1-2.

Noshad A, Hetherington C, Iqbal M. (2019): Impact of AgNPs on seed germination and seedling growth: A focus study on its antibacterial potential against Clavibacter michiganensis

subsp. michiganensis infection in Solanum lycopersicum. Journal of Nanomaterials. Dec 3; 2019.

Paramo LA, Feregrino-Pérez AA, Guevara R, Mendoza S, Esquivel K. (2020): Nanoparticles in agroindustry: Applications, toxicity, challenges, and trends. Nanomaterials. Sep; 10 (9):1654.

Park HJ, Kim SH, Kim HJ, Choi SH. (2006): A new composition of nanosized silica-silver for control of various plant diseases. The plant pathology journal. 22 (3):295-302.

Salachna P, Byczyńska A, Zawadzińska A, Piechocki R, Mizielińska M. (2019): Stimulatory effect of silver nanoparticles on the growth and flowering of potted oriental lilies. Agronomy. Oct;9 (10):610.

Sharma J, Singh VK, Kumar A, Shankarayan R, Mallubhotla S. (2018): Role of silver nanoparticles in treatment of plant diseases. InMicrobial biotechnology (pp. 435-454). Springer, Singapore.

Singh R, Gupta AK, Patade VY, Balakrishna G, Pandey HK, Singh A. (2019): Synthesis of silver nanoparticles using extract of Ocimum kilimandscharicum and its antimicrobial activity against plant pathogens. SN Applied Sciences. Dec;1 (12):1-2.

Singla J, Krattinger SG, Wrigley CW, Faubion J, Corke H, Seetharaman K. Biotic stress resistance genes in wheat.

Sondi I, Salopek-Sondi B. (2004): Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science. Jul 1; 275 (1):177-82.

Tak YK, Pal S, Naoghare PK, Rangasamy S, Song JM. (2015): Shape-dependent skin penetration of silver nanoparticles: does it really matter?. Scientific Reports. 20; 5(1):1-1.

Terra AL, Kosinski RD, Moreira JB, Costa JA, Morais MG. (2019): Microalgae biosynthesis of silver nanoparticles for application in the control of agricultural pathogens. Journal of Environmental Science and Health, Part B. Aug 3; 54 (8):709-16.

Vijayalakshmi D. (1999): Abiotic stresses and its management in agriculture. Stresses in Plants. India: Springer.

Yamanaka M, Hara K, Kudo J. (2005): Bactericidal actions of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. Applied and environmental microbiology. Nov; 71 (11):7589-93.

Yan A, Chen Z. (2019): Impacts of silver nanoparticles on plants: a focus on the phytotoxicity and underlying mechanism. International journal of molecular sciences. Jan; 20(5):1003

ROLE OF COPPER OXIDE NANOPARTICLES IN AGRICULTURE

Anu*[1], Ambalika Sharma[2] and Richa[3]

[1]Department of Physics, Career Point University Hamirpur (H.P.)

[2]Department of Chemistry, Career Point University Hamirpur (H.P)

[3]Department of Microbiology, Career Point University Hamirpur (H.P.)

*Corresponding author E-mail: anubasu550@gmail.com

Abstract:

Nanoparticles (NPs) have piqued the interest of scientists due to their unique properties and numerous applications. According to current research, CuO NPs have been shown to be necessary micronutrients and to enhance plant growth. Copper is an essential element for plant growth and development. Agricultural fertilisers and herbicides are required for CuO NPs to grow. This chapter clearly proved the effects of CuO NPs on cultivated root growth, crop plants by reducing seed germination, plant growth and morphology, and microbial interactions. The data will be used by researchers to set boundaries and make future plans.

Introduction:

Thanks to recent breakthroughs in nanotechnology, researchers are concentrating their efforts on generating nano-sized metal oxide particles with the necessary shape and size. The features of these metal oxide NPs have evolved to varied morphologies because they have different applications in many domains such as medicine, agriculture, and environmental remediation [Siddiqi *et al.,* 2020]. It's a nanometer-scale technology that works with atoms, molecules, and macromolecules with material sizes ranging from 1-100 nm to create and use materials with a wide range of properties. These nanomaterials [Singh *et al.*, 2017] have one or more external dimensions as well as an internal structure that allows them to be used in a number of applications. Due to their high surface-to-volume ratio and other physiochemical properties such as colour, solubility, strength, diffusivity, toxicity, magnetic, optical, thermodynamic, and others, as well as their industrial use, these materials are observed to have unique properties, which opens up new possibilities but also introduces new risks and concerns [Mourdikoudis *et al.,* 2018]. Nanoparticles (NPs) with a particle size of less than 100 nm are a fast expanding research topic with various applications in a variety of sectors. The global market for NPs is expected to reach $25.26 billion by 2022 [Rajput *et al.,* 2020].

Agriculture is the most important source of food for us to live a better life, as well as a source of support for growing economies. Agriculture currently faces a number of issues, including unexpected climate change, soil pollution from a variety of dangerous environmental contaminants such as fertilisers and pesticides, and dramatically increased food demands as the global population grows [Pouratashi and Iravani, 2012]. Absorption on agricultural plants may taint food materials, providing a health concern to humans, as metal oxide NPs are produced and used more widely in agriculture. Crop plants are crucial in the production of food [Waser *et al.,* 2013], gas sensors [Li *et al.,* 2008], solar cells [Siddiqui *et al.,* 2020], pollution remover [Mahmoud *et al.,* 2021], and catalysis [Gawande *et al.,* 2016]. Between 2020 and 2025, the world is expected to consume at least 200,000-830,000 kg of CuO NPs per year. CuO NPs most essential application has greatly improved the likelihood of their discharge [Yang *et al.,* 2020].

New Cu fungicides are being developed using CuO NPs [Li *et al.,* 2017]. Food crop root systems could be exposed to CuO NP via waste streams as a result of the growing use of recycled wastewater for irrigation [Sun *et al.,* 2014]. NPs can disrupt food crop growth by limiting root growth directly or indirectly by interfering with root processes like water transfer. When compared to other metal oxide NPs (e.g., TiO_2, Fe_2O_3), CuO NPs have been shown to have negative effects on the growth of a variety of aquatic and terrestrial plant species, including food crops [Du *et al.,* 2016]. In previous studies of CuO NP's effects on plants, roots performed better than shoots.

Because of their extensive antibacterial action, CuO NPs have aroused the curiosity of scientists all around the world. CuO NPs are important for plant growth and development. CuO NP-based fertilisers and herbicides have also been employed in agriculture. Because of their small size, CuO NPs are easily absorbed by plants. CuO NPs have long been used in food packaging to inhibit the growth of bacteria that cause food spoilage. CuO is now less expensive and more commonly available as a result. CuO NPs can thus be used at a low cost in a range of agricultural applications. The main purpose of this review is to go over the various applications of CuO NPs in agriculture and food. The antibacterial and plant-protective properties of CuO NPs have been investigated. Other important subjects discussed include the mechanism of NPs' interaction with plant diseases, CuO NPs as a plant nutrient and their phytotoxicity, and so on. The use of Cu and CuO NPs in food and agriculture is the topic of this review. The antibacterial and pesticidal properties of CuO NPs are also explored.

Root Growth Affected by CuO NPs:

NPs are being employed in a new field of research to increase crop quality and growth while also compensating for sterile soils, as the world's population grows and the environment

deteriorates [Vassell *et al.,* 2019]. The fast rise in NP production and its many uses pose new threats to an anthropogenically altered ecosystem and, as a result, to humans. Crop plants are necessary and play an important part in food production. As demonstrated in Figure 1, CuO NPs have the following effect on root growth:

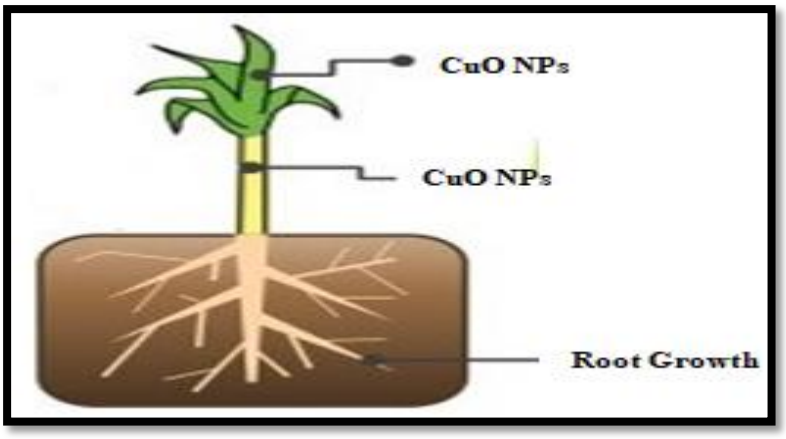


Figure 1: Effect of CuO NPs on Root/ Plant Growth

CuO NPs' Effects on Crop Plants

The effects of CuO NPs on plant form, germination, and yield quality, as well as transpiration and translocation in plant tissues, have been studied in a number of researches. CuO NPs have been found in recent studies to have a negative impact on seed germination and plant growth in a range of crop species, including wheat (*Triticum aestivum*) [Thakur *et al.,* 2021], kidneybean (*Phaseolus vulgaris*) [Apodaca *et al.,* 2017], maize (*Zea mays*) [Wang, Z *et al.,* 2012] spinach (*Spinacia oleracea*) [Singh and Kumar (2016], onion (*Allium cepa*) [Zorawar Singh and Iqbal Singh (2019], mustard (*Brassica juncea* L.) [Nair and Chung (2015], [Nair and Chung (2014] tomato (*Solanum lycopersicum*), soybean (*Glycine max*), carrot (*Daucus carota*) [Ebbs *et al.,* 2016], sweet potato (*Ipomoea batatas*) [Bradfield *et al.,* 2017], radish (*Raphanus sativus*) [Lukatkin *et al.,* 2014] and zucchini (*Cucurbita pepo*) etc. Figure 2 depicts plant growth on a variety of crop species, as shown below. NPs affect plant growth by lowering germination rates, decreasing biomass, and shortening root and shoot lengths, changing photosynthesis and transpiration rates, and boosting chromatin condensation and lipid peroxidation [Singh *et al.,* 2017]. It should be noted that NP accumulation and absorption are affected by concentrations and time of exposure [Karlsson *et al.,* 2009]. Jain et al. have investigated that the differences in NP phytotoxicity are caused by seed size and surface architecture differences [Jain *et al.,* 2017].

Figure 2: Plant Growths on Various Crop Plants

Seed Germination Effects of CuO NPs:

Germination is the process of a plant contained within a seed growing and developing into a seedling. The production of radicles and plumages is also due to the reactivation of the seed's metabolic machinery. Figure 3 depicts the seed germination process. Seed germination is influenced by both internal and external forces. The most important exterior factors are the right temperature, water, oxygen or air, and sometimes light or darkness. Seed germination indexes and elongation are the initial step in measuring the success of crop growth with metal and metal oxide NPs. Plants that have been exposed to NPs contain water channel genes protein activation for better cell growth protein, and for better cell growth by regulation cell cycle; These NP effects represent an increase in seed germination and plant growth [Priyanka *et al.,* 2019]. [Wierzbicka and Obidzinska, 1998] Seed germination is the start of a physiological process. The radicle is the first tissue to come into contact with metals after the seed coat is ruptured [Kranner and Colville, 2011]. CuO NPs affected mustard seed germination and seedling growth, according to Zafar et al. Cu oxide is the most common type of Cu around the root zone [Zafar *et al.* 2017]. Studies have also suggested that NPs absorbed by roots are not transferred to shoots, either because they are concentrated in the epidermis and exodermis, or because they are trapped in the epidermis and exodermis [Shaw *et al.,* 2013].

Figure 3: Seed Germination Process

CuO Influence on Plant Growth and Morphology:

The availability of soil nutrients is controlled by plant shape, which is crucial for annual species' early growth. Plant growth and root characteristics of unidentified Andean chenopod species were compared to the hypothesis that plants in low-resource environments have properties that aid in resource collection. The morphology of individual plants controls the balance between resource intake and resource consumption over the course of a plant's life cycle. Plant morphologies and organ allometry have evolved as a result of natural and human selection, resulting in systematic variation in plant growth, biomass allocation, and morphological traits among species [Alvarez-Flores *et al.,* 2014]. In a phylogenetic sense, plant morphology is a unifying science that focuses on fundamental themes that transcend systematic boundaries, and the German tradition of plant morphology is a unifying science that concentrates on convergences rather than homologies [Kaplan, 2001]. Plant species, growth conditions, exposure time, NP concentration, type, and size all affect NP toxicity. Cu ions, in small concentrations, have been shown to boost plant development and function as a microelement [Karlsson *et al.,* 2009]. The root shape of wheat growing in sandy soil was altered by copper NPs [Shaw *et al.,* 2014]. The green synthesis of CuO NPs, on the other hand, could be advantageous [Kasana *et al.,* 2017].

Mechanism of CuO NPs on Microbes Interaction:

The antibacterial activities of CuO NPs have been investigated, although the mechanism of antimicrobial action is unknown. Only a few publications have been published on the mechanism of CuO NPs' antibacterial action. CuO NPs interact with microbial cell walls because they have a strong affinity for the carboxyl group on the surface of the bacteria [Rai *et al.*, 2018]. The antibacterial action of NPs attribute to the formation of reactive oxygen species (ROS), membrane damage, loss of enzyme activity, protein dysfunction, and other causes [Vega-

Jiménez *et al.*, 2019]. Cu ions are released by CuO NPs when they come into contact with a bacterial cell, which are then absorbed by the cell [Slavin *et al.*, 2017].

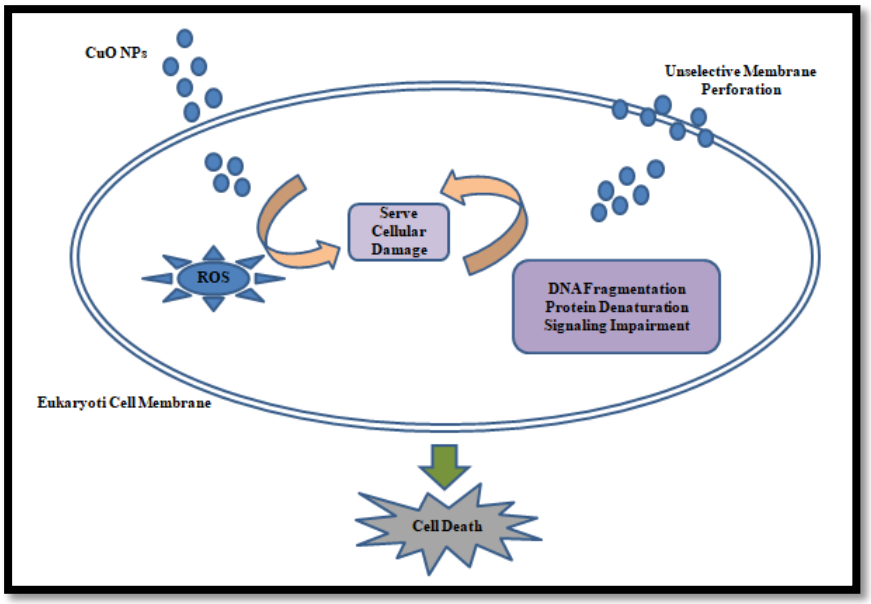


Figure 4: Mechanisms of CuO NPs on Microbes Interaction

The microbicidal effect of CuO NPs is influenced by particle size, electrostatic interaction between the microbial cell and the NPs, microbial cell wall and membrane composition, and the hydrophobic or hydrophilic character of the NPs [Khanna *et al.*, 2021]. Many Cu/CuO NPs generated from plants that have antibacterial properties also have antioxidant properties. Antibacterial activity was also established by the antioxidant activities of CuO/Cu NPs from Allium sativum extract and Allium eriophyllum Boiss leaf extract [Zhao, *et al.*, 2020) and Velsankar *et al.*, 2020].

Conclusion:

The role of Cu NPs in a variety of plant species has been well documented, but there are also a number of promising researches accessible. As a result, the usage of CuO NPs in the environment must be carefully examined. CuO NPs, their use, dispersion, and release in the environment are the subject of an increasing number of research. Finally, many researchers discovered that when CuO NPs are used in excess of a certain concentration, they have negative effects on plants, and that employing CuO NPs at a higher concentration can disturb plant metabolism, lowering yield. As a result, a thorough understanding of CuO NPs in agriculture is necessary for their safe application. CuO NPs are therefore advantageous to agriculture. CuO NPs help agriculture and the food industry by allowing them to make and store food for extended periods of time with minimum waste, helping them to feed the world's growing population. According to the findings of this study, CuO NPs have a wide range of agricultural applications.

CuO NP is necessary for the infection of host plants and are also employed to make a strong antibiotic. CuO NPs were discovered to be an excellent antibacterial option for preventing bacterial disease under greenhouse settings. CuO NPs could be employed as a new plant protection strategy in the future, according to our findings.

Increasing production and application of nanoparticles (NPs) in agriculture, absorption on crop plants may contaminate edible materials and consequently impose a threat to human health. Crop plants are essential and play a critical role to provide food materials. In recent years, significant research has been focused on studying the effects of NPs on variouarious commercial products, including agrochemicals, paints, semiconducting compounds, sensors, catalyzers, and antimicrobial products, which leads to their growing release into terrestrial and aquatic ecosystems (Keller *et al.,* 2017). These emerging contaminants can make their way into soil through direct applications of nanofertilizers or nanopesticides containing copper nanoparticles or through biosolid amendments from wastewater treatment (Lazareva and Keller, 2014; Kah, 2015). Hence, the high reactivity of copper nanomaterials and their established antimicrobial properties raise some concerns about the potential consequences on microbial processes driving soil fertility in agro-ecosyste

References:

Alvarez-Flores, R., Winkel, T., Degueldre, D., Del Castillo, C. and Joffre, R., (2014): Plant growth dynamics and root morphology of little-known species of Chenopodium from contrasted Andean habitats. Botany, 92(2), pp.101-108.

Apodaca, S. A., Tana, W., Dominguezb, O. E., Hernandez-Viezcasc, J. A., Peralta-Videaa, J. R., and Gardea-Torresdey, J. L. (2017): Physiological and biochemical effects of nanoparticulate copper, bulk copper, copper chloride, and kinetin in kidney bean (*Phaseolus vulgaris*) plants. Science of the Total Environment, 599(600), 2085-2094.

Bradfield, S. J., Kumar, P., White, J. C., and Ebbs, S. D. (2017): Zinc, copper, or cerium accumulation from metal oxide nanoparticles or ions in sweet potato: yield effects and projected dietary intake from consumption, Plant Physiology and Biochemistry, 110, 128-137.

Chen, G., Langlois, J.M., Guo, Y. and Goddard, W.A., (1989): Superconducting properties of copper oxide high-temperature superconductors, Proceedings of the National Academy of Sciences, 86(10), pp.3447-3451.

Du, W., Tan, W., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Ji, R., Yin, Y., Guo, H. (2016): Interaction of metal oxide nanoparticles with higher terrestrial plants: Physiological and biochemical aspects. Plant Physiology and Biochemistry, 28(3), pp.1-13.

Ebbs, S. D., Bradfield, S. J., Kumar, P., White, J. C., Musante, C., and Ma, X. (2016): Accumulation of zinc, copper, or cerium in carrot (*Daucus carota*) exposed to metal oxide nanoparticles and metal ions, Environmental Science Nano, 3, 114-126.

Gawande, M.B., Goswami, A., Felpin, F.X., Asefa, T., Huang, X., Silva, R., Zou, X., Zboril, R. and Varma, R.S., (2016): Cu and Cu-based nanoparticles: synthesis and applications in catalysis, Chemical Reviews, 116(6), pp.3722-3811.

Hawthorne, J., Musante, C., Sinha, S.K. and White, J.C., (2012): Accumulation and phytotoxicity of engineered nanoparticles to *Cucurbita pepo*. International journal of phytoremediation, 14(4), pp.429-442.

Jain, N., Bhargava, A., Pareek, V., Akhtar, M. S., and Panwar, J. (2017): Does seed size and surface anatomy play role in combating phytotoxicity of nanoparticles? Ecotoxicology. 26(2), pp.238-249.

Kaplan, D.R., (2001): The science of plant morphology: definition, history, and role in modern biology. American Journal of Botany, 88(10), pp.1711-1741.

Karlsson, H. L., Gustafsson, J., Cronholm, P., and Moller, L. (2009): Size-dependent toxicity of metal oxide particles-a comparison between nano- and micrometer size. Toxicology Letters, 188, pp.112-118.

Karlsson, H. L., Gustafsson, J., Cronholm, P., and Moller, L. (2009): Size-dependent toxicity of metal oxide particles-a comparison between nano and micrometer size. Toxicology Letters, 188, 112-118.

Kasana, R. C., Panwar, N. R., Kaul, R. K., and Kumar, P. (2017): Biosynthesis and effects of copper nanoparticles on plants. Environmental Chemistry Letters, 15, 233-240.

Khanna, K., Kohli, S. K., Handa, N., Kaur, H., Ohri, P., Bhardwaj, R., Yousaf, B., Rinklebe, J. and Ahmad, P., (2021): Enthralling the impact of engineered nanoparticles on soil microbiome: A concentric approach towards environmental risks and cogitation. Ecotoxicology and Environmental Safety, 222, pp.1-17.

Kranner, I. and Colville, L. (2011): Metals and seeds: biochemical and molecular implications and their significance for seed germination, Environmental and Experimental Botany, 72(1), pp.93-105.

Li, Y., Liang, J., Tao, Z. and Chen, J., (2008): CuO particles and plates: synthesis and gas-sensor application. Materials Research Bulletin, 43(8-9), pp.2380-2385.

Li, Y., Yang, D., Cui, J., (2017): Graphene oxide loaded with copper oxide nanoparticles as an antibacterial agent against *Pseudomonas syringae* pv. Tomato, RSC Advances, 7(1), pp.38853-38860.

Lukatkin, A., Egorova, I., Michailova, I., Malec, P. and Strzałka, K., (2014): Effect of copper on pro-and antioxidative reactions in radish (*Raphanus sativus* L.) in vitro and in vivo. Journal of Trace Elements in Medicine and Biology, 28(1), pp.80-86.

Mahmoud, A.E.D., Al-Qahtani, K.M., Alflaij, S.O., Al-Qahtani, S.F. and Alsamhan, F.A., (2021): Green copper oxide nanoparticles for lead, nickel, and cadmium removal from contaminated water, Scientific Reports, 11(1), pp.1-13.

Miralles, P., Church, T. L., and Harris, A. T. (2012): Toxicity, uptake and translocation of engineered nanomaterials in vascular plants. Environmental Science & Technology, 46, pp.9224-9239.

Mittal, D., Kaur, G., Singh, P., Yadav, K. and Ali, S.A., (2020): Nanoparticle-based sustainable agriculture and food science: recent advances and future outlook, Frontiers in Nanotechnology, 2(1), pp.1-38.

Mosa, K.A., El-Naggar, M., Ramamoorthy, K., Alawadhi, H., Elnaggar, A., Wartanian, S., Ibrahim, E. and Hani, H., (2018): Copper nanoparticles induced genotoxicty, oxidative stress, and changes in superoxide dismutase (SOD) gene expression in cucumber (*Cucumis sativus*) plants. Frontiers in plant science, 9(1), pp. 1-13.

Mourdikoudis, S., Pallares, R.M. and Thanh, N.T., (2018): Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties, Nanoscale, 10(27), pp. 12871-12934.

Nair, P. G., and Chung, I. (2014): A mechanistic study on the toxic effect of copper oxide nanoparticles in soybean (*Glycine max* L.) root development and lignification of root cells. Biological Trace Element Research, 162(1), pp.342-352.

Nair, P.M.G. and Chung, I.M., (2015): Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.), Ecotoxicology and Environmental Safety, 113, pp.302-313.

Peng, C., Duan, D., Xu, C., Chen, Y., Sun, L., Zhang, H., Yuan, X., Zheng, L., Yang, Y., Yang, J., Zhen, X., Chen, Y., & Shi, J. (2015): Translocation and biotransformation of CuO nanoparticles in rice (*Oryza sativa* L.) plants, Environmental Pollution, 197, 99-107.

Pouratashi, M. and Iravani, H., (2012): Farmers' knowledge of integrated pest management and learning style preferences: implications for information delivery, International Journal of Pest Management, 58(4), pp.347-353.

Priyanka, N., Geetha, N., Ghorbanpour, M. and Venkatachalam, P., (2019): Role of engineered zinc and copper oxide nanoparticles in promoting plant growth and yield: present status and future prospects. In Advances in Phytonanotechnology, pp.183-201.

Rai, M., Ingle, A.P., Pandit, R., Paralikar, P., Shende, S., Gupta, I., Biswas, J.K. and da Silva, S.S., (2018): Copper and copper nanoparticles: Role in management of insect-pests and pathogenic microbes. Nanotechnology Reviews, 7(4), pp.303-315.

Rajput, V., Minkina, T., Mazarji, M., Shende, S., Sushkova, S., Mandzhieva, S., Burachevskaya, M., Chaplygin, V., Singh, A. and Jatav, H., (2020): Accumulation of nanoparticles in the soil-plant systems and their effects on human health, Annals of Agricultural Sciences, 65(2), pp.137-143.

Shaw, A. K., and Hossain, Z. (2013): Impact of nano-CuO stress on rice (*Oryza sativa* L.) seedlings. Chemosphere, 93, pp.906-915.

Shaw, A. K., Ghosh, S., Kalaji, H. M., Bosa, K., Brestic, M., Zivcak, M., and Hossain, Z. (2014): Nano-CuO stress induced modulation of antioxidative defense and photosynthetic performance of syrian barley (*Hordeum vulgare* L.) Environmental and Experimental Botany, 102, 37-47.

Siddiqi, K.S. and Husen, A., (2020): Current status of plant metabolite-based fabrication of copper/copper oxide nanoparticles and their applications: a review, Biomaterials Research, 24(11), pp.1-15.

Siddiqui, H., Parra, M.R., Pandey, P., Qureshi, M.S. and Haque, F.Z., (2020): Utility of copper oxide nanoparticles (CuO-NPs) as efficient electron donor material in bulk-heterojunction solar cells with enhanced power conversion efficiency, Journal of Science: Advanced Materials and Devices, 5(1), pp.104-110.

Singh, A., Singh, N. B., Hussain, I., Singh, H., and Yadav, V. (2017): Synthesis and characterization of copper oxide nanoparticles and its impact on germination of *Vigna radiata* (L.) R. Wilczek. Tropical Plant Biology, 4(2), 246-253.

Singh, D., and Kumar, A., (2016): Impact of irrigation using water containing CuO and ZnO nanoparticles on *Spinach oleracea* grown in soil media. Bulletin of Environmental Contamination and Toxicology, 97, 548-553.

Singh, T., Shukla, S., Kumar, P., Wahla, V., Bajpai, V.K. and Rather, I.A., (2017): Application of nanotechnology in food science: perception and overview, Frontiers in microbiology, 8(1), pp. 1-7.

Singh, Z., and Singh, I., (2019): Copper Oxide Nanoparticles Synthesized at Different pH Pose Varied Genotoxic Effects in *Allium cepa*. Asian Journal of Biological Sciences, 12(1), pp.616-625.

Slavin, Y.N., Asnis, J., Hafeli, U.O. and Bach, H., (2017): Metal nanoparticles: understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology, 15(1), pp.1-20.

Sun, T.Y., Gottschalk, F., Hungerbuhler, K. and Nowack, B. (2014): Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials, Environmental Pollution, 185(1), pp.69-76.

Thakur, N., Chrungoo, S., Rana, S., Kaur, S. and Pathak, A., (2021): Effect of copper nanoparticles on in-vitro seed germination of wheat (*Triticum aestivum*) varieties. International Journal of Pharmaceutical Sciences and Research, 12(8), 4307-4313.

Vassell, J., Racelis, A. and Mao, Y., (2019): Effects of CuO Nanoparticles on the Growth of Kale, ES Materials & Manufacturing, 5(1), pp.19-23.

Vega-Jimenez, A.L., Vazquez-Olmos, A.R., Acosta-Gio, E. and Álvarez-Perez, M.A., (2019): In vitro antimicrobial activity evaluation of metal oxide nanoparticles, Nanoemulsions Properties of Fabrication Applied, pp.1-18.

Velsankar, K., Kumar, R.M. A., Preethi, R., Muthulakshmi, V., Sudhahar, S. (2020): Green synthesis of CuO nanoparticles via *Allium sativum* extract and its characterizations on antimicrobial, antioxidant, antilarvicidal activities. Journal of Environmental Chemical Engineering, 8(5), pp.1-12.

Wang, Z., Xie, X., Zhao, J., Liu, X., Feng, W., White, J.C., and Xing, B. (2012): Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.) Environmental Science & Technology, 46(1), 4434-4441.

Waser, O., Hess, M., Guntner, A., Novak, P. and Pratsinis, S.E., (2013): Size controlled CuO nanoparticles for Li-ion batteries, Journal of power sources, 241(1), pp.415-422.

Wierzbicka, M., & Obidzinska, J. (1998): The uptake of lead on seed imbibition and germination in different plant species, Plant Science, 137(1), pp.155-171.

Yang, Z., Xiao, Y., Jiao, T., Zhang, Y., Chen, J. and Gao, Y.,(2020): Effects of copper oxide nanoparticles on the growth of rice (*Oryza Sativa* L.) seedlings and the relevant physiological responses, International Journal of Environmental Research and Public Health, 17(4), pp.2-11.

Zafar, H., Ali, A., and Zia, M. (2017): CuO nanoparticles inhibited root growth from Brassica nigra seedlings but induced root from stem and leaf explants. Applied Biochemistry and Biotechnology, 181, pp.365-378.

Zhao, H.W., Su, H.T., Ahmeda, A., Sun, Y.Q., Li, Z.Y., Zangeneh, M. M., Nowrozi, M., Zangeneh, A., Moradi, R. (2020): Biosynthesis of copper nanoparticles using *Allium eriophyllum* Boiss leaf aqueous extract; characterization and analysis of their antimicrobial and cutaneous wound-healing potentials. Applied Organometallic Chemistry, pp. 1-18.

BILE SALT TOLERANCE AND ADHESION MECHANISM OF PROBIOTIC BACTERIA

Deeksha Sharma*, Suman Kapila and Rajeev Kapila

ICAR- National Dairy Research Institute,

Karnal-132 001 (Haryana) India

*Corresponding author E-mail: amaraiberis@gmail.com

Abstract:

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Most of the commercially available probiotics include Lactobacillus and *Bifidobacterium* bacteria species. These probiotic bacteria adhere to the organism's gut and promote the health of the organism improving gastrointestinal discomfort, antagonizing the effect of harmful microorganisms, and secreting antimicrobial proteins, and modulating the immune system. In order to impart these beneficial effects, probiotic bacteria must survive the gastrointestinal transit resisting the acid and bile salt toxicity in the stomach and small intestine respectively. Probiotic bacteria used a different mechanism to tolerate these stress conditions. One of the mechanisms used by bacteria to tolerate bile salt toxicity is by bile salt hydrolyses. These enzymes are secreted by probiotic bacteria during the bile salt toxicity and are found to be involved in the de-conjugation and efflux of the bile salts. Another one of the important criteria required by probiotic bacteria to impart beneficial effect is their ability to adhere to the intestinal mucosa and consequently colonize themselves in the gastrointestinal tract. Bacterial adhesion to the intestinal surfaces could be occurred by physical binding of the bacteria with the intestinal mucosa cells and another specific mechanism used by probiotic bacterial species for the attachment is by secretion of specific adhesion proteins. In the present work, we review and discuss the bile tolerance mechanism specifically the bile resistance process that occurred through bile salt hydrolyses. In addition, we also discussed the adhesion-promoting proteins of the probiotic bacteria.

Keywords: Probiotics, Bile tolerance, BSHs, Mub, and MapA

Introduction:

Historically, lactic acid bacteria were used to improve the storage quality, flavor of consumable foods, and nutritive value, without knowing the existence of microorganisms. Noble

Laureate Elie Metchnikoff first recognized the beneficial effect of lactic acid bacteria of yogurt and hypothesized that these bacteria played a crucial role in prolonging human life and later he wrote the book, *the prolongation of life* (Morelli, 2000). The term 'probiotic' was given in 1960 by Lilley and Stillwell for the microorganism which stimulates the growth of useful microorganisms (Lilley and Stillwell, 1965). According to the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) (FAO/WHO 2001, 2002) probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host. Bacterial strain extensively used as probiotic microorganisms for humans includes Lactobacillus and *Bifidobacterium (Sanchez et al., 2012).* There are different types of mechanisms are reported that are used by probiotics to beneficially affect the human health such as inhibition of pathogenic bacteria, immunomodulatory effects, induced intestine barrier function against harmful bacteria and increased the metabolic function (Guarner *et al.,* 2012). Several health benefits are put forth by probiotic products including antimicrobial activity and treatment of gastrointestinal infections, acute diarrhea, antibiotic-associated diarrhea, rotaviral diarrhea, improvement in inflammatory bowel diseases, anti-*Helicobacter pylori* activity, lactose intolerance, antimutagenic properties anticarcinogenic properties, reduction in serum cholesterol, immune system modulation (Yan and Polk, 2006). To be used as a probiotic, a bacterial strain should possess some important characteristics includes non-pathogenic nature, acid tolerance, bile tolerance, ability to adhere to the intestine surface and produce antimicrobial substances (FAO/WHO, 2002; Begley, 2005; Sarkar, 2010, Ganguly *et al.,* 2011; Seale and Millar, 2013; Tulumoglu *et al.,* 2013; Anwar *et al.,* 2014). Therefore, a probiotic bacterium must endure the acid and bile salt challenges during the gastrointestinal transit and adhere itself to the intestinal surface to promote the health of the host. The exact mechanism by which probiotic bacteria tolerate the bile salt toxicity and survive in the intestine is still a mystery. However, with the advancement of omics techniques, several proteins and gene networks found to be involved in bile resistance of the probiotic bacteria is unrevealed. In addition, one of the important criteria responsible for the survival and beneficial effect of the probiotic bacteria is adhesion and intestine colonization ability. It prolongs the transit time of probiotics in the gut and consequently increases the beneficial effects. This colonization promotes probiotic action, increases the production of metabolites (Monteagudo-Mera *et al.,* 2019). Thus, in this review, we would like to summarize the current knowledge on the mechanisms used by probiotic bacteria to counteract the effect of bile acids specifically at the molecular level. In addition, we also discussed the different bacterial proteins involved in the adhesion of probiotic bacteria in the gastrointestinal tract.

What are bile tolerance and adherence and why these properties are needed for probiotic bacteria??

Bile tolerance is the ability of the microorganism to resist the bile toxicity in the small intestine and survive in the gastrointestinal tract (GIT) of the host (Simon and Gorbach, 1987; Marteau, 1993). Probiotic bacteria should possess this ability to colonize themselves in the GIT after that only probiotic bacteria could exert their beneficial impacts. Probiotic bacteria or microorganism ingested through feed commence their journey through the mouth and finally reach the intestine and colonize themselves. During this journey, they passed through the stressful acidic condition of the host's stomach (Lankaputhra and Shah, 1995). After successfully passing through the stomach acid, probiotic bacteria reach the small intestine where they come across the bile salts, a good probiotic bacteria must resist the bile salt toxicity because it determines its ability to survive in the small intestine and as a result its ability to play its functional role as a probiotic. Bile is yellow-green in color and present in an aqueous solution form. Its constituents include bile acids, cholesterol, phospholipids, and the pigment biliverdin (Carey *et al.,* 1994). After resisting both the stress conditions probiotic bacteria finally adhere to the small intestine and exert their beneficial effects (Gilliland, 1984, 1987). Therefore, it is very crucial for the survival of probiotic bacteria that they have acid and bile tolerance or resist the acid and bile toxicity and reach alive at the active site of the host's gut. There are several phenotypic and molecular factors which involve in the resistance of probiotic bacteria. Probiotic bacteria use different mechanisms to counteract this harmful effect of bile such as increase activity of multidrug resistance (MDR) transporters to export bile, production of alkaline compounds, more specifically, ammonia, by the arginine deiminase (ADI) pathway and other is increased activity of bile salt hydrolases. In this chapter, we are specifically discussing the bile salt hydrolyses that are involved in bile tolerance. And some adhesion proteins are involved in the colonization of the probiotic bacteria. Adherence to the intestinal mucosa transiently or permanently is also an essential requirement for probiotics to avoid their clearance from the colon and is promoted by the expression of key proteins *Mub* (mucus-binding protein) and *Map-A* (mucus-adhesion promoting protein) present on the surface of probiotics (Ramiah *et al*. 2007). Precedent studies showed the expression of various probiotic factors involved in the bile tolerance and adhesion of bacteria. Such as *Bsh* (bile salt hydrolase) involve in the bile tolerance mechanism, and cell surface proteins of bacteria are involved in the adhesion of bacteria encoded by *Mub* (mucus binding protein), *MapA* (mucous binding protein) gene (Bhat *et al.,* 2019).

The Bile salt hydrolyses:

One of the mechanisms employed by bacteria is the activity of bile salt hydrolases (BSHs) which involve in the bile salt deconjugation and protect the intestinal epithelium from

bile salt toxicity. BSHs belong to the chologlycine hydrolase family of enzymes, and it is observed that the production of BSHs is an adaptation to bile-containing environments (Begley *et al.,* 2005). BSH involve in the deconjugation of glycine and taurine from bile salts, and the corresponding unconjugated acids can be further metabolized by other gut bacteria (De Boever *et al.,* 2000). BSHs have been purified and characterized from various microorganisms *Lactobacillus acidophilus* O16, *L. salivarius* (BSH1), *L. casei* J57 V, *Bifidobacterium, Enterococcus, and Clostridium.* BSHs have generally been found to be located intracellularly, are oxygen insensitive, and have slightly acidic pH optima (usually between pH 5 and 6) Christiaens *et al.,* 1992; Corzo and Gilliland, 1999a & b and Taranto *et al.,* 1999). Likewise, Kaya *et al.* (2017) cloned the *Bsh* gene of *Lactobacillus rhamnosus* E9 in *the E. coli* BLR strain and examined its activity after purification using different bile acids. This gene of *L. rhamnosus* has an ORF of 1014 nucleotides which encoded protein of 37 Kd having 338 amino acids. Several experiments show that BSHs are highly specific, act only on a specific substrate. A study by Moser and Savage (2001) found that L. *buchneri* JCM1069 expressed only taurodeoxycholic acid hydrolase activity but not taurocholic acid hydrolase activity. But some other studies contradicted their results and suggested that BSHs recognize bile acids on both the cholate steroid nucleus and the amino acid groups (glycine/taurine) (McAuliffe *et al.,* 2005). However, the majority of kinetic data available in the literature theorized that substrates are predominantly recognized at the amino acid moieties, so most BSHs are more efficiently acting on glyco-conjugated bile salts than tauro-conjugated bile salts (Coleman and Hudson, 1995; Tanaka *et al.,* 2000; Kim *et al.,* 2005). Further analysis of probiotic strain reveals that many possess more than one '*Bsh'* homolog. The presence of these different *Bsh* homologs should confer some advantage to the strains. Each *Bsh* may respond differently to different bile. Auliffe *et al.* (2005) experimented on *L. plantarum* WCFS1 and *L. acidophilus* NCFM and observed four and two *Bsh* gene homologs respectively in the above strains. The *Bsh* deconjugates the bile acid and release amino acids that could be used as carbon, nitrogen, and energy sources, glycine may be metabolized to ammonia and carbon dioxide, and taurine may be metabolized to ammonia, carbon dioxide, and sulfate.

De Smet *et al.* (1995) analyzed the role of *Bsh* in bile detoxification by mutating the *Bsh* gene in *L. Plantarum* and observed that cells become significantly more sensitive to bile and bile salts. Tsai *et al.* (2014) performed several *in vitro* and *in vivo* experiments and found the ability of LAB to reduce cholesterol levels by increasing *Bsh* gene activity. However, the exact mechanism of Bsh enzyme involves in the resistance of bile tolerance is not known. It has been suggested that Bsh enzyme convert the acidic protonated (non-dissociated) form of bile salts into weaker unconjugated parts and prevent intracellular acidification. This is supported by several

experimental findings showed that BSH-positive cells can tolerate bile salts toxicity by forming the weaker unconjugated counterparts. Hence it is concluded that microbial BSHs function in the detoxification of bile salts which ultimately increases their intestinal survival and persistence of BSH positive strains. Therefore BSH can be declared as a good candidate marker for studying the bile tolerance mechanism. Several experiments were done to monitor *Bsh* gene expression of the probiotic bacteria. In a related study, the expression of the *Bsh* gene after in*vivo* and *invitro* administration of *L. Plantarum* strain Lp0237 and Lp0075 showed the increased expression by six folds (Bron *et al.,* 2004). Duary *et al.* (2011) reported that *Bsh* gene expression was significantly up-regulated by two-fold in two indigenous *Lactobacillus Plantarum* strains during *in vitro* conditions adjusting different bile concentrations and incubation time. But these expression trends were different for both strains. Thus, the expression of *the Bsh* gene was strain and incubation time-dependent. Chandran *et al.* (2013) monitored *Bsh* gene expression during *in vivo* experiment and found 41.6folds up-regulated relative gene expressions when Lp91 was administered to mice over the control. These all studies indicate the importance of *Bsh*gene in the tolerance of bile salts.

Bacterial adhesion property:

Bacterial adhesion knack Colonization in the intestinal tract is the well-analyzed prerequisite character for probiotic bacteria (Ouwehand *et al.,* 1999). Most studies reported on the adhesion of probiotic bacteria to intestinal cell lines (Adlerberth *et al.,* 1996; Dunne *et al.,* 2001). Even though colonization may be a useful indication of adhesion, but it is not always an indication of the ability of the bacteria to adhere to mucus covering the intestinal cells (Ouwehand *et al.,* 1999). The mucus layer serves a dual role; It may stop the adhesion of pathogenic bacteria, and by this means protecting the intestinal cells, and also serves as a nutrient source and matrix for colonization of probiotic bacteria (Nielsen *et al.,* 1994). For the proliferation of probiotic cells in the small intestine, adhesion to the mucosa is a prerequisite. Probiotics use various mechanisms for adhesion to the intestinal cells. Probiotics commonly used a sortase-dependent anchoring mechanism via the LPXTG-motif (leucine, proline, X represents any amino acid, threonine, and glycine). Proteins showing this mechanism have a carboxyl-terminal LPXTG motif, a hydrophobic region, and a tail of charged amino acids. The LPXTG sequence is covalently linked to the peptide cross-bridges of bacterial peptidoglycan (PG) via a membrane-associated sortase enzyme (Paterson and Mitchell, 2004; Ton-That *et al.,* 2004). The Molecular-level study of adhesive proteins in lactobacilli has not received much attention. Most of the research on adhesion focuses principally on phenotypic aspects such as *in vitro* adhesion of potential probiotic cells to adhesion models such as cell lines (Caco-2 and HT-29) or mucus preparations. Adhesion proteins characterized to date include the mucus binding protein (Mub)

(Roos and Jonsson, 2002) mucus adhesion-promoting protein (MapA) (Satoh *et al.,* 2000*)* and some other surface layer proteins (Slp), the aggregation promoting factors (apf1 and apf2) and elongation factor Tu (EF-Tu) (Granato *et al.,* 2004).

Mucus binding protein:

The mucus binding protein (MUB) is a high molecular mass (358 kDa), a cell surface protein isolated and characterized from *L. reuteri* 1063 strain(Roos and Jonsson, 2002) which was isolated from the small intestine of a pig and showed strong adherence to mucus preparations prepared from the small intestine of pigs and hens. Proteinase k treated strain eradicated the adhesion to mucus, signifying that binding was mediated by a proteinaceous substance. Mub isolated from *Lactobacillus* was similar in domain organization, characterized from other adhesions protein of Gram-positive bacteria. Its structure showed two different types of repeats present in 6 to 8 copies responsible for the adherence to intestinal mucus (Roos and Jonsson, 2002). These include an N terminal signal peptide, targeting the protein for secretion & C-terminal sortase recognition site, or anchoring sequences containing the LPXTG domain. Mub is an extraordinary protein due to some of its specific features, such as it is one of the largest bacterial cell surface adhesion proteins identified, and others are *Mub* gene structure which has possibly two translation start sites, and its membrane anchor sequence contains a cysteine residue at the C terminus. It is proposed that mub is a multifunctional protein because it possesses two different types of amino acid residue repeats (MBP-Mub1 and MBP-Mub2) and an N-terminal region of >500 amino acids. Ramiah *et al.,* (2008) monitored the *Mub* gene expression of *L. plantarum* during *an in-vitro* experiment giving different growth conditions by adjusting mucin, bile, and pancreatin in MRS broth and found 80–140-fold up-regulated expression in the presence of mucin, but it was suppressed 7–30-fold under normal gut physiological conditions containing bile and pancreatin. In the related study, the relative expression of *the Mub* gene significantly upregulated up to 8.29 ± 0.33 folds in LP91 strain when MRS broth was supplemented with 0.01% mucin along with 0.3% each of bile and pancreatin (, pH 6.5), (Duary *et al.,* 2011). Similarly, Chandran *et al.,* (2012) reported 12.8 folds increase in expression of *the Mub* gene in mice during *in vivo* study. These reported studies suggest that *Mub*gene plays an important role in the bacterial adhesion.

Mucus adhesion-promoting protein:

Mucus adhesion-promoting protein (MapA) is an oligomeric protein containing two or more polypeptide chains covalently bound to the cell surface of the bacteria (Johansson *et al.,* 1993). MapA was isolated from *L. reuteri* 104R, originally thought to be *L. fermentum* 104R. The method by which this protein anchors to the cell surface is not known yet. MapA has a molecular mass of 26kDa and a theoretical pI of 9.7. MapA showed great similarity with the

collagen-binding protein, CnBP, of *L. reuteri* NCIB 11951 and the bacterial surface protein, BspA, of *L. fermentum.* BspA and CnBP are predictable for their binding potential to type 1 collagen and fibronectin which are mammalian receptors for several pathogens. Miyoshi *et al.,* (2006) observed the binding of purified *MapA*to Caco-2 cell lines. Ramiah *et al.,* (2008) observed the gene expression during *in vitro* experiment and found *MapA* was upregulated 6–8-fold when incubated in the presence of mucin and up to 25-fold when exposed to physiological concentrations of pancreatin and bile compared to MRS grown controls. *MapA* was suggested to aid *L. reuteri* DSMZ 20016 in colonizing mice tissue. The relative expression profile of *the MapA* gene in Lp9 at different conditions showed that it was significantly upregulated to 17.27 ± 1.32 folds when MRS broth was supplemented with 0.01% mucin. (Duary *et al.,* 2011). Chandran *et al*. (2012) observed upregulated *MapA* gene expression only up to 1.3 folds during *in vivo* experiments. These all studies suggest the role of *MapA*gene in the bacterial adhesion. Overall, both the *Mub,* and *MapA*genes could be used as the potential molecular marker to study the adhesion property of the probiotic bacteria.

Conclusion:

Overall, the present review showed the importance of the bile tolerance ability of the probiotic bacteria and factors involved in the adhesion of the probiotic bacteria in GIT. This review shed the light on the molecular mechanism of bile tolerance and adhesion ability of the probiotics and established the relevance of the molecular marker genes of the bacteria.

References:

Adlerberth, I., Ahrne, S. I. V., Johansson, M. L., Molin, G., Hanson, L. A., andWold, A. E. (1996). A mannose-specific adherence mechanism in *Lactobacillus plantarum* conferring binding to the human colonic cell line HT-29. Applied and Environmental Microbiology, 62(7), 2244-2251.

Anwar A.A., Thikra A.A., Saeed A.M. (2014). Adhesion, autoaggregation and hydrophobicity of six *Lactobacillus* strains. British Microbiology Research Journal, 4:381–391

Begley, M., Gahan, C. G., and Hill, C. (2005). The interaction between bacteria and bile. FEMS microbiology reviews, 29(4), 625-651.

Bhat, M. I., Singh, V. K., Sharma, D., Kapila, S., and Kapila, R. (2019). Adherence capability and safety assessment of an indigenous probiotic strain *Lactobacillus rhamnosus* MTCC-5897. Microbial pathogenesis, 130, 120-130.

Bron, P. A., Molenaar, D. O. U. W. E., de Vos, W. M., andKleerebezem, M. I. C. H. I. E. L. (2006). DNA micro-array-based identification of bile-responsive genes in *Lactobacillus plantarum*. Journal of Applied Microbiology, 100(4), 728-738.

Carey, M.C. and Duane, W.C.(1994). Enterohepatic circulation. The liver: biology and pathobiology, 3, pp.719-767.

Chandran, A., Duary, R. K., Grover, S., andBatish, V. K. (2013). Relative expression of bacterial and host specific genes associated with probiotic survival and viability in the mice gut fed with *Lactobacillus plantarum* Lp91. Microbiological research, 168(9), 555-562.

Christiaens, H., Leer, R. J., Pouwels, P. H., andVerstraete, W. (1992). Cloning and expression of a conjugated bile acid hydrolase gene from *Lactobacillus plantarum* by using a direct plate assay. Applied and environmental microbiology, 58(12), 3792-3798.

Coleman, J. P., and Hudson, L. L. (1995). Cloning and characterization of a conjugated bile acid hydrolase gene from *Clostridium perfringens*. Applied and environmental microbiology, 61(7), 2514-2520.

Corzo, G. and Gilliland, S.E. (1999). Bile Salt Hydrolase Activity of Three Strains of *Lactobacillus acidophilus*1. Journal of Dairy Science, 82(3), pp.472- 480.

Corzo, G. and Gilliland, S.E., 1999. Measurement of Bile Salt Hydrolase Activity from *Lactobacillus acidophilus* Based on Disappearance of Conjugated Bile Salts1. Journal of dairy science, 82(3), pp.466-471.

De Boever, P., andVerstraete, W. (1999). Bile salt deconjugation by *Lactobacillus plantarum* 80 and its implication for bacterial toxicity. Journal of Applied Microbiology, 87(3), 345-352.

De Smet, I., Van Hoorde, L., VandeWoestyne, M., Christiaens, H., andVerstraete, W. (1995). Significance of bile salt hydrolytic activities of lactobacilli. Journal of Applied Bacteriology, 79(3), 292-301.

Duary, R. K., Batish, V. K., and Grover, S. (2012). Relative gene expression of bile salt hydrolase and surface proteins in two putative indigenous *Lactobacillus plantarum* strains under in vitro gut conditions. Molecular biology reports, 39(3), 2541-2552.

Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., and Collins, J. K. (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. The American journal of clinical nutrition, 73(2), 386s-392s.

Food and Agriculture Organization of the United Nations/World Health Organization FAO/WHO Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. 2001. Available online: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf.

Food and Agriculture Organization of the United Nations/World Health Organization FAO/WHO (2002). Guidelines for the Evaluation of Probiotics in Food. Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food; London, Ontario, Canada.

Ganguly, N. K., Bhattacharya, S. K., Sesikeran, B., Nair, G. B., Ramakrishna, B. S., Sachdev, H. P. S., and Hemalatha, R. (2011). ICMR-DBT guidelines for evaluation of probiotics in food. The Indian journal of medical research, 134(1), 22.

Gilliland, S. E. (1987). Importance of bile tolerance in lactobacilli used as dietary adjunct. Biotechnology in the feed industry. TP Lyons. Alltech Feed Co, Lexington, KY, 149-155.

Gilliland, S. E., Staley, T. E., and Bush, L. J. (1984). Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. Journal of dairy science, 67(12), 3045-3051.

Granato, D., Bergonzelli, G. E., Pridmore, R. D., Marvin, L., Rouvet, M., andCorthésy-Theulaz, I. E. (2004). Cell surface-associated elongation factor Tu mediates the attachment of *Lactobacillus johnsonii* NCC533 (La1) to human intestinal cells and mucins. Infection and immunity, 72(4), 2160-2169.

Guarner, F., Khan, A. G., Garisch, J., Eliakim, R., Gangl, A., Thomson, A., and Kim, N. (2012). World gastroenterology organisation global guidelines: probiotics and prebiotics october 2011. Journal of clinical gastroenterology, 46(6), 468-481.

Johansson, M. L., Molin, G., Jeppsson, B., Nobaek, S., Ahrne, S., andBengmark, S. (1993). Administration of different *Lactobacillus strains* in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. Applied and environmental microbiology, 59(1), 15-20.

Kaya, Y., Kök, M. Ş., andÖztürk, M. (2017). Molecular cloning, expression and characterization of bile salt hydrolase from *Lactobacillus rhamnosus* E9 strain. Food Biotechnology, 31(2), 128-140.

Kim, G. B., Brochet, M., and Lee, B. H. (2005). Cloning and characterization of a bile salt hydrolase (bsh) from *Bifidobacterium adolescentis*. Biotechnology letters, 27(12), 817-822.

Lankaputra, W. E. V., and Shah, N. P. (1995). Survival of *b. acidophilus and Bi, dobacterium spp* in the presence of acid and bile. Cultured Dairy Products Journal, 30(2), 7.

Lilly, D. M., and Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. Science, 147(3659), 747-748.

Marteau, P. M., Minekus, M., Havenaar, R., and Huis, J. H. J. (1997). Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. Journal of dairy science, 80(6), 1031-1037.

McAuliffe, O., Cano, R. J., andKlaenhammer, T. R. (2005). Genetic analysis of two bile salt hydrolase activities in *Lactobacillus acidophilus* NCFM. Applied and environmental microbiology, 71(8), 4925-4929.

Miyoshi, Y., Okada, S., Uchimura, T., and Satoh, E. (2006). A mucus adhesion promoting protein, MapA, mediates the adhesion of *Lactobacillus reuteri* to Caco-2 human intestinal epithelial cells. Bioscience, Biotechnology, and Biochemistry, 70(7), 1622-1628.

Monteagudo-Mera, A., Rastall, R. A., Gibson, G. R., Charalampopoulos, D., andChatzifragkou, A. (2019). Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Applied microbiology and biotechnology, 103(16), 6463-6472.

Morelli, L. (2000). In vitro selection of probiotic lactobacilli: a critical appraisal. Current Issues in Intestinal Microbiology, 1(2), 59-67.

Moser, S. A., and Savage, D. C. (2001). Bile salt hydrolase activity and resistance to toxicity of conjugated bile salts are unrelated properties in lactobacilli. Applied and Environmental Microbiology, 67(8), 3476-3480.

Nielsen, O. H., Jøorgensen, S., Pedersen, K., andJustesen, T. (1994). Microbiological evaluation of jejunal aspirates and faecal samples after oral administration of bifidobacteria and lactic acid bacteria. Journal of Applied Bacteriology, 76(5), 469-474.

Ouwehand, A. C., Salminen, S., andIsolauri, E. (2002). Probiotics: an overview of beneficial effects. Lactic acid bacteria: genetics, metabolism and applications, 279-289.

Paterson, G. K., and Mitchell, T. J. (2004). The biology of Gram-positive sortase enzymes. Trends in microbiology, 12(2), 89-95.

Ramiah, K., van Reenen, C. A., and Dicks, L. M. (2008). Surface-bound proteins of *Lactobacillus plantarum* 423 that contribute to adhesion of Caco-2 cells and their role in competitive exclusion and displacement of Clostridium sporogenes and Enterococcus faecalis. Research in microbiology, 159(6), 470-475.

Roos, S., and Jonsson, H. (2002). A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus componentsThe GenBank accession number for the sequence reported in this paper is AF120104. Microbiology, 148(2), 433-442.

Ruiz, L., Margolles, A., and Sánchez, B. (2013). Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. Frontiers in microbiology, 4, 396.

Sánchez, B., Ruiz, L., Gueimonde, M., Ruas-Madiedo, P., and Margolles, A. (2012). Toward improving technological and functional properties of probiotics in foods. Trends in Food Science and Technology, 26(1), 56-63.

Sarkar, S. (2010). Approaches for enhancing the viability of probiotics: a review. British Food Journal.

Satoh, E., Leer, R. J., Rojas, M., Conway, P. L., andPouwels, P. H. (2000). The gene encoding the adhesion promoting protein MapA from *Lactobacillus reuteri* 104R is part of an operon

whose expression is controlled by a mechanism of transcription attenuation, involving cysteine. Genebank Accession Number AJ, 293860.

Seale J.V., and Millar M. (2013). Probiotics: A new frontier for infection control. Journal of Hospital Infection, 84:1–4.

Simon, G. L. (1987). Intestinal flora and gastrointestinal function. Physiology of the gastrointestinal tract, 1729-1747.

Tanaka, H., Hashiba, H., Kok, J., andMierau, I. (2000). Bile salt hydrolase of *Bifidobacterium* longum—biochemical and genetic characterization. Applied and environmental microbiology, 66(6), 2502-2512.

Taranto, M. P., Sesma, F., and De Valdez, G. F. (1999). Localization and primary characterization of bile salt hydrolase from *Lactobacillus reuteri*. Biotechnology Letters, 21(11), 935-938.

Ton-That, H., Marraffini, L. A., and Schneewind, O. (2004). Protein sorting to the cell wall envelope of Gram-positive bacteria. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1694(1-3), 269-278.

Tsai, C. C., Lin, P. P., Hsieh, Y. M., Zhang, Z. Y., Wu, H. C., and Huang, C. C. (2014). Cholesterol-lowering potentials of lactic acid bacteria based on bile-salt hydrolase activity and effect of potent strains on cholesterol metabolism in vitro and in vivo. The Scientific World Journal, 2014.

Tulumoglu, S., Yuksekdag, Z. N., Beyatli, Y., Simsek, O., Cinar, B., andYaşar, E. (2013). Probiotic properties of *lactobacilli* species isolated from children's feces. Anaerobe, 24, 36-42.

Yan, F., and Polk, D. B. (2006). Probiotics as functional food in the treatment of diarrhea. Current Opinion in Clinical Nutrition and Metabolic Care, 9(6), 717-721.

EVALUATION OF PRODUCTIVENESS OF SOILS OF CAUVERY DELTA DISTRICTS USING HEBER SOIL QUALITY INDEX

Shanmuganathan M[1] and Rajendran A[2*]

[1]Department of Chemistry, Panimalar Engineering College, Chennai, Tamilnadu, India

[2]Department of Chemistry, Sir Theagaraya College, Chennai, Tamilnadu, India

*Corresponding author E-mail: annamalai_rajendran2000@yahoo.com

Abstract:

Soil quality plays an important role in the assessment of sustainable land-use systems. Assessment of soil quality will always help farmers to apply correct fertilizers in correct proportions to their soil. This will definitely help them achieve maximum yield with minimum expenditure. Not only that, they can conserve their immediate environment. In the current investigation, an effort has been made to utilize Heber Soil Quality Index (HSQI) to examine the appropriateness of a soil of Thanjavur and Thiruvarur districts, Tamilnadu, India for the vegetation of rice and sugarcane as these are the major cash crops being cultivated. The overall HSQI values of all samples ranged from 71.76 – 79.9 divulging an information that the quality of soils inspected in this task are good for the effective farming of sugarcane and rice. Even though more than thirty-five parameters are available, only twelve parameters were taken into consideration for designing the HSQI as those parameters are interrelated one way or other. Hence, it is a time saving method and gives lot information about the inherent nature of the soil with special reference to the plantation of sugarcane and rice. The main objective of this paper is to enlighten the researchers to frame one such index for other cash crops using minimal indicators.

Keywords: Heber soil quality index, rice, sugarcane, potassium, nitrogen, soil texture

Introduction:

In considering soil quality, attempts have been made to examine the factors that indicate good soil health or soil quality, to reach consensus on the definition, upon the key soil attributes that translate into variables to be examined, on their data value ranges, their value limits, threshold values, comparability and to aggregate or integrate the variables/values in such a way as to develop meaningful indices that characterize the quality/health of varying soils in various world regions, across nations, or in local areas, and at the farm level. The creation of soil quality

models is a difficult task even for specialists, due to high number of variables that are normally considered (Evenson et al. 2006). Furthermore, models are often difficult for farmers to understand (Dorfman and Karali 2009). Therefore, the development of simple models with a few variables can facilitate understanding and respond to local farmer's needs. But, to generate these models, simple methods and indices of soil quality assessment are needed. Soil quality plays an important role in the assessment of sustainable land-use systems (Klemens et al. 2003; Sepp et al. 2005). Research supervisors and scholarsof Bishop Heber College, India have devised a Soil Quality Index which is popularly known as Heber Soil Quality Index (HSQI).Commonly to assess the fertility of a soil with reference to the vegetation of sugarcane and rice, as many as thirty-five parameters have to be considered. But the determination of the quantity of all these indicators is a time consuming one. As these thirty-five parameters are interrelated in one way or other, twelve factors are considered more than sufficient to assess the fertility of a soil. Taking this as an advantage, HSQI was formulated. Herein, an effort has been attempted to use the HSQI to assess the suitability of a soil of a chosen area for better farming of rice and sugarcane. The twelve factorstaken into consideration werepH, available phosphorus (kg/ha), available nitrogen (kg/ha), available potassium (kg/ha), water holding capacity (WHC) (%),soil organic matter (SOM) (%),electrical conductance (EC) (mmho/cm), texture, bacterial content (SPC/g), total hardness (mg/L), chloride (mg/L), and bulk density (g/cm^3) (Gugan et al.2001; Muthuvel et al. 1992).

Experimental:

Study area:

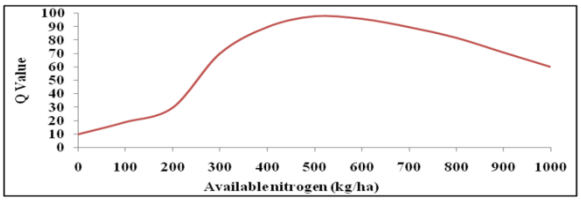
Thanjavur district lies between 9º 50' and 11º 25' North latitude and 78º 45' and 79º 25' East longitude. The geological formation of the major area of Thanjavur district was found to contain Alluvial and Tertiary deposits. Soils in these areas are found to be with red, black and brown colors. Rice and sugarcane are the major crops being cultivated in these areas. Thanjavur is designated as the rice bowl of Tamilnadu.

Thiruvarur is located between 10 °20' and 11 °07' Latitude (N-S) and between 79 °15' and 79 ° 45' Longitude (E-W). Sample were taken from fifteen places of in and around Thanjavur and Thiruvarur district such as Oothukkadu,Paappakkudi, Perungudi, Poonthottam, Pulavarnatham, Puliyakkudi, Rajendiranallur, Saaranatham, Vaniyankarambai, Thenkuvalavelei, Arithuvaramangalam, Chandrasekarapuram, Uthamanathapuram, Veeranamand Velangudi.

Sampling method:

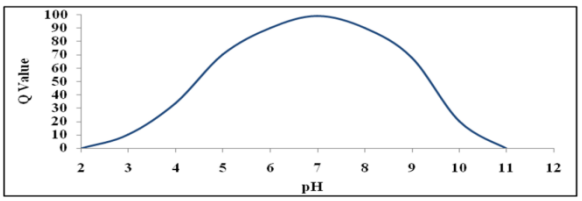
Soil samples were collected from the aforementioned area. Initially, the bulk or junk part of the soil was taken out and thrown off from each sampling site. Using a spade, Soil samples were collected from the four corners and middle place (at least 15 cm) from five places of each sampling area. Five sub samples collected from each sampling area was thoroughly mixed and from the mixture, 1Kg of the composite sample was taken for lab analysis. These composite samples were thoroughly cleaned to ensure that they contain no strange materialssuch as pebbles, stones and roots. The unruffled soil of each sampling site was taken in a dirt-free cloth bag and labeled with the required details. Before, the samples were subjected to lab analyses for the above said twelve parameters (Table 1), the composite soil samples of each area were crushed using timberhammer and separated to attain soil units of 2-mm dimension.

To frame the HSQI table, statistical results were gathered from agricultural scientists and other eminent of this research zone. They were advised to: (i) assemble the chosen twelve factors in their order of merit, (ii) award scoring on a 10 - point balance with '0' showing the lowest rating and '10' the highest, (iii) allot weighting curve value (Q -Value) (Table 1) and (iv) sketch the diagram for each factoras per their permissible and tolerance limits. 'Q' values of each sample were calculated from Figure 1-12.



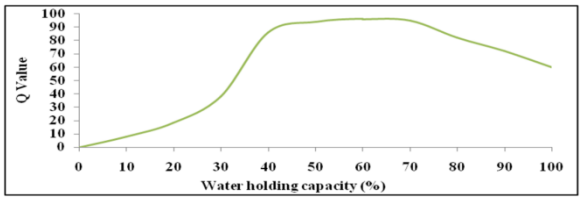
If Available 'N' > 1000; Q = 60

Figure 1: Std 'Q' graph for Available 'N'



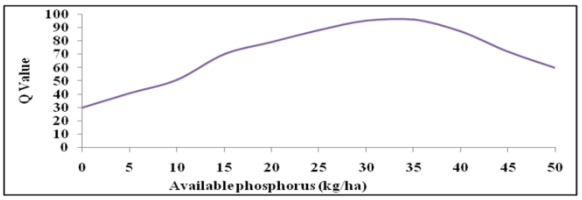
If pH > 11; Q = 0

Figure 2: Std 'Q' graph for pH



If WHC > 100; Q = 60

Figure 3: Std 'Q' graph for WHC



If Available 'P' > 50; Q = 60

Figure 4: Std 'Q' graph for Available 'P'

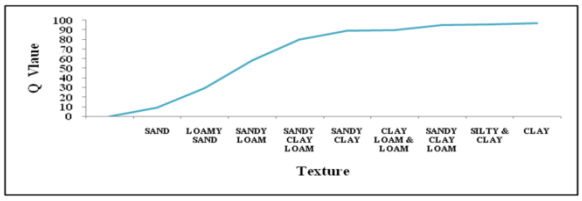
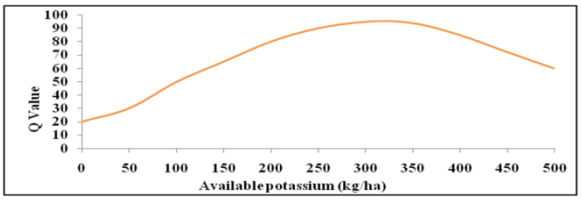
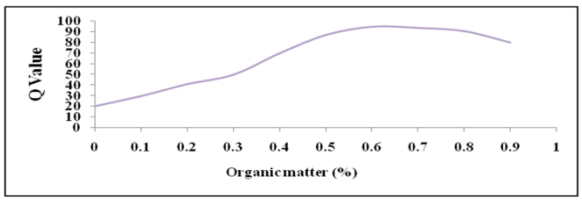


Figure 5: Std 'Q' graph for Texture



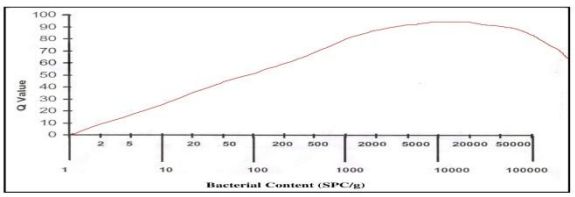
If Available 'K'> 500; Q = 60

Figure 6: Std 'Q' graph for Available 'K'



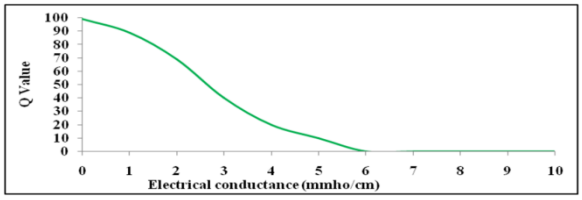
If OM > 1; Q = 70

Figure 7: Std 'Q' graph for OM



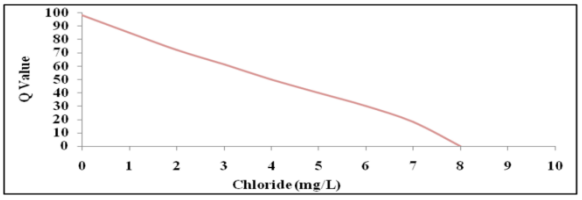
If BC > 10^5; Q = 60

Figure 8: Std 'Q' graph for BC



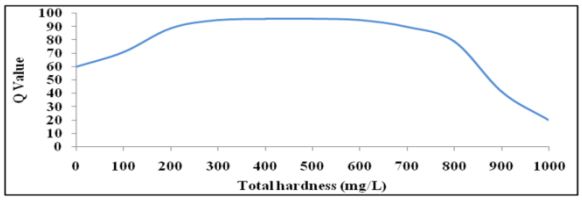
If EC > 6; Q = 0

Figure 9: Std 'Q' graph for EC



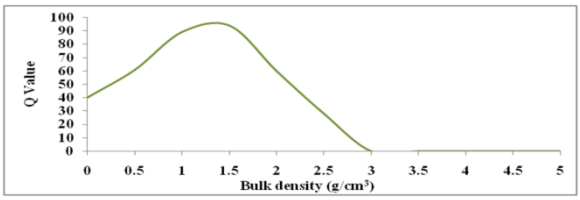
If Cl^-> 8; Q = 0

Figure 10: Std 'Q' graph for Cl^-



If TH > 1000; Q = 20

Figure 11: Std 'Q' graph for TH



If BD > 3; Q = 0

Figure 12: Std Q graph for BD

Table 1: Methods of determination, optimum range and weighting factor various Parameters

Parameter	Methods	References	Optimum Range	References	Weighting Factor
Physical-WHC (%)	Weight loss method	Soil testing procedure manual, 2008	40 – 55	Majumdar 2000	0.093
Texture	International pipette method	Singh *et al.*,1999	Clay and Clay loam	Thiyagarajan and Kalaiyarasi 2011	0.089
Chemical-Available 'N' (Kg ha^{-1})	Alkaline permanganate method	Subbiah and Asija, 1956	>328	Yadav *et al.*, 1998	0.095
pH	Electrometric method	Davis and Freitas 1970; Singh *et al.*,1999	4.5 – 8.0	Thiyagarajan and Kalaiyarasi 2011	0.095
Available 'P' (Kg ha^{-1})	Olsen's method	Olsen and Sommers, 1982; Davis and Freitas, 1970	> 30	Yadav *et al.*, 1998	0.090
Available 'K' (Kg ha^{-1})	Flame photometer method	Ghose and Bajaj, 1993; Knudsen and Peterson, 1982; Somawanshi *et al.*,1994	>305	Yadav *et al.*,1998	0.087
OM (%)	Walkley and Black method	Page *et al.*,1982	0.34 - 0.95	Mandal *et al.*,2013	0.084
EC (mmho/cm)	Digital conductometric method	Davis and Freitas, 1970; Singh *et al.*,1999	< 1	Dahnke and Whitney 1988; Smith and Doran 1996	0.076
Cl^- (mg L^{-1})	Titrimetric method	Davis and Freitas, 1970; Jackson, 1967	< 4	Horneck *et al.*,2011	0.075
TH (mg L^{-1})	Titrimetric method	Nelson, 1982	< 1.5	Tucker 1984; Wurts and Durborow 1992	0.070
BD (g cm^{-3})	Clod Method	Kadam and Shinde, 2005	1.23 – 1.5	Mandal *et al.*, 2013	0.069
Bilogical-BC (SPC g^{-1})	Standard plate count method	-	10^8 – 10^9	Hoorman and Islam 2010	0.082

Table 2: Soil properties of various locations of Oothukkadu, Paappakkudi, Perungudi, Poonthottam and Pulavarnatham

Parameter	Weighting Factor	Oothukkadu			Paappakkudi			Perungudi			Poonthottam			Pulavarnatham		
		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI	
			'Q' Value	Total		'Q' Value	Total		'Q' Value	Total		'Q' Value	Total		'Q' Value	Total
Physical-WHC (%)	0.093	43.76	92	8.56	46.24	93	8.65	45.38	92	8.56	47.65	94	8.74	43.81	92	8.56
Texture	0.089	SC	58	5.16	CL	92	8.19	SCL	94	8.37	CL	92	8.19	CL	92	8.19
Chemical-Available 'N' (Kg ha^{-1})	0.095	126	22	2.09	150.5	24	2.28	196	30	2.85	196	30	2.85	238	44	4.18
pH	0.095	7	99	9.41	8	90	8.55	7.5	96	9.12	6.4	96	9.12	7	99	9.41
Available 'P' (Kg ha^{-1})	0.090	73.25	60	5.40	235.75	60	5.40	32.5	96	8.64	32.5	96	8.64	187	60	5.40
Available 'K' (Kg ha^{-1})	0.087	150	65	5.66	262.5	92	8.00	112.5	56	4.87	112.5	56	4.87	275	92	8.00
OM (%)	0.084	0.87	84	7.06	0.83	88	7.39	0.71	94	7.90	0.74	94	7.90	0.57	94	7.90
EC (mmho/cm)	0.076	0.14	96	7.30	0.44	94	7.14	0.10	99	7.52	0.10	99	7.52	0.12	99	7.52
Cl^- (mg L^{-1})	0.075	2.2	70	5.25	2.6	66	4.95	3.4	56	4.20	1.2	82	6.15	2.8	63	4.73
TH (mg L^{-1})	0.070	41	64	4.48	49	66	4.62	37	63	4.41	29	63	4.41	57	66	4.62
BD (g cm^{-3})	0.069	1.27	94	6.49	1.24	94	6.49	1.16	94	6.49	1.34	94	6.49	1.35	94	6.49
Biological-BC (SPC g^{-1})	0.082	$15x10^4$	60	4.92	$17 x10^4$	60	4.92	$4 x10^6$	60	4.92	$1x10^6$	60	4.92	$16x10^5$	60	4.92
Total HSQI				71.76			76.58			77.84			79.80			79.90

Table 3: Soil properties of various locations of Puliyakkudi, Rajendiranallur, Saaranatham, Vaniyankarambai and Thenkuvalavelei

Parameter	Weighting Factor	Puliyakkudi			Rajendiranallur			Saaranatham			Vaniyankarambai			Thenkuvalavelei		
		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI	
			'Q' Value	Total		'Q' Value	Total		'Q' Value	Total		'Q' Value	Total		'Q' Value	Total
Physical-WHC (%)	0.093	48.25	94	8.74	46.3	92	8.56	44.38	92	8.56	45.2	92	8.56	46.81	92	8.56
Texture	0.089	CL	92	8.19	CL	92	8.19	CL	92	8.19	CL	92	8.19	CL	92	8.19
Chemical-Available 'N' (Kg ha^{-1})	0.095	203	38	3.61	154	24	2.28	146.25	24	2.28	210	36	3.42	178.5	26	2.47
pH	0.095	7.8	94	8.93	7.3	98	9.31	8	90	8.55	7.3	98	9.31	7.5	98	9.31
Available 'P' (Kg ha^{-1})	0.090	113.75	60	5.40	243.75	60	5.40	92	60	5.40	75	60	5.40	162.5	60	5.40
Available 'K' (Kg ha^{-1})	0.087	912.5	60	5.22	225	84	7.31	237.5	88	7.66	612.5	60	5.22	387.5	87	7.57
OM (%)	0.084	0.47	84	7.06	0.53	92	7.73	0.52	90	7.56	0.55	92	7.73	0.44	78	6.55
EC (mmho/cm)	0.076	0.91	90	6.84	0.65	92	6.99	0.27	96	7.30	0.92	92	6.99	0.58	94	7.14
Cl^- (mg L^{-1})	0.075	2.8	63	4.73	3.6	54	4.05	1.4	80	6.00	18.8	0	0.00	2.4	68	5.10
TH (mg L^{-1})	0.070	73	68	4.76	65	68	4.76	37	64	4.48	253	93	6.51	53	64	4.48
BD (g cm^{-3})	0.069	1.37	94	6.49	1.34	94	6.49	1.41	94	6.49	1.29	94	6.49	1.31	94	6.49
Biological-BC (SPC g^{-1})	0.082	$18x10^5$	60	4.92	$28x10^5$	60	4.92	$27x10^5$	60	4.92	$35x10^4$	60	4.92	$53x10^4$	60	4.92
Total HSQI				74.88			75.98			77.37			72.73			76.18

Table 4: Soil properties of various locations ofArithuvaramangalam, Chandrasekarapuram, Uthamanathapuram, Veeranam and Velangudi

Parameter	Weighting Factor	Arithuvaramangalam			Chandrasekarapuram			Uthamanathapuram			Veeranam			Velangudi		
		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI		Test Result	HSQI	
			'Q' Value	Total		'Q' Value	Total		'Q' Value	Total		'Q' Value	Total		'Q' Value	Total
Physical-WHC (%)	0.093	49.42	94	8.74	47.32	93	8.65	47.41	93	8.65	45.83	92	8.56	47.18	93	8.65
Texture	0.089	CL	92	8.19	CL	92	8.19	CL	92	8.19	CL	92	8.19	SCL	95	8.46
Chemical-Available 'N' (Kg ha^{-1})	0.095	192.5	30	2.85	221	36	3.42	154	24	2.28	203	32	3.04	181.5	28	2.66
pH	0.095	7.5	98	9.31	7.7	94	8.93	7	99	9.41	7.9	92	8.74	7.4	98	9.31
Available 'P' (Kg ha^{-1})	0.090	203.25	60	5.40	260	60	5.40	406.25	60	5.40	325	60	5.40	250.8	60	5.40
Available 'K' (Kg ha^{-1})	0.087	400	85	7.40	562.5	60	5.22	212.5	84	7.31	106.25	54	4.70	252.45	90	7.83
OM (%)	0.084	0.66	95	7.98	0.68	94	7.90	0.83	88	7.39	0.75	93	7.81	0.76	93	7.81
EC (mmho/cm)	0.076	0.56	96	7.30	0.17	97	7.37	0.43	96	7.30	0.19	97	7.37	0.24	95	7.22
Cl^- (mg L^{-1})	0.075	2.6	65	4.88	2.6	65	4.88	2	72	5.40	2.6	66	4.95	1.4	80	6.00
TH (mg L^{-1})	0.070	49	65	4.55	73	68	4.76	53	66	4.62	41	64	4.48	72	68	4.76
BD (g cm^{-3})	0.069	1.31	94	6.49	1.34	94	6.49	1.26	94	6.49	1.41	94	6.49	1.29	94	6.49
Biological-BC (SPC g^{-1})	0.082	$53x10^4$	60	4.92	$36x10^4$	60	4.92	$17x10^5$	60	4.92	$32x10^4$	60	4.92	$3x10^6$	60	4.92
Total HSQI				77.99			76.12			77.34			74.64			79.50

Results and Discussion:

Test results of samples are given in the tables 2 – 4. The total HSQI values of all samples are found in the range of 71.76 – 79.9, which indicates that the fertile nature of samples examined in this work is good for the effective farming of sugarcane and rice.Among the various samples investigated, sample 5 (Pulavarnatham) was found to have high total HSQI value, 79.9 (Table 2). For this sample, the test results of the parameter such as pH (7), water holding capacity (43.81 %), texture (clay loam), available 'K' (275 kg/ha) and organic matter (0.57 %) are found to be excellent in good agreement with the best possible values required for the best vegetation of sugarcane and rice (Table 1). The HSQI values of WHC, pH, available potassium texture, and organic matter were found to be extremely good with 8.56, 9.41, 8.00, 8.19, and 7.90 respectively. The parameters such as available phosphorus (187 kg/ha), electrical conductance (0.12 mmho/cm) and bulk density (1.35 g/cm^3) contribute appreciably to the fertility of this soil sample with the HSQI values of 5.40, 7.52 and 6.49 respectively. Available nitrogen, bacterial content, chloride and total hardness do not significantly contribute to the quality of this sample.

The sample 1 (Oothukkadu) registered with the low HSQI, 71.76 (Table 2) which suggests that this sample is also rated good for the vegetation of rice and sugarcane. The pH (7), WHC (43.76 %), organic matter (0.87 %), electrical conductance (0.14 mmho/cm) and bulk density (1.27 g/cm^3) were found to be good as per the most favorable value required for the best plantation of sugarcane and rice. The HSQI values of pH, WHC, organic matter, EC and bulk density were found to have 9.41, 8.56, 7.06, 7.30 and 6.49 respectively. The parameters such as available phosphorus (73.25 kg/ha), texture (sandy clay loam), available 'K' (150 kg/ha) and chloride (2.2 mg/L) contribute significantly to the fertility of this soil with HSQI values of 5.40, 5.16, 5.66 and 5.25 respectively. The test results of available 'N', total hardness and bacterial content and do not contribute much to the quality of the soil. This reveals that this soil sample has low contents of available 'N', available 'P' and available 'K'.

Nitrogen is the most important macro nutrient for plant growth. Nitrogen is available for plants in the form of nitrate and ammonium ions. Deficiency of nitrogen in plants may cause stunted growth because of reduction in cell division. Depending on the severity of deficiency, the plant disease called chlorosis could result in the death or dropping of the older leaves. This is caused by the translocation of nitrogen from the older to the younger tissues. Reduced nitrogen lowers the protein content of seeds and vegetative parts. In severe cases, flowering is greatly reduced (Silva and Uchida 2000). Excess of nitrogen in soil can cause excess vegetative growth, dark green leaves, lodging, maturity is delayed which increases susceptibility to pest and disease. The availability of nitrogen in soil depends upon soil pH and soil texture. Nitrification occurs to be estimated between pH 6.5 to 8.8. The nitrates leaching ismore rapidly in sandy soils because sandy soils have a lower WHC. The organic matter content of sandy soils is usually lower than those of finer-textured soils. Soil organic matter acts as a slowly available source of nitrogen. The best favorable quantity of the nitrogen for

the better farming of rice and sugarcane is >328 kg/ha (Table 1). Available 'N' of the all soils ranged from 126 – 238 kg/ha indicating a fact that all the samples inspected in this study severely suffer from nitrogen shortfall. All the soil samples are reported as clay loam, rate of leaching is low all the nitrogen makes available for plant intake. pH range of all samples lie in the range of 6.4 – 8, the rate of nitrification is high. Soil organic matterof all soil samples is in the range of 0.44 – 0.87 %, indicates that low available nitrogen in samples.

pH is employed as animportantpointer of the accessibility of other nutrients in the soil.Availability of potassium and phosphorus becomes problematicat pH less than 6 and also the availability of aluminium and manganesebecomes decreased at pH less than 4. If pH is more than 7 most of micro nutrients are unavailable. If pH is between 6 and 7 then the soil is good for plant growth. Adjusting the pH will make these nutrients accessible by plants. SOM can typically buffer plants against the effect of acidity in order that a soil with a lower pH vary can still with success grow plants (Brady and Weil, 1996; Silva and Uchida, 2000). pH of all the tested soil soilsof this examination ranged from 6.4 - 8, which infers that these samples is moderately acidic to moderately alkaline. The range of pH suggested by experts for the better procurement of sugarcane and rice is 4.5 – 8.0 (Table 1). Soil samples2 and 8 (Paappakkudi and Saaranatham) registered high pH (8), which indicates that this sample is basic (Table 2 and 3) and sample 4 (Poonthottam) presented low pH (6.4), which shows that this soil is acidic (Table 1). Other samples showed intermediate pH values.

The amount of water retained by the soil is called as water holding capacity. Water movement is principally in a very descending way by attractive force pull within the giant whole area with restricted sideward and rising movement by capillarity within the little pore area (Duiker and Curran, 2004). Organic matter, that holds 10 times a lot of water than sand, considerably improves the WHC of sandy soils. As a degree of amplification, plants on sandy soils don't use additional water than plants on clayey soils. With the restricted WHC, sandy soils merely would like lighter and a lot of repeated irrigations than clayey soils (Brady and Weil 1996). Water without delay moves below the development zone once an excessive amount of is applied at a time. Clayey Soils have little pore area, presenting it high WHC. However, the shortage of enormous hole area deeply confines water movement. Water is sluggish to penetrate into clayey soil, typically resulting in surface run-off issues. Soil texture according in samples as clay dirt, that shows that each one samples have high water holding capability (Doran *et al.*, 1994). Soil organic matter of tested samples lied in the range of 0.44 – 0.87 % which is responsible for high water holding capacity. WHC of all the soils chosen in this task was found to be between 43.76 and 49.42 %. As per the suggestions of the eminents in this area of research, soils with WHC in the range of 40 – 55 % (Table 1) is fine for the plantation of rice and sugarcane form which better yield is expected. Sample 11 (Arithuvaramangalam) and sample 1 (Oothukkadu) recorded high (49.42 %) (Table 4) and low (43.76 %) (Table 2) water holding capacity values respectively.

Phosphorus is available in plants in the form of orthophosphate ions. It plays a major role in photosynthesis and respiration. It is also important for energy storage and transfer as ADP and ATP(adenosine di- and triphosphate) and DPN and TPN (di- and triphosphopyridine nucleotide). It is a part of the RNA and DNA structures, which are the major components of genetic information. Phosphorusaids in root development, flower initiation, and seed and fruit development. 'P' has been shown to reduce disease incidence in some plants and has been found to improve the quality of crops. It is needed in large quantities during the early stages of cell division; the initial overall symptom is slow, weak, and stunted growths(Brady and Weil 2002). Under severe deficiency, purpling of leaves and stems might seem. Lack of phosphorus will cause delayed maturity and poor seed and fruit development. Phosphorus (P), is directly suffering from soil pH. At pH larger than 7.5, phosphate ions tend to react quickly with Ca (Ca) and magnesium (Mg) to make less soluble compounds. At acidic pH, phosphate ions react with aluminum (Al) and iron (Fe) to again form less soluble compounds. The organic matter (OM) in soil may account for anywhere from 3% to 75% of the total P in a soil. Usually, increased SOM results in higher fixation of iron and Aluminium. This in turnresults in less 'P' fixation by these elements, and more movable (available) P. Generally, low ion exchange capability (CEC) of soils needs higher soil phosphorus tests to provide equivalent quanta of phosphorus to a crop (Brady and Weil, 2008).The best possible amount of available 'P' for better yield of sugarcane and rice is >30 kg/ha (Table 1). Available 'P' of the soils investigated in this work ranged from 32.5 – 406.25 kg/ha which reveals that all soils have surplus phosphorus. All samples have the pH in the range of 6.4 – 8, at this pH availability of phosphorus is high.

Soil texture refers to the size and percentage of the mineral substances in the soil. Explicitly, it refers to the relative proportions of silt, clay and sand (Lipiec and Hatano, 2003). Based on the percentage of each type of mineral, soils can be classified into four groups namely, silty soils, sandy soils, loamy soils and clay soils It allows faster penetrability of water than clays as their grain size is larger (Brady and Weil, 2002, 2008). The imperfection in clay, sandy and silty soils can be rectified by improving the soil structure and adjusting the pH, as essential.Organic matter breaks down quicker in sandy soils than in fine-textured soils, given similar environmental conditions, tillage and fertility management, attributable to a better amount of oxygen available for decomposition within the light-textured sandy soils. The ion exchange capability of the soil will increase with % clay and organic matter and therefore the pH buffering capability of a soil (its ability to resist hydrogen ion concentration amendment upon lime addition), is additionally largely supported clay and organic matter content (Brady and Weil, 2008). Soil texture of soils reported as clay loam which supports high WHC and bulk density values.

Potassium is available in soil in the form of potassium ion for uptake. It is vital for plant growth because it is known to be an enzyme activator that promotes metabolism. It assists in regulating the plants use of water by controlling the opening and closing of leaf stomata. In photosynthesis, it has the role of maintaining the balance of electrical charges at the site of ATP

production. It also promotes the translocation of photosynthates (sugars) for plant growth or storage in fruits or roots. It also involved ATP production and protein synthesis. It has been shown to improve disease resistance in plants, improve the size of grains and seeds, and improve the quality of fruits and vegetables. The most common symptom of potassium deficiency is chlorosis along the edges of leaves (leaf margin scorching) (Hodges, 1995). This occurs first in older leaves, because potassium is very mobile in the plant. Deficiency affects plant growth makes slow. Due to deficiency the stem are weak and lodging. The size of seeds and fruits and the quantity of their production also reduced. Plants imbibe 'K' in the form of K^+ from the soil. They may takeK^+ that is adsorbed onto exchange sites (exchangeable K) or 'K' that is dissolved in the soil water. As a result of this, in dry farms, transferable 'K' tends to be more significant than dissolved K. Because 'K' dissolves instantly, it's extremely portable within the soil. However, it will get at bay between covers of increasing clays. 'K' tends to stay in ionic form among cells and tissues (Bar-Tal *et al.*, 1991).The optimum value of available 'K', for the better vegetation of sugarcane and rice is > 280 kg/ha (Table 1). The samples registered potassium in the range of 106.25– 912.5 kg/ha. The sample 6 (Puliyakkudi) recorded high (912.5 kg/ha) (Table 3) and sample 14 (Veeranam) showed low (106.25 kg/ha) (Table 4) values of available potassium.

SOM is the lifeblood of fertile, fruitful soil. Lack of SOM, agricultural procurement is not sustainable. Beneficial microorganisms like bacteria and earthworms decompose organic matters present in the soil system and convert them into humus (SOM). The process of degradationdischarges nutrients which can be absorbed by plant roots (Wuddivira *et al.,* 2009). The ultimateartifact of decomposition is humus, a black flakysubstance reluctant toundergo further degradation. A multipart chemical material, humus stores plant nutrients, retains humidity and progresses soil structure. The strength of soil structure is associated to the concentration of SOM at the surface, not the whole quantity present in the soil (Nelson and Sommers, 1996). The most favorable range of SOM for the better yield of rice and sugarcane is 0.6 – 0.8 % (Table 1). All the samples of this task were found to have SOM in the range of 0.44 – 0.87 %. The sample 1 (Oothukkadu) showed high (0.87%) (Table 2) and sample 10 (Thenkuvalavelei) presented low (0.44%) (Table 3) SOM. pH of samples lied in the range of 6.4 – 8and the activity of microorganisms make high value of organic matter. Soil texture is reported as clay loam, which is also the reason for high value of organic matter. The bacterial count of the soils of this task ranged from 150000 – 4000000 SPC/g, which causes decomposition to occur rapidly thereby giving high value of organic matter.

Bacteria are unicellular and ultra-microscopic organisms. Some of them are useful to man in one way or the other, while others are very dangerous as they are the basisof various plant and animal illness.Good bacteria help in decomposition of dead SOM of plants and animals and convert them into highly useful humus by the secretion of enzymes (Alegre *et al.,*, 1996). Therefore, these bacteria not exclusively degrade the SOM however also flush out the harmful wastes from the globe and henceserve as nature's scavengers (Day and Bassuk, 1994). A biologically active soil is a healthy soil

should expect a faster decomposition of organic matter (Watson and Kelsey, 2006). Anything that affects the microbes and different living organisms within the soil, as a result, can have an effect on the rate of organic matter break down. A low pH scale indicates an acidic soil, and this may have a significant impact on the decomposition of organic matter. bacteria is most accountable for breaking down organic matter and experience a sharp drop-off in activity once the pH scale drops below 6 (Magdoff and Startsev, 2009). The bacterial content of the soil samples of this study ranged from 150000 – 4000000 SPC/g. The recommended optimal range of bacterial content for a good soil is. 10^7 – 10^8 numbers per gram soil (Table 1). The sample 3 (Perungudi) had high (4000000) (Table 2) and sample 1 (Oothukkadu) showed low (150000) (Table 2) bacterial content values. All other tested soil samples had moderate soil bacteria content. Soil organic matter lied in the range of 0.44 – 0.87 % shows that microorganisms have enough food so that the rate of decomposition is high.

Soil electrical conductivity (EC) is that the ability of soil to conduct electrical current. EC is expressed in (mS/m). Traditionally, soil scientists used EC to measure soil salinity. However, EC measurements even have the potential for estimating variation in a number of the soil physical properties in a field wherever soil salinity is not a problem. Greater the soil porosity, the more easily electricity is conducted (Jung *et al.,* 2005). Soil with high clay content has higher porosity than sandier soil. Compaction normally increases soil EC. Dry soil is much lower in conductivity than moist soil. Increasing concentration of electrolytes (salts) in soil water will dramatically increase soil EC (Fargo *et al.,* 1977). Mineral soil containing high levels of organic matter (humus) and/or 2:1 clay mineral such as montmorilloniteor vermiculite have a much higher ability to retain positively charged ions than soil lacking these constituents(Farahani *et al.,* 2005). The presence of those ions within the wet stuffed soil pores can enhance soil EC within the same way that salinity wills (Devis and Freitas, 1970). As temperature decreases toward the freezing point of water, soil EC decreases slightly (Adviento-Borbe *et al.,* 2006). Below the freezing point soil pores become progressively insulated from one another and overall soil EC declines quickly. EC of the soilsexamined in this area is determined to be in the range of 0.10 – 0.92 mmho/cm. The most required value ofEC recommended by agricultural scientists is < 1 mmho/cm (Table 1). Sample 9 (Vaniyankarambai) recorded high EC value (0.92 mmho/cm) (Table 3) and the samples 3 and 4 (Perungudi and Poonthottam) had low value of electrical conductance (0.10 mmho/cm) (Table 2 and 2). All other tested samples were found to have good electrical conductivity. Soil texture is reported as clay loam, which also supports the high value of electrical conductivity.

Chlorine in the form of Cl^- is essential in photosynthesis, where it is involved in the evolution of oxygen. It also increases cell osmotic pressure and the water content of plant tissues. It reduces the severity of certain fungal diseases (Engel *et al.,* 1997). Deficiency of chlorine occurs in drooping, followed by chlorosis, unduedivisions of lateral roots, bronzing of leaves, chlorosis and necrosis in tomatoes and barley(Heckman 1998). Chloride content of the samples was estimated to be in the range of 1.2 – 18.8 mg/L. The most required level of chloride content for the better cultivation of

sugarcane and riceis< 4mg/L (Table 1). Sample 9 (Vaniyankarambai) tested high value of chloride (18.8 mg/L) (Table 3) and the sample 4 (Poonthottam) showed low (1.2 mg/L) (Table 2) Cl^- content.

Soil alkalinity or salinity could be a condition that results from the accumulation of soluble salts in soil. Alkali conditions are caused primarily by a high concentration of sodium carbonate. The injuries caused by alkaline conditions are additional extravagance than those caused by salinity and include the following: (i) acute impact of the Na^+ in flouting down the soil construction, (ii) poisonous nature of the CO_3^{2-}, (iii) condensed uptake of Ca.The total hardness of the inspectedsoils ranged from 29 - 253 mg/L. The suggested optimum quantity of total hardness is <1.5 mg/L (Table 1). The sample 9 (Vaniyankarambai) had high total hardness (253 mg/L) (Table 3) and sample 4 (Poonthottam) presented low value (28 mg/L) (Table 2). Almost all samples were found with extreme hardness which is an indicator for poor yield.

The oven dry weight of a unit volume of soil comprehensive of pore areas is named bulk density. The bulk density of the soil may be a manifestation of the quantity of holegap within the soil. The bulk density of a soil is usually smaller than its particle density. Other factors impacting the bulk density are they types of natural resources present, the texture and the quantum of SOM. The bulk density of sandy soil is about 1.6 g / cm^3, whereas that of organic matter is about 0.5. Bulk density normally decreases, as mineral soils become finer in texture (Grossman and Reinsch, 2002). The bulk density varies indirectly with the total pore space present in the soil and gives a good estimate of the porosity of the soil. Bulk densities of sandy soil have 1.6 g / cm^3, loam soils 1.4 g / cm^3, silt loam soils 1.3 g / cm^3,Clay 1.1 g / cm^3. Fine textured soils such as silt loams, clays and clay loams generally have lower bulk densities than sandy soilsisdue the fine textured soils are likely to adequate organic matter content, high pore space and low bulk density. However, in sandy soils, organic matter content is generally low. More the organic matter content in soil results in high pore space thereby showing lower bulk density of soil and vice –versa (Campbell and Henshall, 1991). Bulk density of the soilsexamined in this workwas found to have the ranged from 1.16 – 1.41 g/cm^3. The best possible range of bulk density values for the effectivevegetation of sugarcane and rice is 1.23 – 1.50 g/cm^3 (Table 1). Samples8 and 14 (Saaranatham and Veeranam) showed very high bulk density value (1.41 g/cm^3) (Table 3and 4) and sample 3 (Perungudi) registered low value (1.16 mg/cm^3) (Table 2). Soil texture is reported as clay loam, this makes high value of bulk density values. Moderate value of organic matter 0.44 – 0.87% shows moderate value of bulk density values.

Conclusion:

The chief basis of revenue for the farmers residing at Thiruvarur and Thanjavur districts, India, depends on the procurementof cash crops such as sugarcane and rice. Highly useful and newly formulated HSQI was exploited in this study to rate the samples derived from fifteen places of Thanjavur and Thiruvarur district as excellent, good or bad with particular reference to rice and sugarcane farming. The total HSQI values of all the samples studied in this current task were found

to be in the range of 71.76 – 79.9 suggesting that these soil samples are of good.Soil quality index refers to the vibrantfertility of soil those characteristics that are disturbed by soil management. HSQI was found to be high useful, time saving and economically useful one. After this study was conducted, farmers of these study area were informed about the inherent quality of their soils and advised them to apply correct fertilizer in correct proportion in correct time. Not only was that, the state agricultural officers also advised to make use of such soil quality indices for their investigations.

Acknowledgement:

The authors thank the principals and the managements of Sir Theagaraya College, Chennai-21 and the Panimalar Engineering College, Chennai-123 for their constant encouragement and support rendered.

References:

Adviento-Borbe, M.A.A., Doran, J.W., Drijber, R.A.and Dobermann, A.(2006). Soil electrical conductivity and water content affect nitrous oxide and carbon dioxide emissions in intensively managed soils. Journal of Environment Quality,35: 1999-2010.

Alegre, J.C., Pashanasi, B.and Lavelle, P.(1996). Dynamics of soil physical properties in Amazonian ecosystems inoculated with earthworms. Soil Science Society of America Journal,60: 1522-1529.

Bar-Tal A., Feigenbaum, S.and Sparks, D.L.(1991). Potassium salinity interactions in irrigated corn. Irrigation Science,12: 27-35

Brady, N.C.and Weil, R.R.(2008). The Nature and Properties of Soils, fourteenth edition, Prentice Hall, London. pp 345 – 362.

Brady, N.C. and Weil, R.R. (2002). The Nature and Properties of Soils, Thirteenth edn. Prentice Hall. Upper Saddle River, New Jersey. pp 36 – 129.

Brady, N.C. and Weil, R.R. (1996). The Nature and Properties of Soils. Eleventh edn. Prentice Hall. Upper Saddle River, New Jersey. pp 45 – 160.

Campbell, D.J.and Henshall, J.K.(1991).Bulk Density, In: K.A. Smith and C. E. Mullins, Eds., Soil Analysis, Physical Methods. Marcel Dekker, New York. pp 329-366.

Davis, J.and Freitas, F.(1970). In Physical and Chemical Methods of Soil and Water Analysis, FAO of United Nations, Rome. Soil Bulletin,10: III-1, 68-70.

Day, S.D.and Bassuk, N.L.(1994). A review of the effects of soil compaction and amelioration treatments on landscape trees. Journal of Arboriculture,20 (1): 9-17.

Doran, J.W., Coleman, D.C.,Bezdicekm,D.E.and Stewart, B.A.(1994). Defining Soil Quality for a Sustainable Environment. Soil Science Society of America Special Publication Number 35,

Soil Science Society of America, Inc. and American Society of Agronomy, Inc., Madison, Wisconsin.pp 244

Dorfman, J.H.and Kareli, B.(2009). Do farmers hedge optimally or byhabit? A bayesian partial-adjustment model of farmer hedging. In: Ncc-134 Conference on applied commodity price analysis forecasting and market risk management St. Paul. Proceedings. St. Paul: Ag Econ Search. pp 18.

Duiker, S.W.and Curran, W.S.(2004). Cover crops. In E. Martz (ed.). The Agronomy Guide 2004. The College of Agricultural Sciences, The Pennsylvania State University, University Park, PA. pp 109-117.

Engel, R., Bruckner, P.L., Mathre, D.E.and Brumfields, S.K.Z.(1997). A chloride deficiencies leaf spot syndrome of wheat. Soil Science Society of America Journal,61:176-184.

Evenson, D.G., Orlob, G.T.and Lyons, C.(2006). Ground-water quality models: what they can and cannot do. Ground Water, 12: 97-101.

Farahani, H.J., Buchleiter, G.W.and Brodahl, M.K.(2005).Characterization of apparent soil electrical conductivity variability in irrigated sandy and non-saline fields in Colorado. American Society of Agricultural Engineers,48(1):155−168.

Fargo, N.D., Maas, E.V.and Hoffman, G.J.(1977). Crop salt tolerance current assessment. Journal of the Irrigation and Drainage Division,103:115-134.

Grossman, R.B.and Reinsch, T.G.(2002). Bulk Density and Linear Extensibility, In: Dane, J. H., Topp, Eds, G. C., Methods of Soil Analysis, Part (4), Physical Methods, Third Edition, SSSA, Madison., pp 201-228.

Gugan, S., Ramesh Kumar, C., Rajendran, A.,Princy Merlin, J.and Jeyakarchellaraj,J.(2001). Formulation of a new Heber Soil Quality Index for rice and sugarcane cultivation. Pollution Research,19 (3): 485 - 489.

Heckman, J.R.,(1998). Corn stacks root suppression and grain yield response to chloride. Journal of Plant Nutrition 21:149-155.

Hodges, S.C.,(1995). Soil Fertility Basics, Soil Science Extension North Carolina State University Certified Crop Advisor Training. pp 5-75.

Hoorman,J.and Islam, R.(2010). Understanding Soil Microbes and Nutrient Recycling. Fact Sheet SAG – 16 -10. Ohio State University Extension. pp 1-5.

Horneck, D.A., Sullivan, D.M., Owen, J.S.and Hart, J.M.(2011). Soil Test Interpretation Guide. Oregon state university, Extension service. pp. 1-12.

Jackson, M.L.(1967). Chloride Determination, in Soil Chemical Analysis, Prentice Hall of India Pvt. Ltd., New Delhi. 10, 261-263.

Jung,W.K., Kitchen, N.R., Sudduth, K.A., Kremer, R.J.and Motavalli, P.P.(2005). Relationship of apparent soil electrical conductivity to clay pan soil properties. Soil Science Society of America Journal,69: 883-892.

Kadam, J.R.and Shinde, P.B.(2005). Practical Manual on Soil Physics – A method manual, Department of Agricultural Chemistry and Soil Science, P.G.I., Rahuri. pp 24

Klemens, E., Stierhof, T., Dauber, J., Kreimes, K.and Wolters,V.(2003). On the quality of soil biodiversity indicators: abiotic and biotic parameters as predictors of soil faunal richness at different spatial scales. Agriculture, Ecosystems and Environment,98: 273-283.

Knudsen, D.and Peterson, A.G.(1982). Lithium, Sodium and Potassium, P. 225 to 246, In A.L. Page (Ed.) Methods of Soil Analysis. Agronomy Monogram No. 9, American Society of Agronomy, Inc. Soil Sci. Soc. Of America Inc. Publisher Madison WB Consin, USA.

Lipiec, J.and Hatano, R.(2003). Quantification of compaction effects on Soil physical properties and crop Growth. Geoderma, 116: 1-2.

Magdoff, D.H.and Startsev, A.D.(2009). Effects of compaction on aeration and morphology of boreal forest soils in Alberta, Canada. Canadian Journal of soil science,89 (1): 45-56.

Majumdar, D.K.(2000). Irrigation Water Management by, Prentice Hall of India. pp 1-18.

Mandal, K.G., Kundu, D.K., Ravender Singh, Ashwani Kumar, Rajalaxmi Rout, JyotiprakashPadhi, Pradipta Majhi,and Sahoo DK.(2013). Cropping practices, soil properties, pedotransfer functions and organic carbon storage at Kuanria canal command area in India. Springer Plus, 2 (631), 1- 14.

Muthuvel, P., Udayasoorian, C., Natesan, R.and Ramaswami, P.R.(1992). Introduction to soil analysis. pp 10 – 60

Nelson, D.W.and Sommers L.E.(1996). Total Carbon, Organic Carbon and Organic Matter, In: D. L. Sparks, et al. Eds., Methods of Soil Analysis, Part (3), Chemical Methods, SSSA, Madison. pp 995-996.

Nelson, R.E.(1982). Carbonate and Gypsum, In A. L. Page (Ed.). Methods of Soil Analysis, Part-2. Agronomy, 9: 181-197. American Society of Agronomy Ins. Soil Science Society of America Inc., Madison Wisconsin.

Olsen, S.R.,and Sommers, L.E.(1982). Phosphorous in method of soil analysis, Part-2, Chemical and microbiological properties – Agronomy monograph number 9 (2^{nd} Ed.).American Society of Agronomy, Inc, Soil Science Society of America, Inc, Madison, Wisconsin, USA, 24, 421 – 422.

Page, A.L., Miller, R.H.and Keeny, D.R.(1982). Organic Carbon in Methods of Soil Analysis, Chemical and Microbiological properties, American Society of Agronomy, Inc, Soil Science Society of America, Inc, Madison, Wilconsin, USA, , 9(2): 570-571.

Sepp, K., Ivask, M., Kaasik, A., Mikk,M.and Peepson, A.A.(2005). Soil biota indicators for monitoring the stonian agri-environmental programme. Agriculture, Ecosystems and Environment,108:264-273.

Silva, J.A.and Uchida, R.(2000). Biological nitrogen fixation, Nature's partnership for sustainable agriculture production. In: Silva J.A. and Uchida R. (eds.). Plant nutrient management in

Hawaii's soils. Approaches for tropical and subtropical agriculture, College of tropical agriculture and human resources, University of Hawaii at Manoa. pp 121-126.

Singh,Dhyan, Chhonka, P.K.and Pande, R.N.(1999). Electrical Conductivity in Soil, Water analysis Method Manual, IARI, ICAR, New Delhi. 1, 4.2 (b) 14-16.

Smith, J.L. and Doran, J.W.(1996). Measurement and use of pH and electrical conductivity for soil quality analysis. In J.W. Doran and A.J. Jones (ed.) Methods for assessing soil quality. Soil Science Society of America Spec. Publ. 49. SSSA, Madison, WI. pp 169-185.

Somawanshi, R.B., Tamboli,B.D,, Patil, Y.M.and Kadu, P.(1994).Twenty Five years of Research on soil test Crop Response Studies in Maharashtra. 1968 – 1993, M.P.K.V. Res. Publ. No. 14.;

Subbiah, B.V.and Asija, G.L.(1956).A rapid procedure for the estimation of nitrogen in soils. Current Science,25:259-260.

Thiyagarajan, K.,and Kalaiyarasi, R.(2011). Status paper on rice in Tamil Nadu. Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. pp 1-20.

Tucker, C.S.,(1984). Carbon dioxide. in T.L.Wellborn, Jr. and J.R. MacMillan (eds.) For Fish Farmers 84-2. Mississippi Co-operative Extension Service.

Watson, G.W.and Kelsey, P.(2006). The impact of soil compaction on soil aeration and line root density of Quercus palustris. Urban Forestry and Urban Greening,4 (2): 69-74.

Wuddivira, M.N., Stone, R.J.and Ekwue, E.I.(2009).Clay Organic Matter and Wetting Effects on Splash Detachment and Aggregate Breakdown under Intense Rainfall. Soil Science Society of America Journal,73 (1): 226-232.

Wurts,W.A.and Durborow, RM.(1992). Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Southern Regional Aquaculture Center, Publication.pp 464.

Yadav, R.L., Kamta Prasad, K.and Gangwar, K.S.(1998). Prospects of Indian Agriculture with special reference to nutrient management under irrigated systems. In: Long-Term Fertility management through Integrated Plant Nutrient Supply (Eds. A. Swarup, D. Damodar Reddy and R.N. Prasad), Indian Institute of Soil Science, Bhopal, India. pp 1-335.

SOIL ORGANIC CARBON STOCKS UNDER VARIOUS TEMPERATURE AND MOISTURE REGIMES

Priyanka Kumari[1], Kumar Chiranjeeb[2*], Rajani[3] and Tara Man Rai[4]

[1]Department of Soil Science and Agricultural Chemistry,

Bihar Agricultural University, Sabour (Bhagalpur), India

[2]Department of Soil Science, GIET University, Gunupur, Odisha - 765022

[3]Department of PBG, GIET University, Gunupur, Odisha - 765022

[4]Department of Soil Science, Dr. Rajendra Prasad Central Agricultural University,

Pusa, Samastipur (Bihar)-848125

* Corresponding author E-mail: kumar.chiranjeeb3@gmail.com

Abstract:

Soil organic carbon is the key component for enhancing soil quality along with providing sustainable food production as well as regulating the concentration of atmospheric carbon dioxide (Singh *et al*., 2007). Minerals soils are the largest reservoir of carbon with estimate of carbon stock ranging from 1115 to 2200 pg in a meter soil profile (Post *et al*.,1982;) and carbon content is affected by mostly temperature and moisture which also changes the carbon stock in soil. Soils developed under different temperature and moisture regimes, having different vegetation. Total organic carbon stock to a depth of 250 cm in the ice complex of Central Yakutia is 38.7 kg/m^2 (Shepelev *et al*., 2018). Soil organic carbon stocks, soil moisture and active layer of thickness correlated strongly in discontinuous permafrost, whereas no correlation was found between soil organic carbon stocks and active layer thickness in continuous permafrost region (Dorfer*et al*., 2013). As we go downwards, soil organic carbon decreases. High amount of soil organic carbon in combination with soil temperature and moisture regime is found in cryic-udic regime and minimum in thermic-xeric regime. Increase and decrease in soil temperature affect soil organic carbon and mineralization of organic carbon which directly and indirectly affect the crop production.

Introduction:

Soil organic carbon is very important part in soil and it plays an important role in the fertility of soil and helps in the balance of ecosystem, agriculture land other aspects of

sustainable development. Soil organic carbon is about 2.4 times that of terrestrial biomass carbon in which mineralization and sequestration is directly related to the soil nutrient supply and release. It maintains soil quality and also plays an important role in regulating the carbon dioxide concentration in the atmosphere. Soil temperature and moisture plays an important role in soil organic carbon mineralization by affecting the microorganisms present in the soil.The world mineral soils are a large sink of carbon with quantity of carbon stock ranging from 1115 to 2200 pg in a meter soil profile (Post *et al*., 1982). Environmental factors such as moisture and temperature affect the stock of carbon present in the soil. Soil temperature and moisture regimes guide and control soil utilization for the growth of plant.

Soil Moisture Regimes (SMR):

SMR refers to the absence or presence of water in a soil at different times in a year. When the soil moisture tension is less than 1500 kpa it is considered as moist and dry when tension is 1500 kpa (ISSS). The soil moisture regime is the fragmental function of soil, climate and landform. It may be defined in terms of number of days, the soil moisture control section remains moist with moisture tension between 33 kPa and 1500 kPa. On the above criteria soil moisture regimes are ice, permafrost, interfrost, aridic, ustic and udic.

Ice: In this regimes there is permanent ice or snow throughout the year. Eg; artic ice sheet, mountain glacier.

Permafrost: In this moisture regime soil remains below 0 degree celcius for more than two successive years.

Interfrost: It is said to be an intermittent permafrost condition.

Aridic: It is also known as torric regime. Under this soil moisture regime water is not available to plants for more than half cumulative time that the soil temperature at 50 cm below the surface is greater than 5 degree celcius and has no period as long as 90 consecutive days when there is water for plants while the soil temperature at 50 cm is continuously greater 8 degree celcius (ISSS).

Xeric: It is associated with Mediterranean climate when mean annual soil tempreture is less than 22 degree celcius, mean summer soil temperature and mean winter soil temperature differ by greater than 5 degree celcius. Dry in all parts for 45 or more consecutive days within 4 months following summer solstice in 6 or more years out of 10 years. Moist in all parts for greater than 45 consecutive days following winter solstice.

Ustic: This is intermediate between aridic and udic moisture regimes and common in temperate, subhumid or in tropical and subtropical regimes with a monsoon climate.

Udic: Common to soils of humid climate which have well distributed rainfall, or which have enough rain in summer. Not dry in all parts for as long as 45 consecutive days following summer solstice.

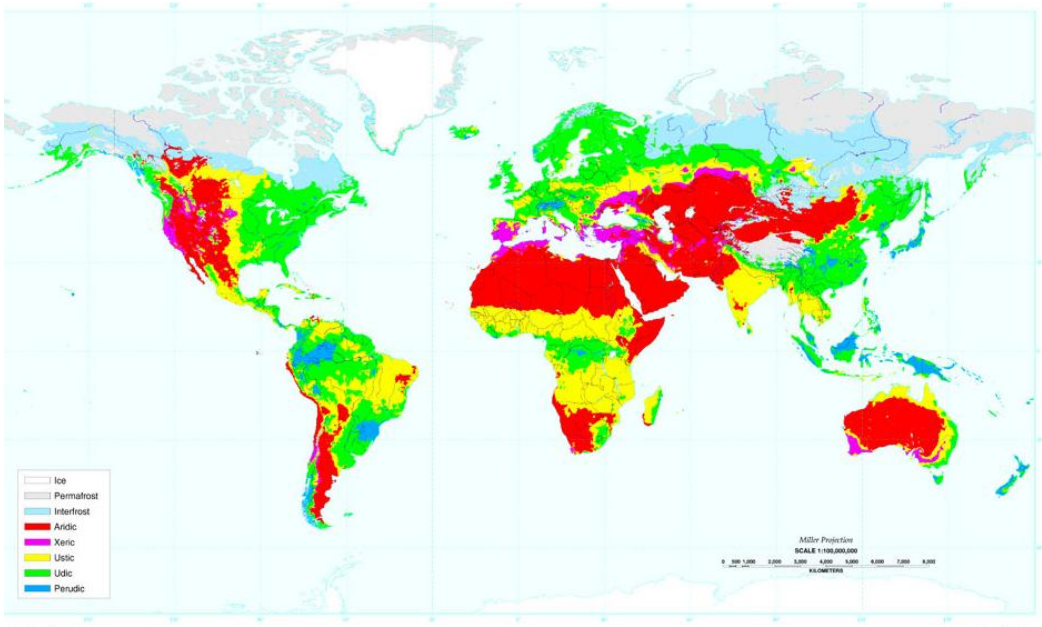


Figure 1: Distribution of soil moisture regimes over the world (by USDA GOV.)

Near polar region in both the hemispheres ice moisture regime is to be found. The whole area of Greenland comes under this moisture regime. As we move downward from the polar region permafrost regime is found. Alaska, Canada and north parts of Russia comes under permafrost regimes where soil temperature is below 0 degree celcius for more than 2 successive years. North part of South Africa (Sahara Desert), most partof Australia , the Thar desert region of India, Pakistan, Mongolia, Kazikistan, Argentina, and western part of norh America comes under aridic moisture regime. About 95% region of India comes under ustic moisture regime. Xeric moisture regime is very less all over the world(Spain, Italy, Turkey, some districts of Australia and north America). Eastern countries of Asia and North America have udic moisture regime.

Soil temperature regimes:

These are the ranges in temperature classes within which biological activities of different degrees prevails. There is no biotic activity between 0 to 5 degree celcius. The temperature range from 5 to 35 degree celcius is important in determining the degree of biological and chemical activities.

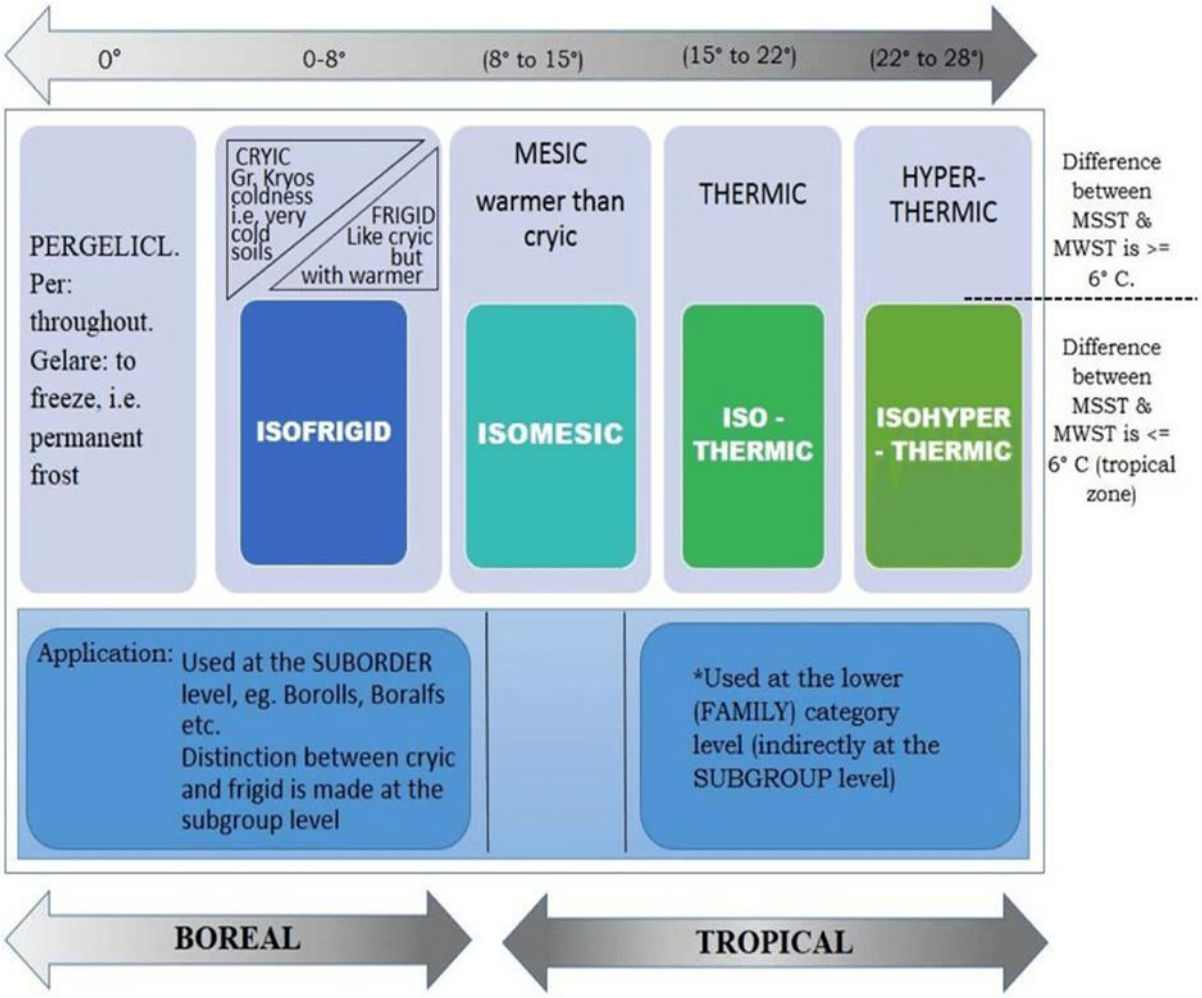


Figure 2: Classification of temperature regimes (from ISSS book)

In all there are six soil temperature regimes viz. pergelic, frigid/cryic, mesic, thermic, Hyperthermic, megathermic. The prefix iso is used if the difference between MST and MWT is less than 5 degree celcius to separate tropical areas. The following diagram shows that the mean annual soil temperature of 8 degree celcius is used to separate frigid soil from mesic soils. Mean annual soil temperature of 22 degree celcius. Permanent frozen region comes under pergelic temperature regime. The region having temperature 0 to 8 degree celcius i.e. very cold region comes under cryic regime. Frigid is same as cryic but warmer in summer. The temperature of mesic regime is between 8-15 degree celcius. Thermic regime has temperature between 15-22 degree celcius.

Soil organic carbon stocks in world:

From above figure it is clear that the maximum soil organic carbon stocks is found near polar regions i.e at higher latitude. At 80^{o}N the soil organic carbon stocks is greater than 500 g/kg at 0-10 cm depth of soil. As we move downward towards equator the soil organic carbon stocks decreases. The soil organic carbon stock between 80^{o} N and 60^{o} N is about 400-500 g/kg

at 0-10 cm depth of soil. 200-300 g/kg soil organic carbon is found in between 40-60^{o} N. the minimum soil organic carbon stock is found near equator i.e in between 0-100 g/kg at 0-10 cm depth of soil. Same trends follow in southern hemisphere.

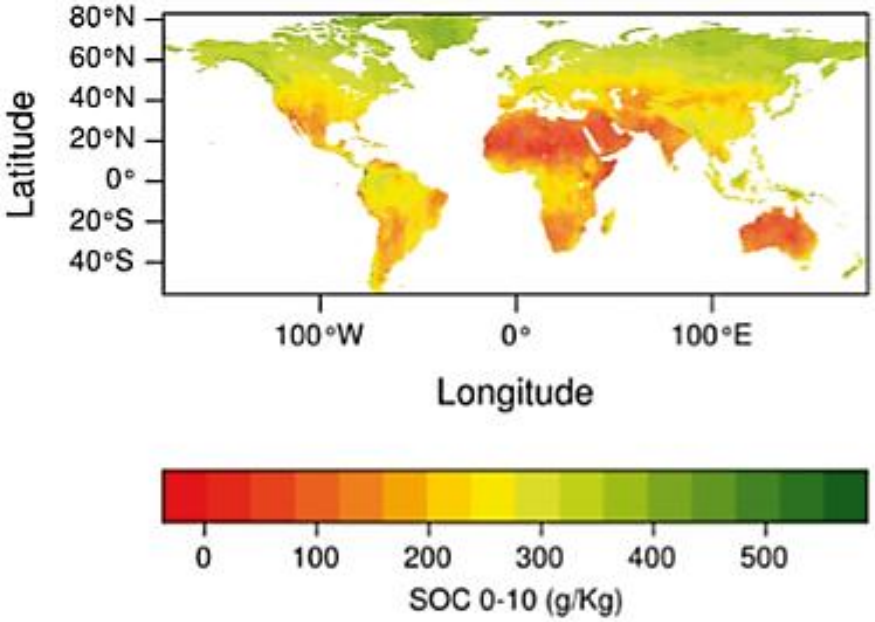


Figure 2.1: distribution of SOC stocks all over the world at different latitude (Malone *et al*., 2015)

Soil organic carbon stocks in India:

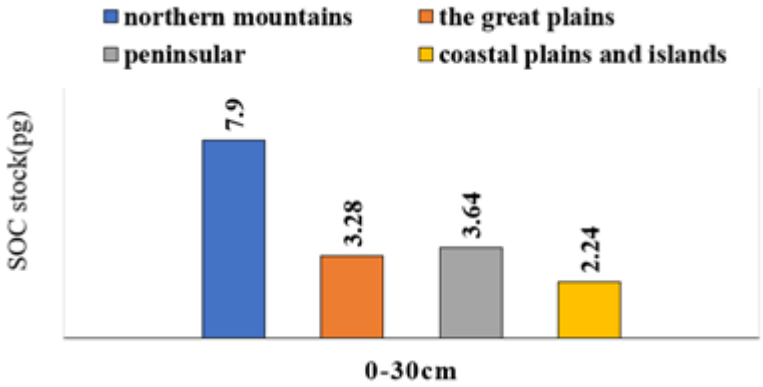


Figure 2.2: SOC stocks upto 30cm depth of soil in different parts of India (Bhattacharyya *et al*., 2000)

The North Mountains are holding 39% of soil organic carbon stock of the country largely due to the thick forest vegetation aided by low rate of organic matter decomposition. This is about 7.9 pgupto 30 cm depth of soil. The great plains of India have 3.28 pg of soil organic

carbon. Peninsular part of India which has temperature range between 28-34^{o} C contains 3.64 pg of soil organic carbon and the coastal plains of India contain 2.24 pg soil organic carbon. There are differences in carbon stocks in different regions of India; this is because the organic carbon stock of a region depends on the distribution of soil type, organic carbon percentage and bulk density of soil.

SOC under various moisture regimes:

Soil organic carbon in high-altitude and high-latitude permafrost ecosystems:

Region	Ecosystem	Depth (cm)	Mean SOC (Kg/m^2)	Study
Russia	Tundra	100	21.8	Post *et al*. (1982)
Siberia, Russia	Tussock tundra	100	30.7	Gundelwein *et al*. (2007)
Brooks range	Dry tundra	100	14	Bockheim *et al*. (1998)
Tibetan plateau	Alpine meadow	30	9.3	Wang *et al*. (2008)
Tibetan plateau	Alpine meadow	30	6.2	Yang *et al*. (2008)
Qinghai, China	Alpine meadow	75	53.1	Wang *et al*. (2002)

From above table it is concluded that with Tundra and Tussock tundra ecosystem soil organic carbon is maximum in case of tussock tundra ecosystem. On comparing Alpine meadow and Alpine steppe of Qinghai and Tibetan plateau , the alpine meadow has high soil organic carbon due to high primary production and favorable condition for preservance of soil organic matter such as high moisture content. It is also situated at high altitude and has good vegetative growth and low temperature due to which decomposition rate is low and so the loss of soil organic carbon is low in this area.

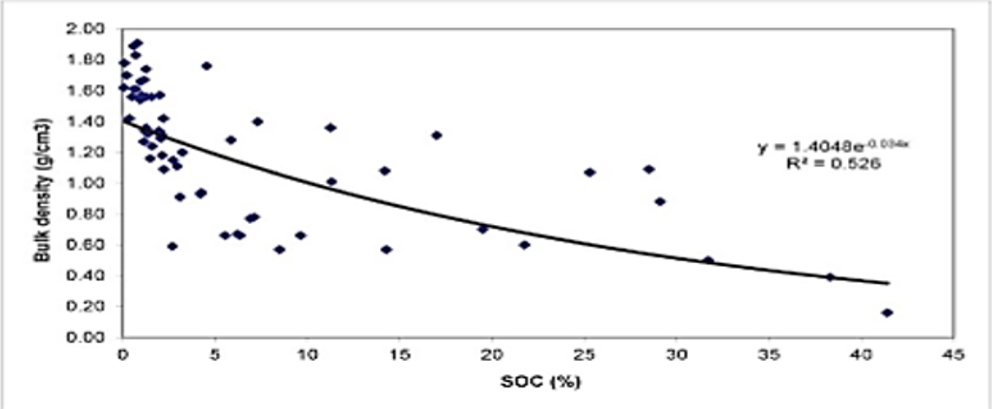


Figure 3.1: Relation between soil organic carbon and bulk density in alpine soils of permafrost regime

A study was done by Bockein and Munroe in the year 2014, he stated that bulk density of forest is strongly correlated with organic carbon content. It is very difficult to calculate bulk density in this region but this graph shows that as the bulk density of soil decreases the soil organic carbon percentage decreases.

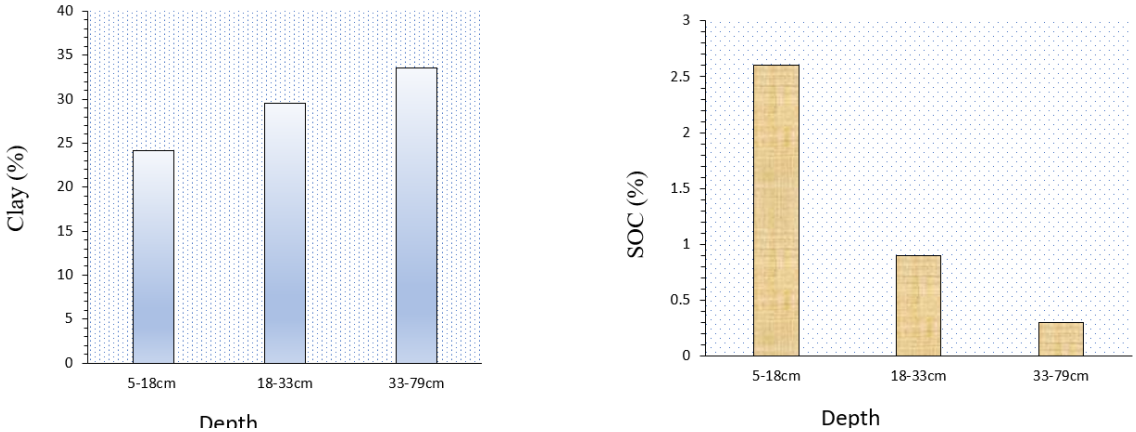


Figure 3.2: Relation between amount of clay, depth and soil organic carbon in permafrost regime (Bockheim and Munroe, 2014)

In first graph we see that as we go downward the earth surface the clay% increases. It is about 34% in case of 33-79 cm depth of soil and least at surface of soil. In second graph as soil depth increases the soil organic carbon percentage decreases, maximum in case of upper surface of soil. Hence from both the graph it is concluded that clay % is inversely related to soil organic carbon % in permafrost regime.

Soil organic carbon stocks in cryic regime:

Soil organic carbon stocks of cryic regime are similar to permafrost regime because both the regimes are found in colder areas. So soil organic carbon is more at high altitude regions.

Mean soil organic carbon density in aridic regime up to 25 cm depth of soil (Singh *et al*., 2006)**:**

Great groups	Carbon density (kg/km^2)
Vertisol	2600
Entisol	2620
Alfisol	1680
Inceptisol	1350
Aridisol	1290

Carbon density varies according to great groups of soil in aridic zone of Rajsthan. A high soil organic carbon in vertisol followed by Entisol, Alfisol, Inceptisol as compared to Aridisol could be explained by higher rainfall, larger vegetation input and higher clay content. Organic carbon associated with sand particle is readily decomposable as compared to that in silt and clay. Clay with higher surface area protects organic carbon from decomposition on developing stable clay-organic complexes.

Effect of cropping pattern on carbon density in aridic regime:

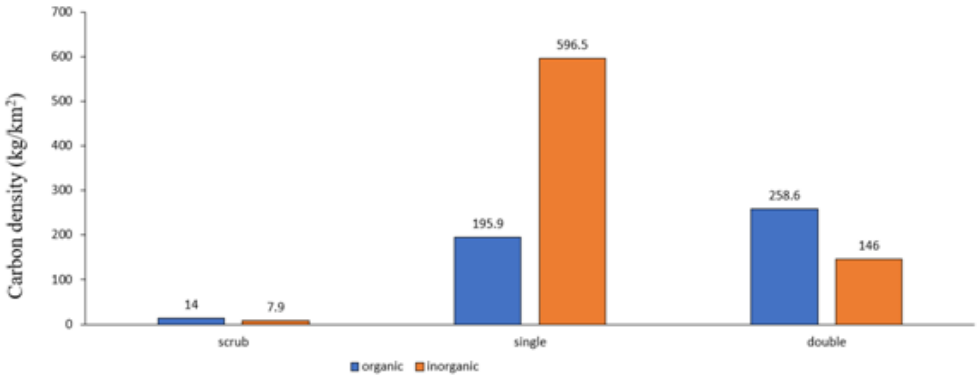


Figure 5.1 Effect of different cropping pattern on carbon density in aridic climate (Singh *et al.*, 2006)

Soil inorganic carbon is maximum in double cropping system followed by single and scrub. Organic carbon is maximum in case of single cropping system followed by double and scrub but the overall carbon density in maximum in case of single cropping system followed by double and scrub.

Soil organic carbon density varied according to soil texture in aridic regime:

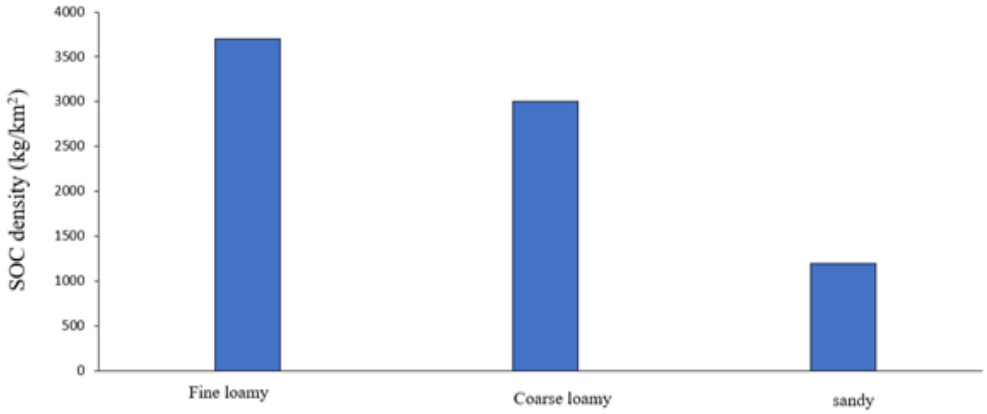


Figure 5.2: Soil organic carbon density variation according to soil texture in aridic regime (Singh *et al.*, 2006)

Mean soil organic carbon varied with soil texture and depth. It is highest in case of fine loamy followed by coarse loamy and least in sandy soil. Soil organic carbon is maximum in case of loamy due to presence of more clay % it form a clay complex and is case of sand it is readily decomposable.

Microbial biomass carbon and potassium oxidizable carbon under thermal regime:

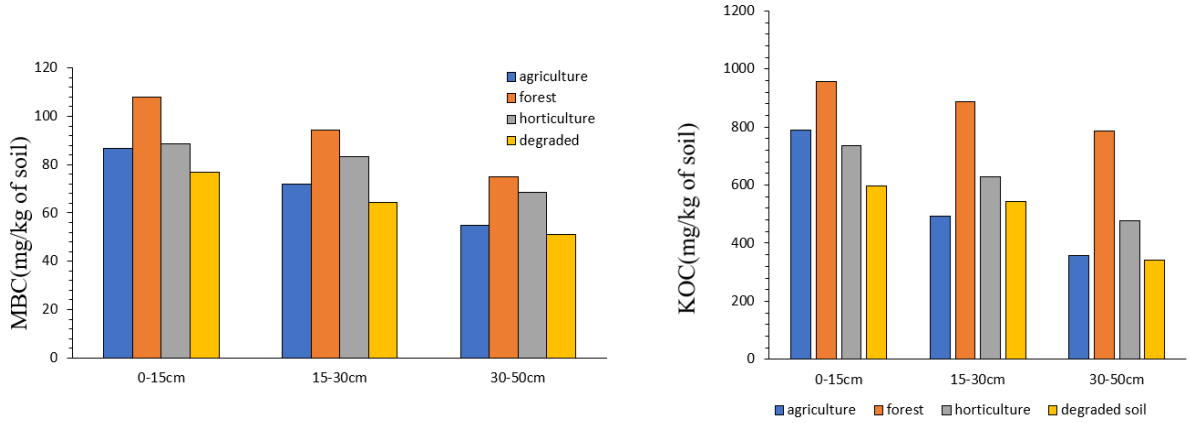


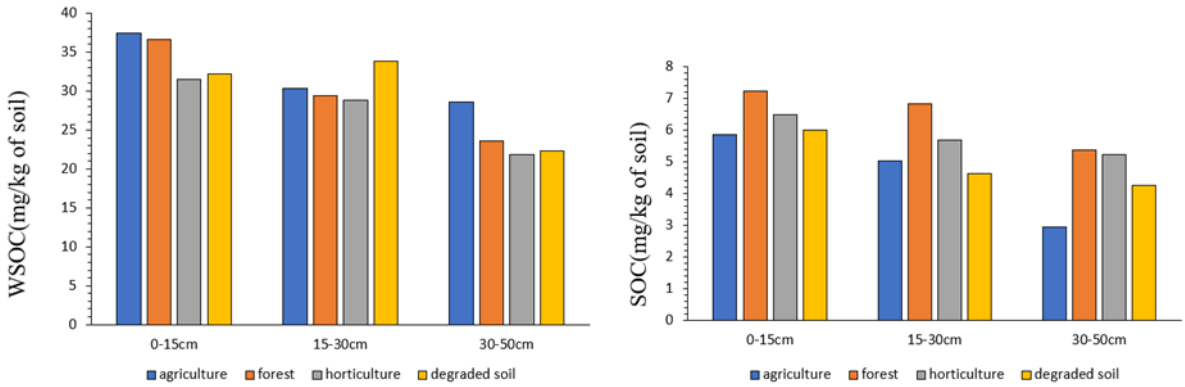
Figure 6: Variation of microbial biomass carbon and potassium oxidisable carbon under (Sharma *et al*., 2014)

The above graph shows that the land uses system affect the MBC and KOC in thermal regime. Low concentration of MBC and KOC in agricultural land use system can be attributed to tillage practices. Cultivation period has negative effect on labile carbon. Tillage reduces MBC content by affecting the moisture content. MBC was higher in tree based system as compared to monocropping. Low MBC in degraded soil may be the result of poor vegetation resulting in poor microbial activity. It is same in case of KOC and follow the trend : forest>agriculture>horticulture>degraded.

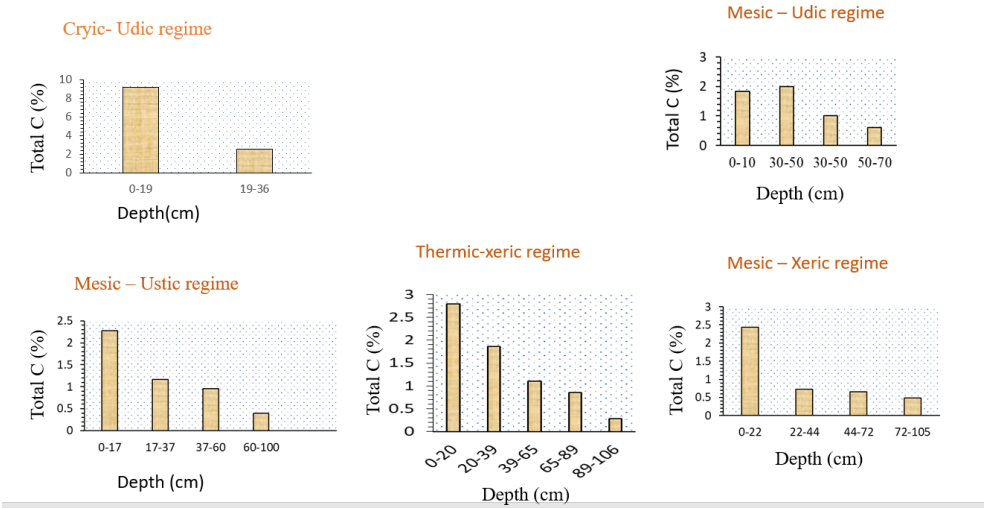
Water soluble carbon and soil orgainc carbon stocks under thermal regime:

Unlike other active fractions water soluble carbon did not seems to vary with land use system based upon weighed mean value. Although within different locations there were wide variations in the water soluble content in all the land use system. Boyer and Groffman suggest that while agricultural soils generally have lower level of total and microbial carbon than forest soils, they may support equal or greater rates of microbial activity than forest soil due to increased production of water soluble carbon. In case of soil organic carbon, forest soil have 40% more soil organic carbon as compared to agriculture. Degraded soil has least soil organic carbon because

agricultural field suffered from repeated biomass removal and degraded land has no any biomass inputs **(**Sharma *et al*., 2014)



Soil organic carbon in different combinations of temperature and moisture regimes



(Filcheva *et al*., 2017)

The above study was done by Filcheva in the year 2017. He established a relationship between the total organic carbon percentage and depth of soil in combination of different soil and temperature regimes. The soil having more organic carbon percentage has maximum soil organic carbon stocks. From the above graph we conclude that maximum soil organic carbon stocks in cryic-udic regime.

Conclusion:

Soils developed under more cool and moist conditions accumulate more organic carbon and those developed under warm and dry regimes.Soil organic carbon content varies significantly according to soil temperature and moisture regimes and follow the trend udic>ustic>xeric>aridic> and cryic>mesic>thermic.Soil organic carbon stocks are affected by land use, soil types and soil texture under various soil moisture and temperature regimes.

References:

Bhattacharyya, T., Pal, D. K., Mandal, C., &Velayutham, M. (2000). Organic carbon stock in Indian soils and their geographical distribution. current science-Bangalore. **79**(5), 655-660.

Bockheim, J. G., & Munroe, J. S. (2014). Organic carbon pools and genesis of alpine soils with permafrost: a review. Arctic, antarctic, and alpine research. **46(**4), 987-1006.

Dorfer, C., Kuhn, P., Baumann, F., He, J. S., & Scholten, T. (2013). Soil organic carbon pools and stocks in permafrost-affected soils on the Tibetan Plateau. PloS one. **8**(2), e57024.

Gundelwein A, Mu¨ller-Lupp T, Sommerkorn M, Haupt ETK, Pfeiffer E, et al. (2007). Carbon in tundra soils in the Lake Labaz region of arctic Siberia. European Journal of Soil Science. 58 (5), 1164–1174. Available: http://dx.doi. org/10.1111/j.1365-2389.2007.00908.x.

Hristov, B., and Filcheva, E. (2017). Soil organic matter content and composition in different pedoclimatic zones of Bulgaria. Eurasian Journal of Soil Science. **6**(1), 65-74.

Malone, B. P., McBratney, A. B., Minasny, B. &Laslett, G. M. (2009). Mapping continuous depth functions of soil carbon storage and available water capacity. Geoderma. 154, 138–152.

Post, W. M., W. P. Emanuel, P. J. Zinke, and A. G. Stangenberger. (1982). Soil carbon pools and world life zones. Nature**.** 298, 156-159.

Sharma, V., Hussain, S., Sharma, K. R., and Arya, V. M. (2014). Labile carbon pools and soil organic carbon stocks in the foothill Himalayas under different land use systems. Geoderma. 232, 81-87.

Shepelev, A., Fedorov, A., and Cherepanova, A. (2018). Quantitative and qualitative composition of soil organic matter in samples of ice complex from central Yakutia, eastern Siberia. Applied and Environmental Soil Science.

Singh, S. K., Singh, A. K., Sharma, B. K., &Tarafdar, J. C. (2007). Carbon stock and organic carbon dynamics in soils of Rajasthan, India. Journal of Arid Environments. 68(3), 408-421.sss

Yang, Y., Fang, J., Tang, Y., Ji, C., Zheng, C et al. (2008). Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. Global Change Biology. 14, 1592–1599.

Wang, G., Qian, J., Cheng, G. and Lai, Y. (2002). Soil organic carbon pool of grassland soils on the Qinghai-Tibetan Plateau and its global implication. The Science of the Total Environment 291, 207–217.

Wang, G., Li, Y., Wang, Y. and Wu, Q. (2008). Effects of permafrost thawing on vegetation and soil carbon losses on the Qinghai-Tibet Plateau, China. Geoderma. 143, 143–152.

EFFECT OF AGROFORESTRY PRACTICES ON CLIMATE CHANGE WITH AMELIORATION OF SALT AFFECTED SOILS

Megha Bhadani[1], Somya Kumari Bhadani[2], Kumar Chiranjeeb*[3] and Rajani[4]

[1]Department of Soil Science, Dr.Rajendra Prasad Central Agriculture University, Pusa, Samastipur (Bihar) - 848125

[2]Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh

[3]Department of Soil science, GIET University, Gunupur, Odisha-765022

[4]Department of PBG, GIET University, Gunupur, Odisha-765022

*Corresponding author E-mail: kumar.chiranjeeb3@gmail.com

Abstract:

Agroforestry is the practice of growing food crops with tree crops and/or livestock animals sustainably on the same piece of land as well as support the alternative form of biological reclamation of salt affected soils apart from sustainable production, continuous income generation, and regular employment along with ensuring food and nutrition security. The soil chemical properties along with different physical properties are enhanced by this agro forestry system. Various multipurpose tree species such as *Acacia species* and *Acacia nilotica* etc when grown for longer period of time alter the soil pH, EC_e, exchangeable cations, anions in soil system. Agroforestry also proven to increase soil fertility status in long term manner as well as helps in mitigating climate change with supporting the livelihood of farmers.

Keywords: Agroforestry, Climate change, Salt affected soil, Amelioration.

Introduction:

The geographical area of India is 329 million hectare out of which 6.74 million hectares of land area is affected by salts in the states West Bengal, Maharashtra, Rajasthan Gujarat, Uttar Pradesh, Madhya Pradesh, and Tamil Nadu (Biswas and Biswas, 2014). In this era of change in climate with deteriorating soil fertility and depletion of ground water table.Increasing pressure on land resources lead to different types of degradation including salinization that is stated as the process of subsequent increase of salt in soil profiles. It has adverse effect on biological and physico-chemical properties of the soils. Major constraint for planting of deep rooted plants is the occurance of hard kankar (calcite) pan at a depth of about 90 cm (Garg *et al.,* 1996; Jain and Singh, 1998). Tree growth in alkali soils is not suitable due to inability of roots to penetrate this

calcite pan. Sustainable and holistic use of these lands can substantially contribute to demand elevation for food, fodder, fuel, and timber in India. Deterioration of alkali soils can be prevented by planting of trees and grasses. Saline soil also influences the growth, development and production of crops, fruit and tree. Salinity is an exhaustive and emerging problem especially in arid and semiarid regions. It has direct link with the significant yield losses incurring from the existing land uses. A great threat to world agriculture involves production of 70% more food for an additional 2.3 billion people by 2050 worldwide. Of the total land area approximately 76 Mha salt-affected lands are affected by humaninduced salinization and sodification. Agricultural productivity in saline soils is directly correlated with osmotic and specific ion effects (Munns and Tester, 2008) and decline in the physical properties in sodic soils (Abrol *et al.,* 1988). Expensive inputs make it costlier to practice conventional agricultural that limits the farmers to go for cultivation of arable crops. A huge amount of tree germplasm were analysed and found trees of the genus Prosopis followed by Acacia and Casuarina to be highly promising for the rehabilitation of salt lands. Similarly *Brichiaria mutica* are reported as promising grasses for growing in salt lands. Woody tree species are toleratant abiotic stresses better than annual species (Dagar and Yadav, 2017).

Alternative land use potential options for saline ecologies are forestry and agroforestry systems (Dagar *et al.,* 2016). More recently, some systematic research efforts have been made to establish trees and grasses on salt lands, but no attempts to grow trees and grasses together in salt lands have been made. One study report of a long-term field trial stated *P. juliflora* was grown 'with' and 'without' Leptochloa grass for 8 years on a salt land with an initial pH of 10.4. The efforts are also made to solve challenges that may be in present or future in the eye of agroforestry approaches for farm diversification at large and economic sustenance in particular. The current and future challenges in agroforestry systems evaluations with headway road map is also duly highlighted and discussed. *Different tree species like Terminalia arjuna, Casuarina equisetifolia, Prosopis cineraria, Albizia lebbeck, Prosopis juliflora*; and fruit trees such as *Zizyphus jujuba, Emblica officinalis, Syzygium cumini,* and *Tamarindus indica are grown to* ameliorate the salt affected soils with the help of different agroforestry systems *such as* agrisilvicultural system, silvipastoral system and agri-horisilvicultural system.

Agroforestry not only helps in climate change mitigation but also climate change adaptation. Agroforestry system is one of the most economically and ecologically sound practices with increment of overall farm productivity along with soil enrichment through litter

fall, maintaining different environmental services such as climate change and its mitigation (carbon sequestration), phytoremediation, watershed protection and biodiversity conservation. Agroforestry is recognized as an afforestation activity that help in sequestering carbon dioxide (CO_2) to soil, conservation of biodiversity, protection of croplands, works as a windbreak, and supports food and feed to human and livestock, supplies pollen for honey bees, wood for fuel, and timber for shelters construction. The role of agroforestry in climate change mitigation might be recognized to its full potential worldwide by solving various financial, technical, and institutional barriers. Leguminous tree crops increases the fertility status and also the productivity of soil. Agroforestry practice has much more potential to increase carbon sequestration of agriculture dominated landscapes significantly than the mono crop agriculture.

Agroforestry: Key to mitigate climate change:

Agroforestry is a land management system . It serves as a tool for both climate change adaptation and mitigation, and also addresses various challenges that smallholder farmers are facing. Agroforestry can generate multiple livelihood and environmental benefits, mitigate climate change and help farmers to adapt to extreme and variable weather (Ripple *et al.,* 2019). This leads to increased soil fertility, reduced soil erosion and flood and pest control. Smallholder farmers are much more benefitted by agroforestry such that increased farm productivity and less usage of conventional fertilizers and chemicals for pest management, leading to increased income. Immediate action is required to limit the temperature increase to 1.5 degree (IPCC, 2019) and reduce the climate change risks (e.g. severe droughts, flooding and diseases).Climate change has broad impacts on agricultural systems, triggering soil erosion, crop failure, loss of biodiversity along with reduced soil moisture, pest damages and economic losses. Paris Agreement focuses on reducing the carbon emission and can be fulfilled by forests, trees and agriculture. Potential bad impacts of climate change include rise in sea levels; enhanced frequency and intensity of wildfires, floods, droughts, and tropical storms and disturbance of coastal marine and other ecosystems. Trees have important roles in decreasing vulnerability, boosting resilience of farming systems and maintaining households against climate associated risks. Agricultural practices lead to the decrease in carbon stocks mainly due to disposition of aboveground biomass as it involves burning and decomposition with loss of soil carbon both in form of CO_2 and by erosion. Tropical deforestation contributes as much as 25% of the net annual CO_2 emissions worldwide. The current atmospheric CO_2 concentration is about 388ppm & estimated to increase to approximately 470 – 570 ppm until year 2050.

Carbon sequestration and agroforestry:

Soil carbon sequestration implies removal of atmospheric carbon dioxide by plant and storage of fixed carbon as soil organic matter .The aim is to increase the soil organic carbon density in the soil, stabilize the soil organic carbon by encapsulating it within the stable micro-aggregates so as to save the compounds of carbon from microbial process. . Forestry, change in land use, and agriculture account for 25–30% of global anthropogenic GHG emissions to the atmosphere (FAOSTAT, 2013). The agricultural sector is responsible for about 10–12% of total non-CO_2 anthropogenic GHG emissions (IPCC, 2007). Global climate change and warming of the atmosphere causes greater variability in rainfall, rise in sea level, and increased incidence of weather events in an extreme mode such as floods and droughts, heavy and intense storms, and decrease in crop yields, endangering the livelihoods of communities. Carbon dioxide (CO_2) is the most important GHG**.** Annual emissions of CO_2 have grown by about 80% between 1970 and 2004, from 21 to 38 Gt, and represented 77% of total anthropogenic GHG emissions since the industrial revolution**.** Approximately 3 times or more organic matter present in soils than the atmosphere and it is easily destroyed by land degradation. Soil serves as a carbon sink or source that depends on land use, soil properties and, local climate.

About 23% of the world's total emission of greenhouse gases (GHGs, e.g. CO_2, N_2O and CH_4) come from agriculture, forestry and other land use (Forster *et al.,* 2007). Agroforestry trees provide carbon input such as root biomass, litter and pruning in addition to improving land cover in agricultural fields. Terrestrial carbon stock is 25% of global carbon stock and amount of carbon sequestered totally depends on the agroforestry system of one area, the structure and function that is largely decided by the environmental and socio-economic factors. Agroforestry in degraded land offers greater opportunity to sequester more carbon and seek carbon credits.

Carbon sequestration- above ground:

Carbon storage aboveground is defined as the inclusion of Carbon into plant parts either in the part of harvested product or in the in situ remaining living parts Nair *et al.* (2010). The reincorporated of aboveground biomass (AGB) that is not removed from the site is eventually get into the soil as in form of plant residues and organic matter. Prunning of trees synchronizes the time of release of nutrients from the litter with crop demand. Agroforestry systems on humid and tropical sites have higher potential to carbon sequestration than the arid, semiarid, and temperate sites. Studies performed in different parts of the world stated about carbon sequestration potential of different AFS in the range of 0.29 to 15.21 Mg C ha^{-1} $year^{-1}$ in above ground and 30 to

300 Mg C ha^{-1} up to 1 m of soil depth. Existing trees on farmers' fields add some income to small and marginal farmers and also help in mitigating global warming by enhancing carbon sequestration potential of Indian agriculture.

Carbon sequestration- below ground:

The different pools of carbon such as soil organic carbon estimated at 1550pg and soil inorganic carbon amount 750 Pg both to 1m depth (Batjes 1996). Global carbon budgetis significantly affected by every change in soil C pool. Total amount of CO_2–C emitted into the atmosphere from the terrestrial ecosystems is almost calculated to be approx. 136–55 Pg, of which soils account for approx. 78–12 Pg**.**

Roots that contributes as below ground biomass comprises about one-fifth to one-fourth of the overall total living biomass and decay of dead roots constantly add organic matter to the soil that enhance the carbon status of soil**.** Soils under Leucaena leucocephala, Acacia auriculiformis, and Gmelina arborea always show high humification rate, while soils under the canopy of Acacia auriculiformis , Michelia champaca, Dalbergia sissoo and Tectona grandis, show low humification of the whole component of organic matter. The improvements in soil quality have excelled long-term sustainability and productivity of soil (Subba Rao and Saha, 2014).

Carbon sequestration through agroforestry-an effort:

Agroforestry systems on farmers' fields mitigate more than 33% of the total GHG emissions from agriculture sector annually at the country level. In order to develop strategies to sequester organic carbon it is important to study factors that affect soil organic carbon (SOC) fraction stabilization potential and the capacity of individual soils to stabilize additional SOC. Mechanisms including biochemical recalcitrance and formation of mineral complexes associated with organic substances involving fine (clay–silt) soil particles physical protection or inaccessibility enable sequestration of stable soil organic carbon. Fine soil particle is highly stable with long turnover time represents a huge proportion of the soil organic carbon. The mineral particles present in soil are positively correlated with the total SOC. Fine fraction plays a key role in furnishing mineral surface for the organo-mineral complexes Formation. Zero tillage, compost with other organics additions with residue return is enhanced to increase soil organic carbon (SOC) content. Increase in soil organic carbon status by increasing inputs or minimizing losses like for example, stubble retention direct additions of organic materials, namely, composts, manures, and other recycled organic materials. Productivity increase can be reached by different crop intensification practices such as double cropping, multiple cropping, improved

rotations, improved cultivars, and irrigation, use of fertilizers and irrigation water, therefore increase carbon dioxide emission.

Agroforestry - potential mitigation strategy:

Agroforestry when integrated with cropping and livestock systems can enhance carbon sequestration. Different practices like Home gardening, fruit orchards, riverine, hedgerows, woodlots, boundary planting sequester CO_2. The target of carbon sequestration through agroforestry can be achieved through selection, identification, and promoting of suitable agroforestry systems, ease of rules and laws, developing tree species through breeding/biotechnological tools for high carbon sequestration potential, and by providing incentives, credit facility, and insurance cover for the agroforestry practitioners. Agroforestry is considered as cost-effective strategy and have the potential to provide significant mitigation options. Agroforestry help the small farmers to adopt climate change, provides wood products, declines reliance on fertilizer use and enhance soil fertility with reducing soil loss (Buchman, 2008).

Agroforesty- tool to reclaim the salt affected soil:

In India the total geographical area is 328 Mha out of which 6.75 Mha area is salt affected, out of which contribution of 2.92 Mha are saline soils. Agroforestry play a crucial role in biological amelioration and approx. 1125 million hectors of lands are salt affected out of which 76 million hectors are human induced salinization and sodification. Due to high salinity levels one-fifth of total irrigated lands are salt affected. 50% of cultivable lands will be lost by 2050 if the salinization continues at the same rate. Major factors for soil salinity development are weathering of rocks and minerals, inclusion of sea salt in land areas, and transport in wind and rain. Human associated factors for soil salinity development includes increament of water table due to excessive irrigation utilizing underground water, irrigation with saline water and poor drainage. Agroforestry systems for saline reclamation involve multipurpose nitrogen-fixing tree species, halophytes, fruit trees and arable crops of economic importance. Several planting methods are recommended like ridge-trench, subsurface planting with furrow irrigation recommended for saline soils.

Afforestation in saline soils-selection criteria of trees species:

- Multipurpose to cater the demand of firewood, timber, food, fodder, fiber, edible or nonedible oils, medicinal products, paper pulp, ability to fix atmospheric nitrogen, etc.

- Capability to grow in salty conditions with tolerance to frost and occasional flooding.
- High water and nutrient use efficiency.
- Resilience against climate changes by providing income during extreme climate and protection of agriculture crops
- Helpful in checking soil erosion.
- Species should be in the farmers' perspective point of view.
- Greater CO_2 sequestration potential in the saline soil.
- Fast growing, quick returns systems.

Management for salt affected soil:

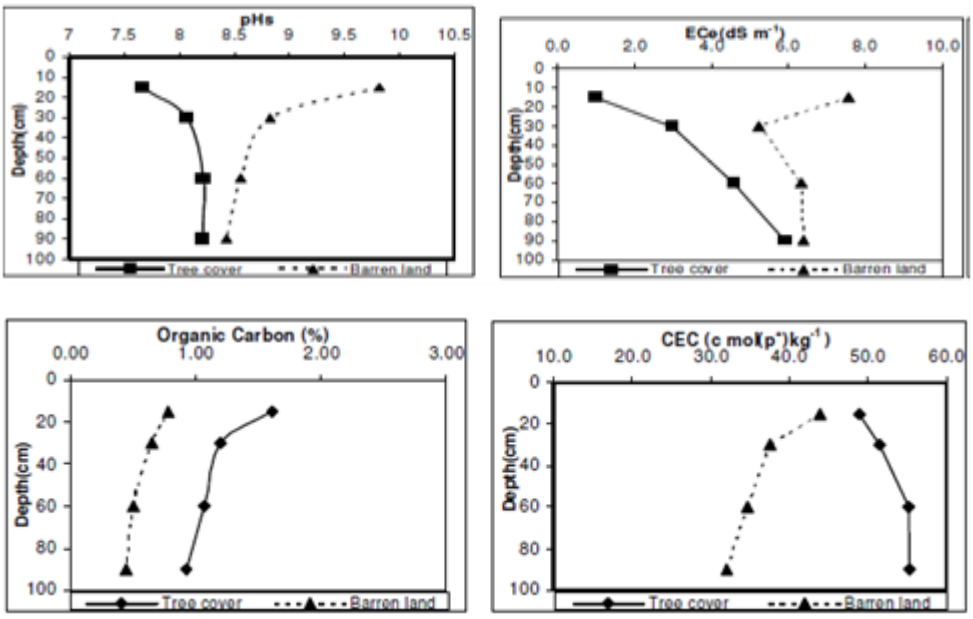
There are various control and management strategies that includes managing the existing situation, reduction of recharge, interception of water in the transmission area with increasing water use in the discharge area and support of strong national policy matter on salinity control and its management implementation are essential for tackling salinity problems.

Two action plans to control and manage soil salinization are (i) Correcting/reclaiming salinity and (ii) Adjust with salinity. First one refers to reclaim or install different drainage systems that allow salts to be removed out of the soil in combination with irrigation water management, developing salinity levels that are acceptable to productive crops. *Prosopis juliflora* is also taken as poor man's fuel wood, which is one of the most tolerant species for saline soil, alkaline soils, and waterlogged areas. It is a leguminous species having stout taproot and laterally spreading root system, acquires bushy nature in the early stages, which provides shade and litter over a relatively large area.

Effect of agroforestry on the availability of nutrient:

Tree such as planting *Prosopis juliflora* for continous ten years significantly reduced pH_s, EC_e, saturated extract cations like Ca, K and Mg contents and major anions like CO_3, Cl , HCO_3 and SO_4. Maximum decrease in sodium content and maximum enhancement in calcium content under different tree species such as *Prosopis juliflora > Acacia nilotica > D. sissoo > Eucalyptus hybrid and Prosopis juliflora > Dalbergia sissoo > Eucalyptus hybrid > Acacia nilotica* respectively. The concentration of carbonates and bicarbonates reduce significantly in surface soil i.e. from 0-15cm. The decrease of bicarbonate levels in soils under tree cover was due to increased litter fall, organic carbon content and root activities. Sulphates and chloride ions increased as the soil depth increased up to 60-90 cm in the planting sites. CEC increased as the depth increased in the planted site. The increase in CEC was due to increase in organic carbon

content of these soils. ESP increased in the planted site as the depth increased due to leaching and accumulation of sodium at lower depths (Jain *et al.*, 2002).



Conclusion:

Salt-affected soil in the world which needs to be managed for future food security. Salt tolerant cultivars of different crops and uses of salt affected lands for biomass production, Carbon & Biodiversity issues, and Ecosystem Services issues would be effective alternative solutions to combat salinity problems. Restricting the exposure of salinity levels in agricultural land and utilizing the same land for agriculture production is crucial. The overall integrated approaches to rehabilitate saline soils with income generation, food and nutritional security and environmental safety for inhabiting masses in arid and semiarid regions are done through agroforestry systems. Carbon sequestration occurs as a result of elevated carbon stocks in belowground biomass and soil.

Reference:

Batjes N.H. (1996). The total carbon and nitrogen in soils of the world. Eur J Soil Sci. 47, 151–163.

Biswas A and Biswas A.(2014). Comprehensive approaches in rehabilitating salt affected soils: A review of Indian perspective. Open Trans Geosci. 1, 13-24.

Buchman N. (2008). Agroforestry for carbon sequestration to improve small farmers' livelihoods, From the NorthSouth Centre Research for development.

Dagar, J. C., & Tewari, J. C. (2016). Agroforestry research developments: anecdotal to modern science. Agroforestry research developments. Nova Publishers, New York, 1-45.

Dagar, J. C., & Yadav, R. K. (2017). Climate resilient approaches for enhancing productivity of saline agriculture. J Soil Salinity Water Qual, 9(1), 9-21.

Dhyani, S.K., Tripathi, R.S. (2000). Biomass and production of fine and coarse roots of trees under agrisilvicultural practices in north-east India. Agrofor Syst. 50(2),107–121.

FAOSTAT (2013). FAOSTAT database. Food and Agriculture Organization of the United Nations. Available at: http://faostat.fao.org/.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey and D.W. et al.(2007). Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon S, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor and HL Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Garg, V.K. and Jain, R.K. (1996). Effect of fuel wood plantation on some properties of sodic wastelands. J Trop Forest Sci. 9,194-205.

IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change 2000: the scientific basis. Oxford University Press, Oxford.

IPCC (2019). IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse gas fluxes in Terrestrial Ecosystems. Intergovernmental Panel on Climate Change.

Jain, R.K and Singh, B.(1998). Biomass production and soil amelioration in a high density Terminalia arjuna plantation on sodic soils. Biomass Bioenergy. 15,187-92.

Munns R. and Tester M. (2008). Mechanisms of salinity tolerance. Annu Rev Plant Biol. 59,651–681.

Nair, P.K.R., Nair, V.D., Kumar, B.M. and Showalter, J.M. (2010). Carbon sequestration in agroforestry systems. Adv Agron 108,237–307.

Ripple, W.R., Wolf, C., Newsome, T.M., Barnard, P. and Moomaw, W.R., (2019). World Scientists' Warning of a Climate Emergency, BioScience. https://doi.org/10.1093/biosci/biz088.

Rao, S.A. and Saha, R. (2014). Agroforestry for soil quality maintenance, climate change mitigation and ecosystem services. Indian Farm. 63(11), 26–29.

PRODUCTION OF NOVEL INTERSPECIFIC AND INTERGENERIC POSSIBILITIES TO POTENTIAL OF SOMATIC HYBRIDIZATION

Mandaloju Venkateshwarlu

Department of Botany,

UC, KU Warangal - 09, T.S. India

Corresponding author E-mail: drvenkat6666@gmail.com

Abstract:

Novel approaches combining a simplified scheme for back crossing has been proposed. (Forster *et al.,* 2007). *In Vitro* fertilization for the production of somatic hybrids in compatibility that inhabit pollen germination and pollen tube growth. The families Solanaceae and Brassiceae contain the most commonly used species for somatic hybridization. Both interspecific and intergeneric hybrids have been obtained. Many disease resistance genes *viz.* potato leaf roll virus, leaf blight, *Verticillium*, *Phytophthora*, etc. have been transferred to *Solanum tuberosumirom* other species where normal crossings would not be possible due to taxonomic or other barriers. Resistance to blackleg disease *(Phoma lingam)* has been found in *B. nigra, B. juncea* and *B. carinata* and after production of symmetric as well as asymmetric somatic hybrids between these gene donors and *B. napus*, resistant hybrids have been developed. Production of novel interspecific and intergeneric crosses between plants that are difficult or impossible to hybridize conventionally. It overcomes sexual incompatibility barriers. For example, fusion between proto-plasts of *Lycopersicon esculentum* (tomato) and *Solanum tuberosum* (potato) Venkateshwarlu M *In Vitro* production of somatic embryo genesis (2020 & 2019). Created the pomato first achieved by Asymmetric hybrids also develop when there is partial hybridization (Fernanda, 2000). These asymmetric hybrids have abnormal or wide variation in chromosome number than the exact total of two species, Somatic regeneration and pollen viability evalution (Kakse, 2010; Ramezan *et al.*, 2011).

Keywords: Potential, Production, Interspecific & Intergeneric, Somatic Hybridization

Introduction:

A protocol for feverse breeding was proposed by wijnker *et al.* (2007). The somatic hybrids were produced by protoplast fusion of *B. oleracea* with *B. napus.* Some of the examples of incorporation of resistance genes via protoplast fusion technique have been listed in Attempts

were made to introduce tolerance in *Brassica napus* against *Alternaria brassiceae* from *Sinapis alba* and beet cyst nematode from *Raphanus sativus* Resistance has been introduced in tomato against various diseases like TMV, spotted wilt virus, insect pests and also cold tolerance reported the incorporation of disease resistance genes from wild species (Carlson, 1972; Solvey *et al*., 1994) *Solanum ochranthum*, a woody vine like tomato relative, to *L. esculentum.* reported that black rot disease caused by *Xanthomonas campestris* is a serious disease in cauliflower (Table-1.0.)

Table 1: Genetic traits transferred via protoplast fusion

Somatic Hybrids	Traits (resistance)
Nicotiana tabacum+ N. nesophila	Tobacco mosaic virus
Solanum tuberosum+ S. chacoense	Potato virus X
N. tabacum+ N. nesophila	Tobacco horn worm
S. tuberosum + S. brevidens	Potato leaf roll virus
	Late blight and PLRV Potato virus Y
S. circalifolium+ S. tuberosum	*Phytophthora*
S. melongena+ S. sanitwongse	*Pseudomonas solanacearum*
S. tuberosum+ S. commersonii	Frost tolerance
B. napus + B. nigra	*Phoma lingam*
B. oleracea+ Sinapis alba	*Alternaria brassiceae*
B. napus+ Sinapis alba	*Alternaria brassiceae*
Citrus sinensis+ Poncirus trifoliate	*Phytophthora*
Lycopersicon esculentum+ L. peruvianum	TMV, spotted wilt virus, cold tolerance
S. lycopersicoides+ L. esculentum	Cold tolerance
Solanum ochranthum+ L. esculentum	Tomato diseases and insect pests
Brassica oleracea+ B. napus	Black rot *(Xanthomonas campestris)*
B. oleracea sp. *capitata+ B. oleracea* (Ogurra CMS line)	Cold tolerance
S. tuberosum+ S. *cammersonii*	Frost tolerance
R. sativa (Japanese radish) + *B. oleracea var. botrytis*	Club rot disease
B. oleracea var. *botrytis+ S, alba + B. carinata*	*Alternaria brassicola* and *Phoma lingam*
Citrull us lanatus+ Cucumis melo	Club rot resistance
Hordeum vulgare+ Daucus carota	Frost and salt tolerance

Solanum melongena+ S. *sisymbrifolium*	Nematode
S. tuberosum+ *S. bulbocastanum*	Nematode
S. tuberosum+ S. *bulbocastanum*	Root knot nematode
Raphanus sativus+ *Brassica napus*	Beet cyst nematode
Sinapis alba+ *R. sativus* + *B. napus*	Beet cyst nematode
Quality characters	
N. rustica+ *N. tabacum*	High nicotine content
B. napus+ *Eruca sativa*	Low erucic acid
Agronomic characters (trarsferred via cybrid formation)	
N. tabacum + *N. sylvestris*	Streptomycin resistance
N. tabacum spp.	Triazine resistance
S. nigrum+ *S. tuberosum*	Triazine resistance
B. nigra + *B. napus*	Hygromycin resistance
B. napus spp.	Triazine resistance
N. tabacum+ *N. sylvestris*	CMS
B. campestris+ *B. napus*	CMS
N. tabacum+ *N. sylvestris*	CMS
B. napus + *B. campestris*+ *Raphanus sativa*	CMS and Triazine resistance
Oryza spp.	CMS
L. esculentum+ *Solanum acaule*	CMS
B. napus+ *B. tournefortii*	CMS

Efficient cell fusion can be achieved between sexually compatible and incompatible parents involving interspecific or intergeneric combination. Power *et al* (1970), Harms (1992), Puite (1992) attempts to overcome conventional breeding barriers by interspecific fusion of rice with four different wild species including *Oryza brachyantha, O. eichingeri, O. officinalis* and *O. perrieri* were more successful (Drew, 1993; Handly, 1986). Mature plants with viable pollen could be obtained in all but the first Oryza combination. The production of fertile interspecific diploid rice hybrid plants as well as cybrids demonstrates that the fusion technology can be extended to graminaceous crops (Plate-1, Table 1).

In Vitro production *Solanum melagina*, to *L. esculentum*, *Solanum nigrum* and *Solanum tuberosum.* Plate -1 Fig: 1-6

1 **3** **5**

2 **4** **6**

Figure 1 & 2: *In Vitro* production of *Solanum tuberosum*

Figure 3 & 4: *In Vitro* production of *Solanum nigrum*

Figure & 6: *In Vitro* production of *Solanum melagina*

The somatic hybrids were produced by protoplast fusion of *B. oleracea* with *B. napus.* Some of the examples of incorporation of resistance genes via protoplast fusion (Zarkal *et al*., 2002; Vasil, 2002; Tudses *et al.*, 2014, 2015) technique have been listed in Attempts were made to introduce tolerance in *Brassica napus* against *Alternaria brassiceae* from *Sinapis alba* and beet cyst nematode from *Raphanus sativus* Resistance has been introduced in tomato against various diseases like TMV, spotted wilt virus, insect pests and also cold tolerance. Kobayashi *et al.* (1996) and Xhu *et al.* (2003) reported the incorporation of disease resistance genes from wild species (Tiwari *et al*., 2010, 2011). *Solanum ochranthum,* a woody vine like tomato relative, to *L. esculentum.* Reported that black rot disease caused by *Xanthomonas campestris* is a serious disease in cauliflower. *Abiotic stress resistance:*

Work related to somatic hybridization for abiotic stress has been mainly done on families Fabaceae, Brassicaceae, Poaceae, and Solanaceae and relates to cold and frost resistance

(Grosser, 2011; Barbara *et al.*, 2015). Developed somatic hybrids between cultivated potato (S. *tuberosum)* and wild relative *(S. acaule)* possessing several disease and early frost resistance characters (Cooking, 1960; Duke, 1981; Galbraith *et al*., 1980).

Somatic hybrids produced between *B. napus* and *Eruca sativa* were fertile and had low concentration of erucic acid content (Ahlowalia, 1991: Amberger *et al*., 1992; Bevers dorf *et al.,* 1977; Bellin, 1987). This hybrid material has been introduced into breeding program. Likewise, nicotine content character has been transferred to *N. tabacum.* Several agriculturally useful traits are cytoplasmically encoded, Butenko (1980) including some types of male sterility and certain antibiotic and herbicide resistance factors (Glinielius *et al*., 1978; Binding, 1974). Reported *Brassica raphanus* cybrids that contain the nucleus of *B. napus*, chloroplasts of atrazine resistant *B. campestris* and mitochondria that confer male sterility from *Raphanus sativus.* Cybridization has been successfully used to transfer cytoplasmic male sterility in rice produced cold tolerant cytoplasmically male sterile (CMS) cabbage *(Brassica oleracea* sp. *capitata)* by the fusion of cabbage protoplasts with cold tolerant ogura CMS broccoli lines. reported that CMS chicory *(Cichorium intybus)* cybrids have been obtained by fusion between chicory and CMS sunflower protoplasts (Christianson, 1983; Baird *et al.*, 1992; Venkateshwarlu, 2020). Resistance to antibiotics, herbicide as well as CMS has been introduced in so many cultivated species (Table 1.1)

3. Production of autotetraploids: Somatic hybridization can be used as an alternative to obtain tetraploids and, if this is unsuccessful, colchicine treatment can be used.

4. Protoplasts of sexually sterile (haploid, triploid, aneuploid, etc.) plants can be fused to produce fertile diploids and polyploids.

5. Hybridization becomes possible between plants that are still in the juvenile phase.

6. Production of heterozygous lines within a single species that normally could only be propagated by vegetative means, e.g. potato and other tuber and root crops.

7. Somatic cell fusion is useful in the study of cytoplasmic genes and their activities: This information can be applied in plant breeding experiments.

8. The production of unique nuclear cytoplasmic combinations: Evidence from numberof somatic hybrids suggests that although two types of cytoplasm are initially mixed during protoplast fusion, resulting in heteroplasmons, eventually one parent type cytoplasm predominates, resulting in cytoplasmic segregation (Ghazi, 1986; Hansen, 1999). Mitochondrial and chloroplast recombination has also been reported to result in unique nuclear-cytoplasmic combinations (Henry, 1998; Kpbayshi, 1996). These unique combinations using protoplasts will aid the development of novel germplasm not obtainable using conventional methods.(Table- 1.0 & 1.1)

Table 1.1: Intergeneric hybrids produced through protoplast fusion.

Plant species with chromosome number	New genes
Raphanus sativus (2n = 18) +*B. oleracea* (2n = 18)	Raphanobrassica
B. oleracea (2n = 18) +*Moricandia arvensis* (2n = 27, 28)	Moricandiobrassica
Eruca sativa (2n = 22) +*B. napus* (2n = 38)	Erucobrassica
Diplotaxis muralis (2n = 42) +*B. napus* (2n = 38)	Diplotaxobrassica
Nicotiana tabacum (2n=24) +*Lycopersicon esculentum* (2n=24)	Nicotiopersicon
Solanum tuberosum (2n = 24) +*L. esculentum* (2n - 24)	Solanopersicon
Datura innoxia (2n = 48) +*Atropa belladona* (2n = 24)	Daturotropa
Oryza sativa (2n = 24) +*Echinochloa oryzicola* (2n = 24)	Oryzochloa
Arabidopsis thaliana (2n = 10) +*B. campestris* (2n = 20)	Arabidobrassica

Problems and limitations of somatic hybridization:

There are limitations, however, to the use of these types of somatic hybridization since plants regenerated from some of the combinations are not always fertile and do not produce viable seeds (Venkateshwarlu, 2021). The successful agricultural application of somatic hybridization is dependent on overcoming several limitations (Venkateshwarlu, 2019).

1. Application of protoplast methodology requires efficient plant regeneration from protoplasts. Protoplasts from any two species can be fused. However, production of somatic hybrid plants has been limited to a few species.
2. The lack of an efficient selection method for fused product is sometimes a major problem.
3. The end-products after somatic hybridization are often unbalanced (sterile, misformed, and unstable) and are therefore not viable, especially if the fusion partners are taxonomically far apart.
4. The development of chimaeric calluses in place of hybrids. This is usually due to the nuclei not fusing after cell fusion and dividing separately. Plants that are regenerated from chimeras usually lose their chimeric characteristics, since adventitious shoots or embryos usually develop from a single cell.
5. Somatic hybridization of two diploids leads to the formation of an amphidiploid which is generally unfavorable (except when tetraploids are formed intentionally). For this reason, in most cases, the hybridization of two haploid protoplasts is normally recommended.
6. Regeneration products after somatic hybridization are often variable due to somaclonal variation, chromosome elimination, translocation, organelle segregation etc.

7. It is never certain that a particular characteristic will be expressed after somatic hybridization.
8. The genetic stability during protoplast culture is poor.
9. To achieve successful integration into a breeding program, somatic hybrids must be capable of sexual reproduction. In all cases reported, somatic hybrids containing a mixture of genes from two species must be backcrossed to the cultivated crop to develop new varieties. All diverse inter generic somatic hybrids reported are sterile and therefore have limited value for new variety development. It may be necessary to use back-fusion or embryo culture to produce gene combinations that are sufficiently stable to permit incorporation into a breeding program.
10. To transfer useful genes from a wild species to a cultivated crop, it is necessary to achieve intergeneric recombination or chromosome substitution between parental genomes (Table 1.2)

Table 1.2: Kao and Michyaluk 8p medium for protoplast culture

Component	Concentration
Mineral Salts (mg/l)	
NH_4NO_3	600
KNO_3	1900
$CaCl_2.\ 2H_2O$	600
$MgSO_4.\ 7H_2O$	300
KH_2PO_4	170
KCl	300
Sesquestrene@330Fe	28
KI	0.75
H_3BO_3	3.00
$MnSO_4.\ H_2O$	10.00
$ZnSO_4.\ 7H_2O$	2.00
$Na_2MoO_4.\ 2H_2O$	0.25
$CuSO_4.\ 5H_2O$	0.025
$CoCl_2.\ 6H_2O$	0.025
Vitamins and carbohydrates (mg/l)	
Inositol	100
Nicotinamide	1

Pyridoxine HCl	1
Thiamine HCl	1
D- Calcium pantothenate	1
Folic acid	0.4
p- Amino benzoic acid	0.02
Biotin	0.01
Choline chloride	1.00
Riboflavin	0.20
Ascorbic acid	2.00
Vitamin A	0.01
Vitamin D_3	0.01
Vitamin B_{12}	0.02
Sucrose and Glucose	0.25
Sucrose	68.4
Glucose	
Hormones (mg/l)	
2, 4-D	0.2
Zeatin	0.5
NAA	1.0
Organic acids (mg/l) adjusted to pH 5.5 with	20
NH_4OH	40
Sodium pyruvate	40
Citric acid	40
Malic acid	
Fumaric acid	
Other sugars and Sugar alchohols (mg/l)	
Fructose	250
Ribose	250
Xylose	250
Mannose	250
Rhamnose	250

Cellobiose	250
Sorbitol	250
Mannitol	250
Amino acids (mg/l)	
Glycine	0.1
Alanine	0.6
Cysteine	0.2
Glutamine	5.6
Glutamic acid	0.6
Aspargine	0.1
Nucleotides (mg/l)	
Adenine	0.10
Guanine	0.03
Thymine	0.03
Uracil	0.03
Hypoxanthine	0.03
Xanthine	0.03
Others	
Casein hydrolysate	250 mg/l
pH	5.6
Coconut water	20 ml/l

Protocol:

Protocol for protoplast isolation and fusion:

Plant material: *Nicotiana tabacum*

1. Detach fully expanded leaves including petiole. Sterilize in 10% sodium hypochlorite solution containing two drops of Tween 20 for 10 min. Wash 3-4x with sterile distilled water and blot dry on sterile tissue paper.
2. Peel the lower epidermis from sterilized leaves with fine forceps and cut into pieces with a fine scalpel.
3. Transfer the leaf pieces (peeled areas only) with lower surface down into 30 ml of 13% mannitol-inorganic salts of cell and protoplast washing media (CPW) solution contained

in a petri dish for 30 min-1 h for plasmolysis. Composition of CPW salt solution is as follows:

KH_2P0_4	27.2 mg/l
KNO_3	101.0 mg/l
$CaCl_2.2H_2O$	1480.0 mg/l
$MgS0_4.7H_2O$	246.0 mg/l
Kl	0.16 mg/l
$CuS0_4.\ 5H_20$	0.025 mg/l
pH	5.8

4. Remove the mannitol-CPW salt solution with a Pasteur pipette and replace it with filter sterilized enzyme mixture in 13% mannitol solution (~ 20 ml). Enzyme mixture contains macerozyme (0.1-0.5%) and cellu-lase (0.5 1%).
5. Gently agitate the leaf pieces with sterile Pasteur pipette to facilitate the release of protoplasts, pushing the larger pieces of leaf material to one side and keeping the petri dish at an angle of 15°C or remove the larger debris by filtering through a 60-80 urn mesh.
6. Transfer the filtrate to a screw cap centrifuge tube.
7. Centrifuge at 100x g for 5-10 min to sediment the protoplasts. Remove the supernatant and resuspend the protoplast pellet in CPW + 21 % sucrose (prepared in CPW) and gently disperse the protoplasts in the solution.
8. Centrifuge again at 100x g for 10 min. The viable protoplasts will float to the surface of the sucrose solution while the remaining cells and debris will sink to the bottom of tube.
9. Remove the band of protoplasts from the top with a Pasteur pipette and transfer into another centrifuge tube. Resuspend in CPW + 10% mannitol. Centrifuge again at 100x g for 10 min to separate the contaminating debris.
10. Repeat the washing procedure at least 3 times.
11. After the final washing add enough MS protoplast culture medium with 9% mannitol to achieve a protoplast density of ~ 5 x 10^4/ml. Kao and Michayluk (1975) media has been widely used for protoplast culture (Table 9.5). Plate the protoplasts as small droplets.
12. (100-150 µl) or a thin layer in small petri dishes for 24 h at 25°C. Keep the cultures at low light intensity of 500 lux for the next 2 days and then for rest of the experiment at 2000 lux.
13. Protoplasts form cell walls and begin to divide after 3-5 days.
14. Protoplasts may also be cultured in an agarose medium by mixing 1.5 ml of protoplast suspension with an equal volume of 1.2 % agarose medium at 45^o C in 35 mm petri dish.

15. Cultures continue to grow in the media and the first colonies become visible after 3-4 weeks.
16. Transfer the colonies to new medium with a reduced mannitol level.
17. Transfer the colonies to a suitable medium for embryo differentiation and plantlet regeneration.

Protoplast fusion:

Fusion treatment: PEG

Material: Green leaf (mesophyll) and cultured cells (albino) of *N. tabacum.*

1. Perform the fusion experiment in continuation with protoplast isolation experiment.
2. Take 4-6 ml of freshly isolated protoplast suspension from two sources in CPW salt medium – 13% mannitol (CPW 13M) with a density of 5 x 10^5/ml.
3. Using a Pasteur pipette place a small drop (-50 µl) of sterile silicone on to the centre of a plastic petri dish (35 mm) and gently lower a cover glass on to the drop of silicone fluid.
4. Pipette -150 µl of protoplast suspension directly onto the middle of cover glass and allow it to settle for 10-15 min. (Protoplasts suspended in CPW 13M for convenience are mixed in screw capped centrifuge tube in a 1:1 ratio).
5. Using a Pasteur pipette add 450 µl of PEG fusion solution drop wise to the edge of the protoplast culture, placing the last drop in the centre. PEG fusion solution contains
   22.5% (w/v) PEG molecular weight 6000
   1.8% (w/v) sucrose
   154 mg/l $CaCl_2$. $2H_2O$
   9.52 mg/l KH_2PO_4
   pH 5.8 (adjust with 1M KOH or IN HCL, autoclaved and stored in dark at 4^oC).
6. Leave the protoplasts undisturbed for 20-40 min at room temperature and then add one drop of washing solution very gradually whilst withdrawing some of the fusion solution from the coalesced drops. Repeat this procedure at 5 min intervals for the next 20 min. during this procedure it is important that the protoplasts are subjected to minimal disturbance. Washing solution: CPW 13M medium to which 0.74g/l $CaCl_2$. $2H_2O$ is added (sterile).
7. Five minutes after the last wash, carefully suck off the solution with a pasteur pipette leaving leaving the protoplasts with a thin film of medium and replace it with culture medium (MS protoplast medium with 9% mannitol). Wash at least 3 times with culture medium.
8. For the culture of protoplasts flood the cover glass with culture medium and scatter several drops of medium on the base of petri dish prior to sealing with parafilm.

9. Incubate the cultures initially in the dark for 24 h at 25^{o}C and then transfer to white light (1000 lux). The fusion products can be easily observed using an inverted microscope.
10. Subsequently transfer the colonies for regeneration.
11. Test the hybridity of fusion product and somatic hybrids as per equipment available in the lab, which has been explained in the text.

Conclusion:

A very efficient tool for the production of completely Intragenic, donor plants, *in vitro* angrogenesis somatic hubridization in crop plants have been gradually developed and constantly improved. The technique is sufficiently elaborated species vegetable crop plants, is gradually also being developed for the plant species. The plants produced are referred as Gynogenic Haploids. *In vitro* production for distant hybrids. The breeding of polyploid species at the diploid level and crossing with related cultivated or wild diploid species

References:

Ahloowalia BS. (1991): Somatic embryos in monocots. Their genesis and genetic stability. Rev. Cytol. Biol. Veget. Bot. 14:223-225.

Amberger LA, Palmer RG, Shoemaker RC. (1992): Analysis of culture-induced variation in soybean. Crop Sci.; 32:1103-1108

Barbara Piwowarczyk, and Anna Pindel, (2015): Determination of an optimal isolation of culture conditions of grass pea protoplasts, Biotechnologia , 96 (2), 192 – 201

Bellincampi D, Morpugo G. (1987): Conditioning factor affecting growth in plant cells in culture; Plant Sci.; 51:83-91

Beversdorf WD, Bingham ET. (1977): Degrees of differentiation obtained in tissue cultures of Glycine species. Crop Sci. 17:307-311.

Binding H. (1974): Cell cluster formation by leaf protoplasts from axenic cultures of haploid.

Butenko R, Kuchko AA. (1980): Physiological aspects of procurement, cultivation and hybridization of isolated potato protoplasts Sov. Plant Physiol. (English Transl.). 26:901-909.

Carlson P. S., H. H. Smith, and R. D. Dearing (1972): Parasexual interspecific plant hybridization, Proc. Natl. Acad. Sci. USA., 69, 2292 – 2294.

Christianson ML, Warnick DA, (1983): Carlson PS. A morpho genetically competent soybean suspension culture. Science. 1983; 222:632-634.

Cocking EC. (1960): A method for the isolation of plant protoplasts and vacuoles; Nature (London). 1960; 187:927-929

Duke JA. (1981): Handbook of legume of world Economics importance. Plenum Press, New York.

Fernanda Januzzi Mendes-da-Gloria, F. de A. A. M. Filho, L. E. A. Camargo, and B. M. J.Mendes (2000)*:* Caipira sweet orange + Rangpur lime : a somatic hybrid with potential for use as root stock in the Brazilian citrus industry, Genet. Mol. Biol., 23(3), 661 – 665.

Forster BP Herberle Bors (2007). The resurgeve of haploids in higher plants. Trends in plant science 12(8) pp: 368-375.

Gaj MD. (2001): Direct somatic embryogenesis as a rapid and efficient system for *in vitro* regeneration of Arabidopsis thaliana. Plant Cell Tiss. Organ Cult. 2001; 64:39-46.

Ghazi TD, Cheema HV, Nabors MW. (1986): Somatic embryo genesis and plant regeneration from embryonic callus of soyabean (*Glycine max* (L). Marr.), Plant physiol. 77:863-868.

Grosser J. W., and F. G. Gmitter Jr., (2011) *:* Protoplast fusion for production of tetraploids and triploids: application of scion and rootstock breeding in Citrus, Plant Cell Tiss. Org. Cult., 104, 343 – 357.

Handley L. W., and T. Kumashiro (1986): Transfer of crop traits by protoplast fusion techniques, Agri. Cell Rep., 6(6), 42 – 46.

Hansen G, Wright MS. Recent advances in the transformation of plants, Trends plant Sci. 1999; 4:226- 231.

Harms C. T., *"*Engineering genetic disease resistance into crops : biotechnological approaches to crop protection*"*, Crop Protec., 11, 291 – 306 (1992).

Henry RJ, Nato A, De Buyser J. Genetic fidelity of plants regenerated from somatic embryos of cereals. In: Jain SM, Brar DS, Ahloowalia BS (EDS) Somaclonal variation and induced mutations in crop improvement. Kluwer Academic Publishers, Dordrecht, Netherlands, 1998, 65-80.

Jaksem (2010). Evolution of Gynogenic Responsiveness and pollen viability of selfed doubled Haploid onion lines and chromosome doubling via somatic regeneration Jouranal of American society for Horticultural Science 135(1) pp: 67-73.

Mandaloju Venkateshwarlu (2020) Shoot induction from Trendril explants of Luffa actangula L A Vegetable crop plant. EJBPS Col.7 Issue:02, pp: 327-329

Mandaloju Venkateshwarlu (2021), Concentration of cytokinine shoot induction from stem node explants of Luffa acutangula (L) International Journal of Current Research Vol.13 Issue 01. Pp 15519-15521.

Puite K. J., *"*Progress in plant protoplast research*"*, Physiologia Plant., 85, 403 – 410 (1992).

R. Drew, *"*Application of tissue culture to plant breeding*"*, Proc. 10th Australiam Conf. on Plant Breeding, Gold Coast, Queensland, Australia, 102 – 115 (1993).

Ramezan Rezazadeh, D. K. Harrison, and R. R. Williams (2011): Intraspecific somatic hybridization of mango (*Mangifera indica* L.) through protoplast fusion, J. of App.Horti.,13(2), 101 – 107.

Solvey Rother, B. Hadelar, J. M. Orsini, W. O. Abel, and R. Reski, (1994): Fate of a mutant macrochloroplast in somatic hybrids, J. Plant Physiol., 143, 72 – 77.

Szarka B., I. Gonter, M. Molnar-Lang, and M. S. Duditso (2002): Mixing of maize and wheat genomic DNA by somatic hybridization in regenerated sterile maize plants, Theor. App. Genet., 105,1 – 7.

Tiwari J. K., D. Sarkar, Poonam, S. Sharma, and J. Gopal (2011): *Solanum tuberosum* (+) *Solanum pinnatisectum* somatic hybrids : a new source of horizontal resistance to potato late blight in India*"*, Sol. Newsletter, 4, 4 – 10.

Tiwari J. K., Poonam, D. Sarkar, S. K. Pandey, J. Gopal, and S. R. Kumar (2002): Molecular and morphological characterization of somatic hybrids between *Solanum tuberosum* L. & *Solanum etuberosum* Lindl.*"*, Plant Cell Tiss. Organ Cult., 103, 175 – 187 (2010).

Tudses N., S. Premjeet, and D. Premjet (2015): Establishment of method of PEG mediated protoplast fusion between *Jatropha curcas and Ricinus communis*, Int. J. Life Sci. Biotech. & Pharma. Res., 4(1), 50 – 56.

Tudses N., S. Premjet, and D. Premjet (2014): Optimal conditions for high yield protoplast isolations of *Jatropha curcas L. and Ricinus communis* L., American – Eurasian J. Agric. Environ. Sc., 14(3), 221 – 230.

Vasil I. K., (2002): Plant Biotechnology and beyond, Proc. 10th IAPTC & B Congress,Orlando, Florida, USA, Kluwer Acad. Publ., Dordrecht, The Netherlands(2002).

Venkateshwarlu M (2021): *In Vitro* multiplication from stem node explants of *Trichosanthes anguiva* L A medicinal important plant (IJSEAS) International Journal of Scientific engineering and Applied Science Vol.6 Issue-10 pp 1-4.

Venkateshwarlu M, Dinesh Khanna N and Ugender T. (2019): Protoplast Isolation of soybean *Glycine max* (L) Merrill from leaf explants. The Pharma Innovation Journal NAAS Rating pp: 368-373.

Xhu C., G. Xia, D. Zhi, F. Xiang, and H. Chem, (2003): Integration of maize nuclearand mitochondrial DNA into the wheat genome through somatic hybridization, Plant Sci., 165, 1001 – 1008.

BACTERIOCIN (NISIN): PRODUCTION AND IT'S APPLICATION

Savita Hulamani*, Nataraj A. Durgannavar and Uma Ballolli

Department of Food Processing and Nutrition,

Karnataka State Akkamahadevi Womens University,

Vijayapura, Karnataka, India

*Corresponding author E-mail: savitahulamani@gmail.com

Introduction:

Bacteriocins are protein chaves toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are generally considered to be narrow spectrum antibiotics. They are phenomenologically analogous to yeast. They are also like paramecium killing factors, and are structurally, functionally, and ecologically divergent. Bacteriocins are various group of antimicrobial peptides and are an important characteristics in the selection of probiotic strains. The vast majority of all bacteria and archaea produce at least one bacteriocin. Lactic acid bacteria (LAB) have wide role in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics. (leory *et al.*, 2004)

In 1925, a scientist called Andre Gratia discovered Bacteriocins for the first time. He was indulged in the process of scouting for ways to kill bacteria, which also resulted in the development of antibiotics and the discovery of bacteriophage, all within a span of a few years. The scientist called his first invention a *colicine* because it killed *E. coli*. Bacteriocins are of interest in medicine because they are made by non-pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body.

Bacteriocins have also been applied for a cancer treatment. They have shown distinct promise as a diagnostic agent for some cancers, but their status as a form of therapy remains experimental and outside the main thread of cancer research Bacteriocins were tested against AIDS drugs (around 1990) but not progressed beyond in-vitro tests on cell lines.

Classification of bacteriocins:

Bacteriocins are divided into four major groups. First three named groups are more describred than the fourth one. This classification is based on their primary structure and mode of action.

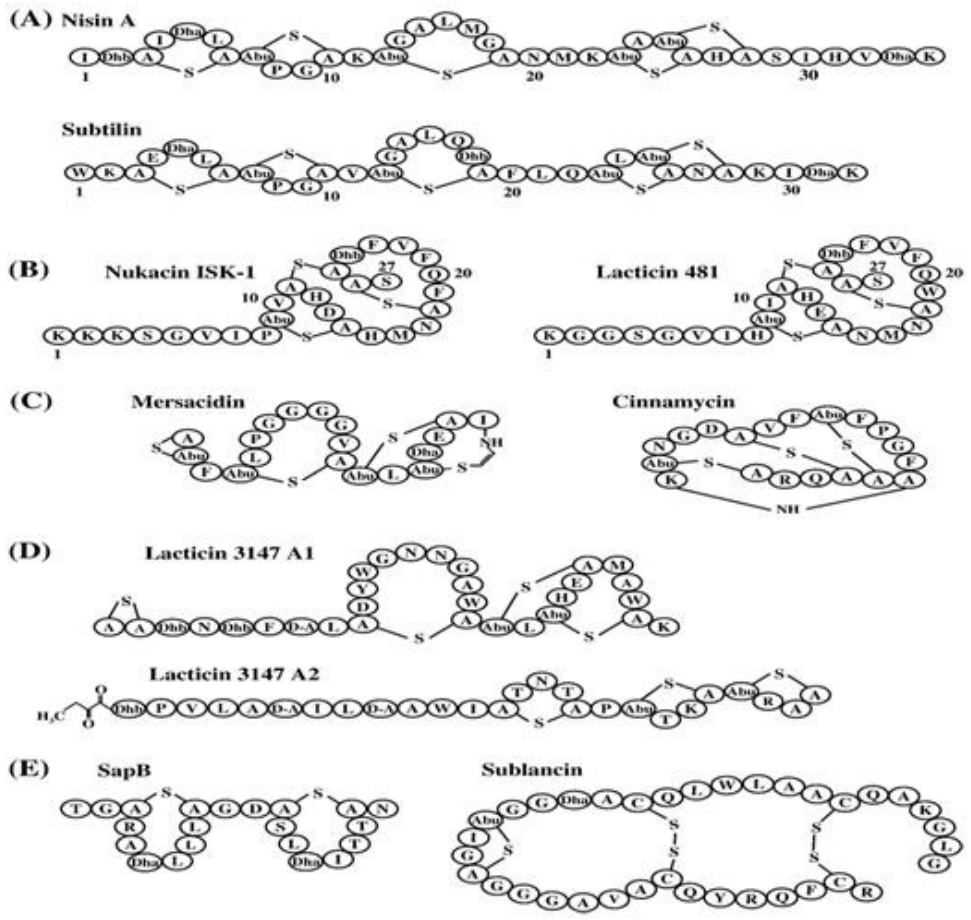


Figure 1: Chemical structure of different bacteriocins

Class I bacteriocins: The class I bacteriocins are small peptide inhibitors and include nisin and other lantibiotics. They are further divided into two subgroups on the basis of structure and charge of the compound: They are

- Group Ia :Which consists of screw-shaped, amphipathic, having small cationic peptides that produce voltage-dependent pores by unspecific interaction with the membrane of the target cell;
- Group Ib : Which consists of anionic or neutral peptides having a globular shape

Class II bacteriocins: This group comprises heat-stable peptides with molecular masses smaller than 10 kDa and with no modified amino acids. Members of this class can be further sub-classified into three groups:

- Group IIa : Consists of anti-listerial peptides showing the consensus sequence YGNGV at their N-terminal sequence. Class IIa bacteriocins have a large potential for use in food preservation as well medical applications, due to their strong antilisterial activity, and broad range of activity.
- The class IIb: Bacteriocins (two-peptide bacteriocins) require two different peptides for activity.

- Class IIc (circular bacteriocins): This class of bacteriocins has a wide range of effects on membrane permeability, cell wall formation and pheromone actions of target cells.

Class III bacteriocins: This group consists of peptidic antibiotics that are heat-Sensitive proteins along with a molecular mass more than 30 k Da.

Class IV bacteriocins: This group of bacteriocins consists of either glycoproteins or lipoproteins that require non-protein moieties for their activity.

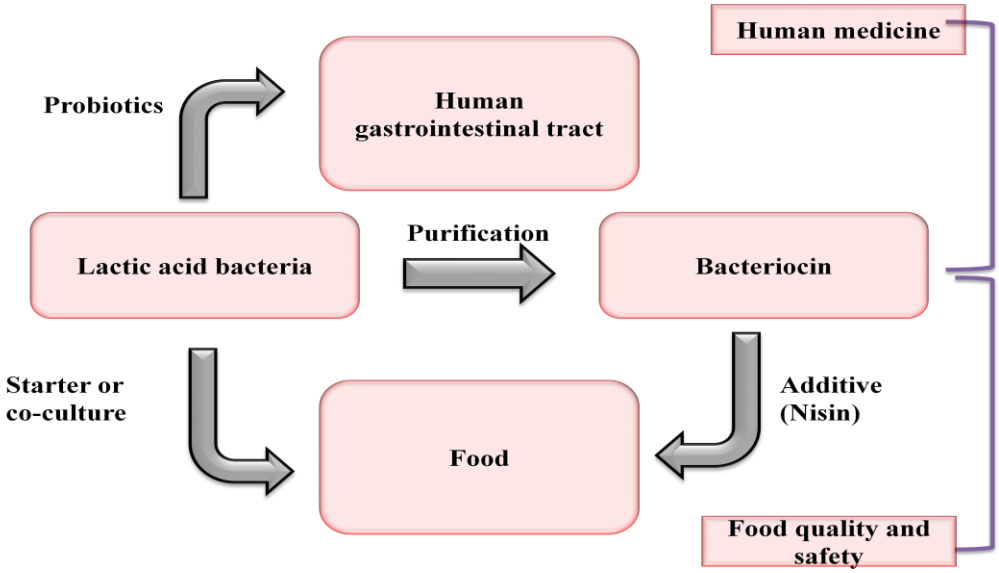


Figure 2: Overview of the application potential of bacteriocin production by LAB in food quality and safety and in medicine, emphasizing their role as food ingredient and in the human gastrointestinal tract

Application of bacteriocins:

- Enterocin AS-48 used in preservation of green asparagus, soybean sprouts.
- Nisin with sodium acetate help in melon preservation.
- Bacteriocins used in preservation milk, cheese, apple juice.
- Nisin A and Pediocin used in preservation of meat spoilage bacteria (L.m,C.b,L.v).
- Nisin is used to preserve cheese and tender coconut water.
- Bacteriocin Preparations in Biocontrol -Effects of syringacin 4A on infection of bean by *P. syringae pv. Phaseolicola* .
- Alcoholic beverages -Nisin can be added to fermenters to prevent or control contamination and can also be used to increase the shelf-life of unpasteurised and bottle-conditioned beers.(LAB).
- Canned foods Nisin is used in canned foods mainly for the control of thermophilic spoilage.
- Peiocin PA-1/ACH – reduce the population of *Listeria* in ice cream mix, sausages mix, ground beef an milk.

- Bacteriocin are also used in sugar processing industries, seed treatment, antibacterial cream, cosmetics, mouth wash and tooth paste.
- Nisin in often used to preserve acidic foods.
- The *Streptococcus. Thermophilus* produced bacteriocin may be especially useful in the control of food spoilage caused by *pediococci* in food processing industries.

Advantages of Bacteriocins:

- Bacteriocins Produce lactic acid that helps to lowers the pH of intestines and inhibiting bacterial villains such as *Clostridium, Salmonella*, *Shigella*, *E. coli*, etc.
- Bacteriocin also decreases the production of a variety of toxic or carcinogenic metabolites.
- Bactereriocin aids in absorption of minerals in human beings especially calcium, due to increased intestinal acidity.
- Bacteriocin also favors in Production of β- D- galactosidase enzymes that break down lactose .
- Bacteriocin Produce a wide range of antimicrobial substances such as acidophilin and bacteriocin etc. that helps to control pathogenic bacteria.
- Bacteriocin also act as fence to prevent harmful bacteria from colonizing the intestines.

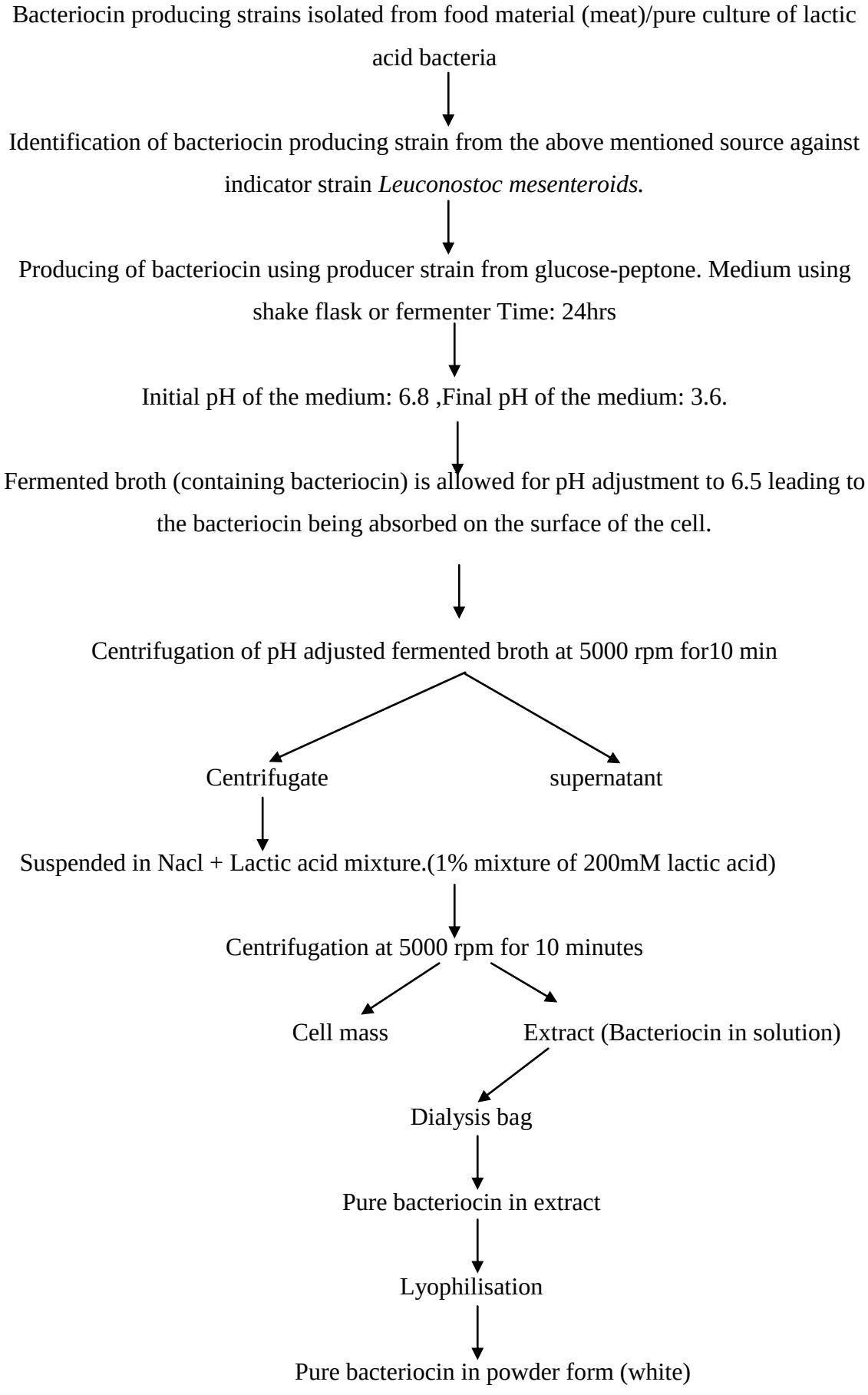
Bacteriocins *vs*. Antibiotics:

Characteristic	Bacteriocins	Antibiotics
Application	Food	Clinical
Synthesis	Ribosomal	Secondary metabolite
Activity	Narrow spectrum	Varying spectrum
Defence from host cell immunity	Yes	No
Mechanism of target cell resistance or tolerance	Usually adaptation affecting cell membrane composition	Usually genetically transferable determinant affecting different sites depending the mode of action
Interaction requirements	Sometimes docking molecules	Specific targets
Mode of action	Mostly pore formation, but in few cases possibly cell wall biosynthesis	Cell membrane or intracellular targets
Toxicity, side effects	Not known	Yes

Application of Nisin in foods:

Products	Level of nisin (mg/kg)	Benefits
Dairy products		
Pasteurized milk	0.05gm/kg	2 times
Second disinfected milk	0.08gm/kg	No bacteria standard
Sour milk and fruit milk	0.05gm/kg	6 days to 1 month
Bacteria-free packing milk	0.05gm/kg	Reduced putrefaction from 0.04% to 0%
No-sugar canned condensed milk	0.08gm/kg	Decrease 10 minutes in heat process time
Flavor milk	0.08gm/kg	6 weeks
Cheese	0.05gm/kg	Solve the putrefaction
Plant protein foods		
Soya-bean milk	0.1gm/kg to 0.15gm/kg	3 times
Lactones bean curd	0.1gm/kg	5 times
Other food preservatives	0.1gm/kg	6 times
Peanut milk	0.1gm/kg	>1 month
Liquid Egg And Egg-contained Products	0.05gm/kg to 0.1gm/kg	7 days to over 1 month
Meat Foods	0.05gm/kg to 0.15gm/kg	Expand the storage period to >3 months
Marine products	0.1gm/kg to 0.15gm/kg	5 times
Canned food	0.1gm/kg	Prolong the food storage to 2 years, saves the energy, improves the nutritional value.
Fruit Juice Drinks	0.05gm/kg to 0.1gm/kg	Prevent the growth and reproduction of the survival alicyclobacillus spores and guarantee the quality of products
Salad Sauce And Dressings	0.05gm/kg to 0.2gm/kg	over 3 times
Beer	0.25 to 1.25	Over 3 times
grape wine	0.1gm/kg	Prevent the pollution caused by lactobacillus brevis, L. casei and Leuconostoc spp.

Flow diagram of bacteriocin production from lactic acid and its purification:



Bacteriocins produced by different Lactobacillus species:

Bacteriocins produced by different Lactobacillus species.	
Substance	**Producing Organism**
Curvalicin	*Lactobacillus curvatus*
Acidocin J1132 β	*Lactobacillus acidophilus*
Plantaricin S β	*Lactobacillus plantarum*
Bacteriocin J46	*Lactococcus lactis*
Lacticin 481 (Lactococcin DR)	*Lactococcus lactis subsp (Streptococcus lactis)*
Lactocin-705	*Lactobacillus paracasei*
Nisin	*Lactococcus lactis subsp (Streptococcus lactis)*
Plantaricin C19	*Lactobacillus plantarum*
Lactocin-S	*Lactobacillus sakei*
Lactococcin MMFII	*Lactococcus lactis subsp (Streptococcus lactis)*
Curvaticin FS47	*Lactobacillus curvatus*
Bavaricin	*Lactobacillus sakei*
Curvacin-A	*Lactobacillus curvatus*
Sakacin-A, Sakacin-P	*Lactobacillus sakei*
Lactococcin-B	*Lactococcus lactis subsp (Streptococcus cremoris)*
Lactobin-A (Amylovorin-L471)	*Lactobacillus amylovorus*
Lactacin-F (lafA)	*Lactobacillus johnsonii*
Plantaricin W α	*Lactobacillus plantarum*
Plantaricin 1.25 β	*Lactobacillus plantarum*
Acidocin B (AcdB)	*Lactobacillus acidophilus*
Reutericin	*Lactobacillus reuteri*

Few of the examples were nisin is incorporated into the product while preparing.

1. **Step wise production of fermented poi from taro corms**

Remove outer skins of taro corms

↓

Cut the corm into pieces

↓

Grind the pieces of corm

↓

Dry the ground taro

↓

Dilute the ground taro with water

↓

Cook the taro and water

↓

Inoculate the cooked taro and water with a bacteriocin producing bacterium

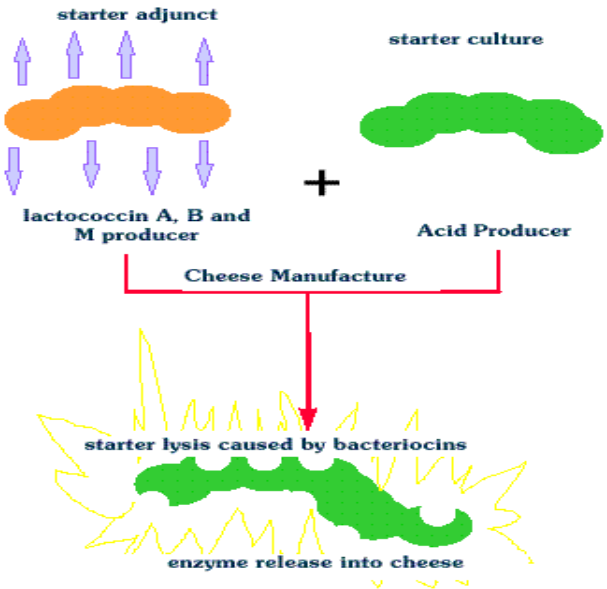
↓

Permit the taro to ferment

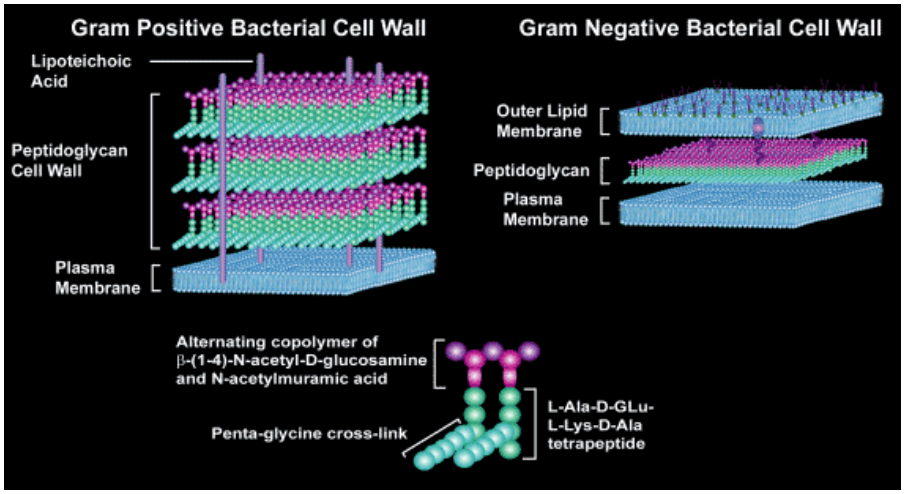
↓

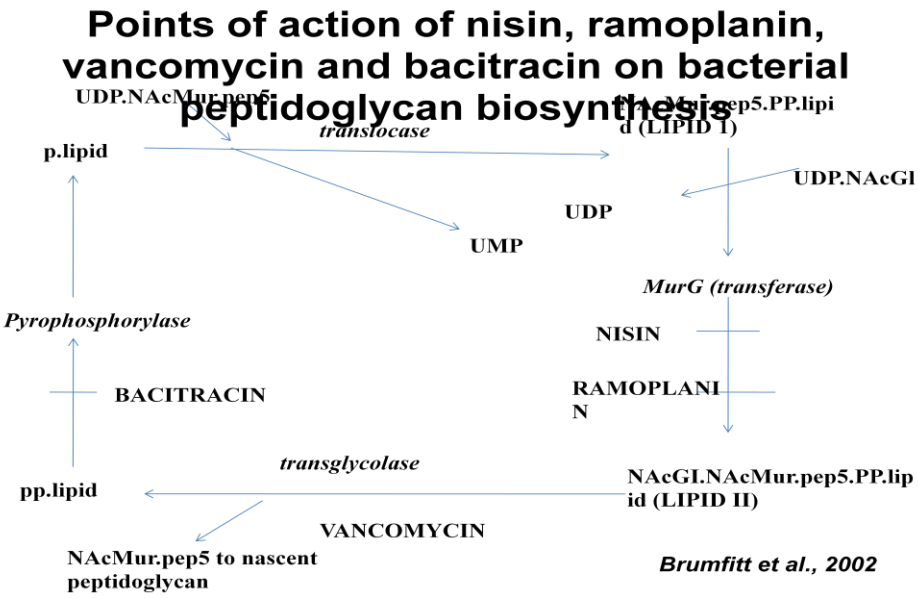
To produce fermented poi

2. **Cheese manufacture:**



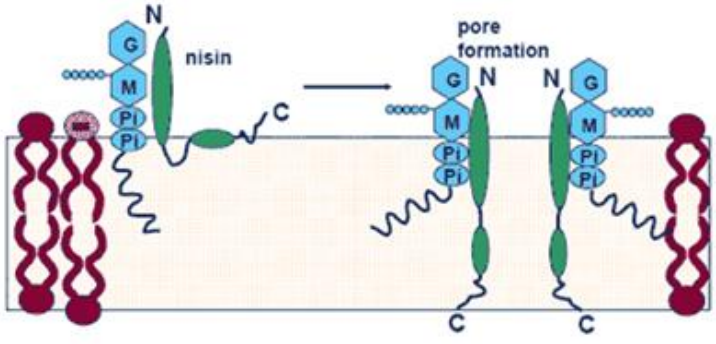
Bacterial cell wall:

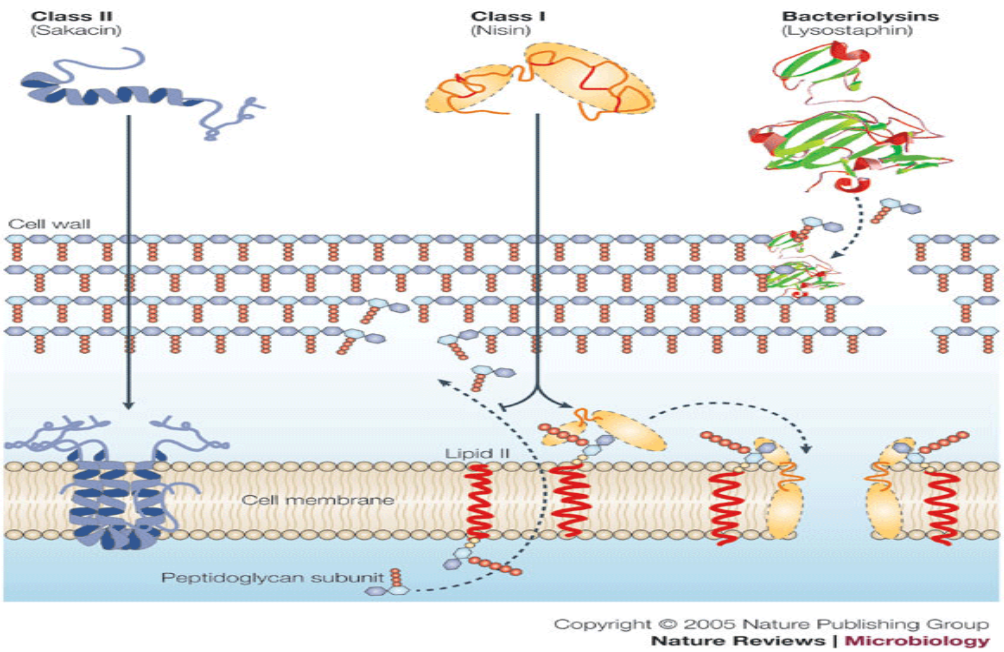




Nisin mode of action against the cytoplasmic membrane:

Nisin binds to the carbohydrate moiety of the cell wall precursor lipid II, using it as a docking molecule prior to pore formation. Diagram of cell membrane showing phospholipid bilayer.



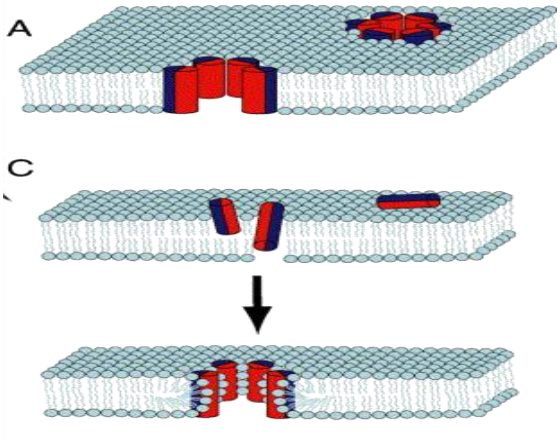


Lactic acid bacteria (LAB) bacteriocins can be grouped on the basis of structure, but also on the basis of mode of action. Like Nisin, members of the class I (or lantibiotic) bacteriocins, have been shown to have a dual mode of action. This Nicin can bind to the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall that is lipid II and therefore prevent correct cell wall production and leading to cell death. Further they can use lipid II as a harbour (docking) molecule to start a process of membrane insertion and pore formation that leads to sudden cell death. Lacticin 3147, A two-peptide lantibiotic can have these dual activities distributed across two peptides, whereas mersacidin has only the lipid-II-binding activity, but does not form pores. In general, the class II peptides have an amphiphilic helical structure, which allows them to insert into the membrane of the target cell, leading to depolarisation and death. Large bacteriolytic proteins (here called bacteriolysins, formerly class III bacteriocins), such as lysostaphin, can function directly on the cell wall of Gram-positive targets, leading to death and lysis of the target cell.

There are two kinds of pore formation mechanism

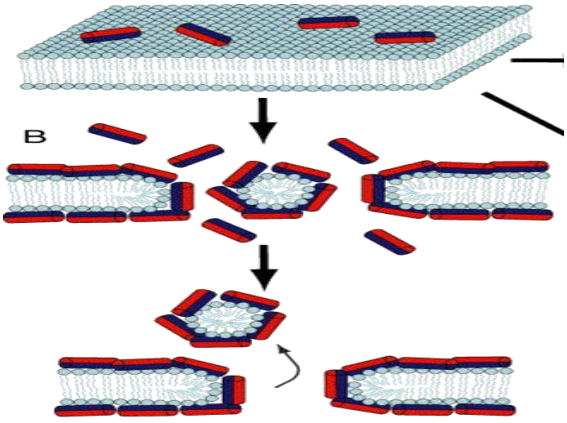
1. **Barrel-stave mechanism**

- In barrel stave mechanism method cationic lantibiotic monomers bind to the membrane surface through electrostatic interactions and are assembled into a preaggregate,
- The pores are formed at a certain membrane potential,The lantibiotic is perpendicular to the membrane



2. Wedge model

- In wedge model pore formation mechanism some surface-bound lantibiotic molecules that bind parallel to the membrane surface and generate local strain, bending the membrane in such a way that the lipid molecules together with the lantibiotic form a pore.



Some of the commercially available Bacteriocins

- Natamycin
- Vasilin™
- Nisaplin™
- Novasin™
- Delvocid™ (Natamycin, Pimaricin, E235)

Methods of Use and Forms

Method of Use	Product Form	Food Products
Application	Dip, Spray	Cheese slices, Deli meats, Cut fruit
Incorporation	Liquid, Powder	Shredded cheese, Ground meat, Beverages
Edible Coatings		Candies
Packaging	Coated film, Impregnated film	Cut meats, Fish, Seafood, Ready-to-Eat (RTE) Foods

Known Nisin Manufacturers Worldwide;

Company	Geographic Location	Producer/Merchant	Products
Peer Manufacturing/Market Competitors			
Danisco Sales $2.9 billion in 2003/04 [Aplin and Barrett (UK), and Genencor, TMI Europe (French biotech)]	Antimicrobial operations in Copenhagen, Denmark Genencor Offices: Palo Alto, CA; Leiden, Netherlands Genencor Manufacturing: Cedar Rapids, Iowa; Rochester, NY; Beloit, WI; Hanko, Finland; Jämsänkoski, Finland; Brugge Belgium; Wuxi, Jiangsu Province, China; Arroyos Prov. de Cordoba, Argentina	Manufacturer R&D involving Nisin at their UK center formerly Aplin and Barrett.	Nisaplin™ (2.5% nisin) off-white powder 1kg polyethylene bottles. Novasin™ (2.5% nisin) light brown powder 550 gm polyethylene bottles.
DSM Sales $10.5 billion in 2004	Netherlands	Producer	Delvocid™ (Natamycin, Pimaricin, E235)
Kerry Bio-Science	Cork, Ireland Locations in Brantford, CA; Cebu, Philippines; Esterol, Malaysia; Menstrie, Scotland; Rochester (MN) and Norwich (NY), US; and Utrecht and Zwijndrecht, Netherlands.	Manufacturer R&D at 2 locations: Naarden, Netherlands and Chicago, US.	Antimicrobials
Other Nisin Providers			
Duke Thomson's International	Madhya Pradesh, India 0091-731-5066802 info@duketoms.com	Manufacturer	Nisin - grey or white powder Delvocid-Natamycin
Profood International, Inc.		Producer	
Sena Health Products and Nutritional Supplements			Nisin Clean – skin and environmental wipes
Xian Medihealth Company Ltd.			Distributor and Exporter
Abana Foodstuff Co., Ltd	Guangzhou, Guang Dong, China (8620) 85542625 gzken@vip.sina.com		Natural Antioxident Nisin

Institutes Engaged for Bacteriocin research in India

- Central Food Technology and Research Institute, Mysore
- National Dairy Research Institute, Karnal
- Institute of Microbial Technology, Chandigarh
- National Dairy Development Board, Anand
- Nestle Pvt Ltd, Panipat

References:

Cao, L. T., Wu, J. Q., Xie, F., Hu, S.H., And Mot, Y., 2007, Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. *J. Dairy Sci*, 90: 3980-3985.

Davies, A. E, Bevis H. E And Broughton J. D, 2003, The use of the bacteriocin, nisin, as a preservative in ricotta type cheeses to control the food- borne pathogen *Listeria monocytogenes*. *Letters in Applied Microbiology*, 24: 343-346.

Deegan, L. H, Paul D. C, Colin, H, And Paul, R, 2006, Bacteriocins: biological tools for bio-preservation and shelf life extension. *International Dairy Journal* 16:1058-1071.

Gautam, N And Nivedita, S., 2009, Bacteriocin: safest approach to preserve food products. *Indian J. Microbiol*. 49: 204-211.

Hagiwara, A. A, Norio Imai, A, Hironao, N. A, Yosuke Toda, A, Mayumi Kawabe, A., Fumio, F. A., Joss Delves-Broughton, B., Kazuo, Y. C., And Shim-Mo Hayashi, D., 2010, A 90-day oral toxicity study of nisin A, an anti-microbial peptide derived from Lactococcus lactis subsp. lactis, in F344 rats . *Food and Chemical Toxicology*, 48: 2421-2428.

Parichat, P. And Pongsak, R, 2010, Synergistic antimicrobial effect of Nisin and ρ-cymene on *Salmonella Enterica serovar typhi* inVitro and on Ready –to-Eat Food. *Biosci. Biotechnol. Biochem.,* 74 (3): 520-524.

Wan, M.N., Norhana, A,B., Susan, E., Poole, C., Hilton, C., Deeth, A. And Gary, A. D., 2012, Effects of nisin, EDTA and salts of organic acids *on Listeria monocytogenes, Salmonella* and native microflora on fresh vacuum packaged shrimps stored at 4^0 C. *Food Microbiology* 31: 43-50.

Wilma, C. D. F., Evandro, L. D. S., Cristina P. D. S. And Antonio, E. R. T., 2008, Anti-staphylococcal Effectiveness of Nisaplin in Refrigerated Pizza Doughs. *Braz. Arch. Biol. Technol*., 51 (3): 595-599.

Leroy F.; De Vuyst L., Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol., 15, 2004, 67–78.

A CRITICAL ANALYSIS OF CHITOSAN ISOLATED FROM CRUSTACEAN WASTE TO EVALUATE ITS BIOMEDICAL PROPERTIES

Pramitha, V. S[1]*., Sajeetha[2], S. B., Alsif, H[2]., Sreejith, P.P.[3], Chaithra Prasad[3], Aiswarya, A[2]., Fathima, J. A. R[2]. and Sahina, B[2]

[1]Department of Aquatic Biology and Fisheries,

University of Kerala, Thiruvananthapuram - 695581

[2]National College of arts and science, Ambalathara, Thiruvananthapuram, India

[3]Department of Zoology, University of Kerala, Thiruvananthapuram-695581

*Corresponding author E-mail: pramivs_vzm@yahoo.com

Abstract:

Chitosan (1-4, 2-amino-2-deoxi-b-D-glucana) is a deacetylated derivative from the biopolysaccharide chitin which is present in insects' exoskeletons, crustaceans shells and fungi cell walls. Chitosan also exhibits properties like biocompatibility, high level of bioactivity,potential biodegradability, reactivity of the deacetylated amino group, permeability and chelation ability. The applicability of chitosan is directly related with its physicochemical features, since the distinct obtaining sources (crustaceans, mollusks, fungi), different processes of extraction and purification will influence changes in the deacetylation degree, molar weight, thermal stability and crystallinity level of chitosan molecule. Chitosan's antibacterial activity, low toxicity and the development of resistance has also reported. Throughout this chapter, our aim is to present and discuss the various biochemical as well as biomedical characteristics of chitosan and its molecular derivatives.

Keywords: Chitosan, Nanoparticle, Shell waste, Chitin, Biodegradable polymers

Introduction:

Biobased and biodegradable polymers are produced from renewable resources and completely degraded to carbon dioxide and water by the action of microorganisms. Chitosan is a linear copolymer consists of β (1→4) - linked 2-acetamido-2-deoxy-β-d-glucopyranose and 2-amino-2-deoxy-β-d-glucopyranose units (Kofuji *et al*., 2005). Chitosan hasbroad applications on several fields like water treatment, spill oil removal, drug delivery, wound healing and enzyme immobilization. Due to its biocompatible, biodegradable and non-toxic nature, it is also known

as a biomaterial (Pradeep, 2004).These properties clearly indicate its great potential in the fields like drug delivery and diagnosis of some diseases like cancer (Al Sagheer and Muslim, 2009).

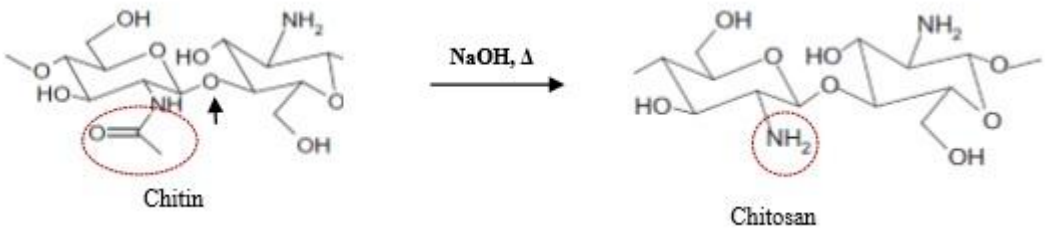
Chitin and chitosan:

Chitin is copolymer of *N*-acetyl-d-glucosamine linked with β-(1-4) glycosidic bond, where *N*-acetyl-d-glucosamine units are predominant in the polymeric chain (Tokura and Tamura, 2007). Chitin is considered as the second-most abundant natural biopolymer after cellulose. Nature produces 10^{11} tons of chitin annually worldwide as a by-product and chitosans are water-insoluble polymers, formed by the deacetylation of chitins. Researchers transformed these complex substances into low-molecular-weight oligosaccharides known as chito oligosaccharides (COSs) (Cheng *et al*., 2011). A wide range of sources and technological approaches for these biologically useful biopolymers have been identified.

Chitin is considered as cellulose derivative and does not occur in organisms producing cellulose andit has acetamide groups (-NHCOCH3) at the C-2 positions. Chitin is estimated to be produced annually almost as much as cellulose. It has become of great interest not only as an under-utilized resource but also as a new functional biomaterial of high potential in various fields (Al Sagheer and Muslim, 2009). Chitin is a white, hard, nitrogenous polysaccharide found in the exoskeleton of the internal structure of invertebrates and the waste of these natural polymers is a major source of surface pollution in coastal areas (Arafat *et al*., 2015).

Structure:

Chitin was found to be the most naturally abundant occurring fiber after cellulose and is structurally similar to cellulose and it contains 2-acetamido-2-deoxy-β-D-glucose units that are linked together via β-(1→4) linkage. Chitin has about 5 - 8 % (w/v) nitrogen content, in the form of primary aliphatic amino groups as found in chitosan (Rinaudo, 2006).



Sources:

Chitin as well as chitosan is carbohydrate derived natural polymers found in the exoskeleton of crustaceans, such as shrimp and marine zooplankton species. Insect's wing and fungi's cell wall are also reported to contain chitin. Shrimp consists of about 45% of raw

material used for processed seafood industry and among them about 30- 40% by weight of raw shrimp is discarded as waste which is composed of its exoskeleton (shells) and cephalothoraxes. Without proper disposals, these have become a big problem for the environment. However, these wastes have the potential to be derived into further materials (Al Sagheer and Muslim, 2009). Beside chitin and chitosan, shrimp shells contain considerable content of astaxanthin, a carotenoid used in fish food additive industry (Pradeep, 2004).

Chitosan is a cationic linear copolymer polysaccharide made up of random distribution of β (1→4) linked 2- amino- 2- deoxy- D- glucose (D-glucosamine) and a natural based polymer obtained by alkaline deacetylation of chitin, exhibiting excellent biological properties such as biodegradation in the human body, immunological, antibacterial and wound healing activity (Jayakumar *et al*., 2005). Studies also indicated the usage of shrimp shell waste to chitosan and it could serve as an effective mode of shell remediation. Chitosan was extracted from shrimp shell waste through the process of deproteinization, demineralisation, decolourization and deacetylation processes. The preparation of chitosan from shell waste was also noted by Arafat *et al*. (2015). Studies by Rhodes and Roller (2000) also revealed about the chitosan film as biofunctional material and well tolerated by living tissues, particularly applicable as edible coatings to prolong shelf-life and preserve quality of fresh foods.

The biopolymer characteristics of chitosan makes it an ideal candidate to create nanoparticles for medical applications, although in order to achieve this, the nanoparticle system needs to be refined to allow an enhanced longevity in the physiological environment. Chitosan nanoparticle (ChNP) could also prepared by the incorporation of polyanion like tripolyphosphate in chitosan solution under continuous stirring and were found to have increased activity compared to the parent chitosan. Chitosan nanoparticles are also obtained by emulsification process and these systems have been extensively studied for drug delivery purposes or modified by the application of different preparation methods (Liu *et al*., 2004).

Studies by Le *et al*. (1997) discussed about chitosan's remarkable contribution to medical related textile sutures, threads and fibres. Biodegradability of chitosan is because of the presence of numerous proteases such as pepsin, lysozyme, papain and others.In the case of colour photography, it has been used as a fixing agent for the acid dyes in gelatin and also acts as an aid to improve diffusion. Use of chitosan in thin layer chromatography for separation of nucleic acids also reported (Rhee *et al*., 1998). Chitin and chitosan might also use as sorbent materials to solid phase extraction of phenol and chlorophenols by using High-Performance Liquid Chromatography (HPLC).

In recent studies, extensive investigations have been carried out to prepare functional chitosan and to increase its solubility in water in order to broaden its application (Goy *et al*., 2009). Studies were also conducted on the application of chitosan in wastewater treatment from agricultural sources. Yep and Terence (2016) were discussed chitosan as a potential filter to treat anionic pollutants like phosphates and nitrates in agricultural wastewater samples. Other reported chitosan derivatives are N-phthaloylation of Chitosan and dendronized Chitosan-sialic acid hybrids (Kurita, 1998). Studies have been conducted on the applications of chitosan in various industries. Beds of flaked chitosan also be used for the purpose of purification of potable water (Sashiwa *et al*., 2001).

Biomedical characteristics of chitosan:

Some of the reported biochemical properties of chitosan include: linear polyamine, reactive amino groups, reactive hydroxyl groups, chelates and many transitional metal ions. Biological properties consists of safe and non-toxicicity, biocompatible - natural polymer, and can adhere to mammalian and microbial cells aggressively and antibacterial (Pradeep, 2004). In agriculture used as a biopesticide, protecting the plants against fungus infections and in wine production, it is used in order to prevent the alteration of wine. On industrial scale, used in the process of water filtration and useful in medicine, for the production of bandages for bleeding reduction (Ono *et al*., 2001).Chitosan also plays a role in the reduction of fat absorption, which makes it useful in the case of diets.

Many studies have been made on chitosan for using it as scaffolding material in tissue engineering. Jarry *et al*. (2001) demonstrated that chitosan can be easily processed into porous scaffolds, films and beads. Kast *et al*. (2003) showed that chitosan-thioglycolic acid (chitosan-TGA) conjugate is a promising candidate as scaffolding material in tissue engineering. Works of Andrady *et al*. (1997) and Kjoniksen *et al*. (1997) were reported that chitin and chitosan do not cause any biological hazard and are inexpensive and these polymers might be suitable for use in the preparation of dosage forms of commercial drugs. According to Guanyi *et al*. (2014) coating of alginate beads with chitosan are also useful for encapsulating probiotics.

Chitosan, has a broad range of antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly susceptible.Antibacterial properties of chitosan facilitate its use in the pharmaceutical products as it limits the threat of infections (Jarry *et al.*, 2001). Kast (2003) reported that not only the antimicrobial property, but also their ability to convey the extrinsic antimicrobial agents to the wounds and burns have made them perfect agent for these applications. Chitosan can also form compatible, tough, and oxygen permeable water absorbent

films and readily degraded by enzymes. Chitosan contains similar structural characteristics as glycosamineglycans and therefore used as suitable material for designing substratum for skin replacement (Andrady, 1997).

In addition, chitosan and chitosan derivatives based systems has considered as promising material for the effective drug delivery systems. Being mucoadhesive polymer, chitosan enhances the residence time of the system and consequently the bioavailability of the drug and leads to the development of target specific carriers. Chitosan and its derivatives can be covalently cross-linked to prepare nano-sized particles as the drug carriers. The cross-linking process involves formation of the covalent bonds between the chitosan chains and functional cross-linking agents that have been widely used for chitosan include bifunctional agents such as PEG dicarboxylic acid, glutaraldehyde or monofunctional agents such as epichlorohydrin (Yao *et al*., 1996).

Chitosan nanoparticles:

Nanotechnology would able to create new materials with wide range of applications (Rabindra *et al*., 2012). The unexpected properties of nanoparticles are largely due to the huge surface area of the material accompanied by an increase in stability and improved functionality. A nanoparticle is a microscopic particle with at least one dimension to less than 100 nm. Chitosan is soluble in acidic conditions - in solution the free amino groups on its polymeric chains can proteonate and giving it a positive charge. Chitosan nanoparticles can be prepared by incorporating a polyanion such as tripolyphosphate (TPP) into a chitosan solution under constant stirring (Rabindra *et al*., 2012). Chitosan based biomaterial possess superior physical and chemical properties such as high surface area, porosity, tensile strength, conductivity and photo-luminescent. It could efficiently retain the bioactivity of macromolecules during preparation and it has been reported that chitosan nanoparticles have an excellent capacity for associating proteins successfully in drug delivery systems to control the releasing process of the drug (Ana Grenha, 2012).

Chitosan nanoparticle preparation:

Preparation of chitosan nanoparticles are carried out by the interaction of oppositely charged macromolecules and Tripolyphosphate (TPP) has often been used to prepare chitosan nanoparticles. The interaction can be controlled by the charge density of TPP and the advantage of this method was attributed to its mild conditions achieved without applying harmful organic solvent, heat or vigorous agitation (Basarkar *et al*., 2009).

Biomedical properties of chitosan nanoparticle:

Chitosan nanoparticles also used for preservative purposes while packaging foods and in dentistry to eliminate caries and can also be used as an additive in antimicrobial textiles for producing clothes for healthcare and other professionals.As antibacterial agents, gene delivery vectors and carriers for protein release and drugs, used as a potential adjuvant for vaccines. These nanoparticles improve antigen uptake by mucosal lymphoid tissues and induce strong immune responses against antigens (Yang *et al.*, 2005). These materials could be used as a wound-healing material for the prevention of opportunistic infection and for enabling wound healing.

Chitosan and chitosan nanoparticle as antibacterial agent:

Chitosan nanoparticles could be prepared under mild conditions, besides can be incorporated with low molecular weight drugs. This characteristic is extremely beneficial for drugs, proteins, genes or hydrophobic molecules that are poorly transported across epitheliaand have many applications in medical and pharmaceutical uses and they have been used successfully in drug delivery systems to control the releasing process of the drug. Hemagglutinationactivity of chitosan nanoparticles were also studied by Rhodes and Roller (2000).

Antibiotic resistant microorganisms have attracted much attention recentlyand there is no single antimicrobial agent that has not exhibited resistance by microorganisms. This new development has forced scientists to formulate novel antimicrobial agents that showed an effect against the continually increasing antibiotic resistant pathogenic microorganisms (Roy *et al*.. 1999). Chitosan, has a broad antimicrobial spectrum to which gram-negative and gram-positive bacteria are highly susceptible. There are several mechanisms which could be suggested to explain this activity and one of them was related to the basic nature of the polymer and the amine content (Zamani *et al*., 2007). Antimicrobial activity of chitosan is well known against a variety of bacteria and fungi. Furthermore, chitosan is also used as a biomaterial widely applied for effective delivery of many pharmaceuticals. According to Li-Ming Zhao (2011) chitosan also suitable for incorporating other antipyretic agents for the long-acting antibacterial wound dressing.

Influence of chitosan on the flocculation of microalgae:

Flocculation is a method of separating algae from the medium by using chemicals. Flocculation, on the other hand takes advantage of aggregating microalgae cells to form larger biomass bulks through interaction of flocculant with the surface charges of suspended solids that

creates large particles. Several flocculants have been reported to induce flocculation of microalgae cells that can be applied to the treatment of larger amount of microalgae. According to their chemical compositions, two classifications are inorganic flocculants and organic flocculants/polyelectrolyte flocculants (Chen, 2011). Commonly used inorganic flocculants include ferric chloride ($FeCl_3$), aluminumsulfate ($Al_2 (SO_4)_3$), and ferric sulfate ($Fe_2 (SO_4)_3$). These multivalent metal salts are effective flocculants and used to flocculate algal biomass (Okuda *et al*., 1997). Pre polymerized metal salts have proved to be efficient over a wider pH range (Irin, 2011). Flocculation by metal salts is not an appropriate method for cheap and sustainable harvesting of microalgae (Ozacarand, 2003).These flocculants are expensive and can kill or prevent the growth of the microalgaeand leave a residue in the water and excess cationic flocculant needs to be removed before it can be reused (Schenk, 2008).

Chitosan has emerged as a favourable flocculating agent in harvesting microalgaedue to its unique combination of properties like biodegradability, biocompatibility, renewability and ecological acceptability in addition to attractive physical and mechanical properties (Varma *et al*., 2004). It has variety of potential applications in wastewater treatment, biomedical engineering and food processing (Hu *et al*., 2013). However, most of the common flocculants have certain drawbacks. Acquiring functional groups provides various chemical and physical features, such as increased selectivity and adsorption capacity (Ganguly *et al*., 2011). The high cationic charge density of chitosan allows it to strongly adsorb negative regions and effectively destabilizethem (Renault *et al*., 2009). As the overall charge of microalgae cells is negative, the positively charged chitosan is adsorbed on microalgae cells andresults destabilizion of the microalgae (Wu *et al*., 2007).

Flocculating agents are classified into inorganic flocculants such as polyaluminum chloride (PAC), synthetic organic flocculants such as polyacrylamide derivatives, natural occurring flocculants such as bio flocculants, nanoparticle such chitosan nanoparticle andnanomagnetite (Fe3O4) (Liu *et al*., 2001). Chemical flocculants are used in water and wastewater treatment industries because of their cost effectiveness and efficient flocculation activities (Goy *et al*., 2009). The use of these chemical flocculants can cause health and environmental problems. Residual aluminium from PAC and acryl amide monomers from polyacrylamide is known to be neurotoxic and carcinogenic toward humans (Yep and Terence *et al*., 2016). Flocculation studies of chitosan by three freshwater algae, *Spirulina*, *Oscillatoria* and *Chlorella*, and one brackish alga, *Synechocystis*, was studied in the pH range 4 to 9 by Ravi chandran and Sivasankara Pillai (2001). Chitosan based materials have been used in the treatment of waste water due to their ability to adsorb heavy metal ionandhave attracted many

research and industry interests, as alternative flocculants, due to their high flocculation performance, ecofriendly, biodegradability and some of them could be produced from agro-industrial wastes also (Kurita, 1998).

Microalgae could be extracted by Fe_3O_4 particles at low pH due to the electrostatic attraction between cells and Fe_3O_4 particles (Sashiwa *et al.,* 2001). Farid *et al*. (2013) developed chitosan nanopolymer and tested it for harvesting of *Nannochloropsis* sp. They reported that the nano-chitosan, resulted in an approximately 40% decrease of flocculant dosage and 9% enhanced biomass recovery (Assisand pessao, 2004). Biocompatible chitosan has been successfully utilized as Fe_3O_4 nanoparticle surface-functionalization material for harvesting *Chlorella* spp (Le *et al*., 1997). Sashiwa *et al*. (2001) reported increased harvesting efficiency with increasing concentration of composites as well as chitosan/magnetic nanoparticle ratio. The reuse of culture medium after microalgae harvesting did not showed any adverse effect on cell growth, indicating the biocompatibility of chitosan coated Fe_3O_4 and the possibility of the economic reuse of residual culture media (Sashiwa *et al*., 2001). Le *et al*. (1997) investigated the bio distribution of chitosan- Fe_3O_4 composite inside cells as a possible eventual influence on the quality of biodiesel produced (Le *et al*., 1997). Unmodified chitosan is not antimicrobially active at pH 7, and does not contain any positive charge on the amino groups (Kjoniksen *et al.,* 1997). Yao *et al*. (1996) indicated that the antimicrobial effect of chitosan also increases with increasing degree of deacetylation.

In this background, the present chapter discussed the importance of extraction of chitosan from shrimp shell biowaste and examined the synthesisof its nanoparticles to analyse its bioactivity scope in various biomedical fields.

References

Al Sagheer, F. and Muslim, S. 2009.Thermal and mechanical properties of chitosan SiO_2 hybrid composites. J. Nanomat: 7 pp.

Ana, G. 2012. Chitosan nanoparticles: A survey of preparation methods. J. Drug Targeting, 20(4): 291-300.

Andrady, A. L. and Xu, P. 1997. Elastic behavior of chitosan films.J. Appl. Polym. Sci., 35: 517pp.

Arafat, A., Sabrin, A. S., Shah, M. M. and Moniruzaman, M. 2015.Preparation and characterization of chitosan from shrimp shell waste.Intl. J. Scien. Engin. Research, 6(5): 538-541.

Assis and Pessoa, J. D. C. 2004."Preparation of Thin Films of Chitosan for use as edible coatings to inhibit fungal growth on sliced fruits," Brazilian Journal of Food Science and Technology, Campinas, 7: 17–22.

Basarkar, A. and Singh, J. 2009. Poly (lactide-co-glycolide)-polymethacrylate nanoparticles for intramuscular delivery of plasmid encoding interleukin-10 to prevent autoimmune diabetes in mice.Pharm. Res., 26: 72-81.

Cheng, N., Cao, X. and Peng, H. T. 2011. Chitosan: A promising biomaterial for tissue engineering and hemorrhage control. Focus on Chitosan Research chapters, 49-82.

Farid, M. S., Ahmad, S., Amir, B.and Bagher, A. 2013. Using nano-chitosan for harvesting microalga Nannochloropsissp. Bio. Res. Tech., 131: 555-559.

Ganguly, K., Aminabhavi, T. M. and Kulkarni, A. R. 2011. Colon targeting of 5-fluorouracil using polyethylene glycol cross-linked chitosan microspheres enteric coated with cellulose acetate phthalate. Indust. Eng. Chem. Res., 50(21): 11797-11807.

Goy, R. C., Britto, D. and Assis, O. B. G. 2009. Review of the antimicrobial activity of chitosan. Pol. Cie. Tecn., 19(3): 241-247.

Guanyi, C., Zhao, L., Yun, Q. and Yuan, L. C. 2014.Chitosan and its derivatives applied in harvesting microalgae for biodiesel production. J. nanoparticles., 21:2-9.

Hu, C. Y., Lo, S. L. and Chang, C. L. 2013. Treatment of highly turbid water using chitosan and aluminum salts. Separation and Purification Technology, 104: 322-326.

Irin, S. S., Trobajo, R., Ibanez, C. and Salvad, J. 2011. Harvesting the microalgae Phaeodactylumtriicornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation.Journal of Applied Phycology, 24(5): 1067-1080.

Jarry, C., Chaput, C., Chenite, A., Renaud, M. A., Buschmann, M. and Leroux, J. C. 2001.Effects of steam sterilization on thermogelling chitosan-based gels.J Biomed. Mater Res., 58(1):127-35.

Jayakumar, R., Prabaharan, M., Rui, L. R. and Joao, F. M. 2005. Graft polymerised chitosan-present status and applications. Carboh.Poly., 62(2): 142-158.

Kast, C. E., Frick, W., Losert, U. and Schnurch, A. B. 2003. Chitosan thioglycolic acid conjugate: a new scaffold material for tissue engineering. Intl. J. Pharma., 256: 183pp.

Kjoniksen, A. L., Nystrom, B., Nakken, T., Palmgren, O. and Tande, T. 1997.Effect of surfactant concentration, pH, and shear rate on the rheological properties of aqueous systems of a hydrophobically modified chitosan and its unmodified analogue.Polym. Bull., 38: 71pp.

Kofuji, K., Qian, C. J., Masumi, N., Ikumi, S., Murata, Y. and Kawashima, S. 2005. Relationship between physicochemical characteristics and functional properties of chitosan.Euro.Poly. J., 41(11): 2784-2791.

Kurita, K. 1998. Chemistry and application of chitin and chitosan.Polym.Degrad. Stab.,59: 117pp.

Le,Y., Anand, S. C. and Horrocks, A. R. 1997. Recent developments in fibres and materials for wound management. Indian. J. Fibre. Text. Res., 22: 337pp.

Li-Ming, Z., Lu-E, S., Zhi-Liang, Z., Jian-Min, C., Dong-Dong, S., Jie, Y. and Zhen-Xing, T. 2011.Preparation and application of chitosan nanoparticle and nanofibers.Brazillian J. Che. Eng., 28(3): 353-362.

Liu, X. F., Guan, Y. L., Yang, D. Z., Li, Z. and Yao, K. D. 2004. Antibacterial Action of Chitosan and Carboxymethylated Chitosan.J. Appl. Polymer Sci., 79: 1324-1335.

Okuda, T., Baes, A. U., Nishijim, W. and Okada, M. 1997.Improvement of extraction method of coagulation active components from Moring aoleifera seed.Water Research, 33(15): 3373-3378.

Ono, K., Ishihara, M., Ozeki, Y., Deguchi, H. and Sato, M. 2001.Experimental evaluation of phospho cross linkable chitosan as a biologic adhesive with surgical applications.Surgery, 130: 844-850.

Ozacarand, M. and Engil, A. S. 2003.Evaluation of tannin biopolymer as a coagulant aid for coagulation of colloidal particles.Colloids and Surfaces, 229(1-3): 85-96.

Pradeep, K. D., Joydeep, D. and Tripathi, V. S. 2004. Chitin and chitosan: chemistry, properties and applications. J. Sc. Indsl. Research., 63: 20-31.

Rabindra, K. N., Subodh, S. P. and Dipak, A. N. 2012.Chiotsan nanoparticles loaded with thiocolchicoside.Der. Pharma. Chemica., 4(4): 1619-1625.

Ravichandran, D., and Sivasankara, V. N. P. 2001.Flocculation of algae using chitosan.J. Appl Phycology, 14: 419-422.

Renault, F., Sancey, B., Badot, P. M. and Crini, G. 2009. Chitosan for coagulation/flocculation processes, an eco friendly approach. European Polymer Journal, .45(5): 1337-1348.

Rhee, J. J. M. and Paeng, K. 1998. Evaluation of chitin and chitosan as a sorbent for the preconcentration of phenol and chlorophenols in water. Analyt. Sci., 14: 1089pp.

Rhodes, J. A. and Roller, S. 2000. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods.Appl. Environ. Microbiol., 66: 80-86.

Rinaudo, M. 2006. "Chitin and chitosan: Properties and applications," Progress in Polymer Science, 31: 603-632.

Roy, K., Mao, H. Q., Huang, S. K. and Leong, K. W. 1999. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat. Med., 5: 387-391.

Sashiwa, H., Shigemasa, Y. and Roy, R. 2001. Chemical modification of chitosan: synthesis of dendronized chitosan-sialic acid hybrid using convergent grafting of preassembled dendrons built on gallic acid and tri (ethylene glycol) backbone. Macromole., 34: 3905pp.

Schenk, P. M., Thomas, S. R. and Stephens, E. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bio Energy Research, 1: 20-43.

Tokura, S. and Tamura, H. 2007.Sulfated chitin and chitosan as novel biomaterial.Int. J. Biol. Macromol., 40(3): 175-181.

Varma, A. J., Deshpande, S. V. and Kennedy, J. F. 2004. Metal complexation by chitosan and its derivatives.Carbohydrate Polymers, 55(1): 77-93.

Wu, X., Ge, X., Wang, D. and Tang, H. 2007.Distinct coagulation mechanism and model between alum and high Al13-PACl.Colloids and Surfaces, 305(1-3): 89-96.

Yang, T. C., Chou, C. C. and Li, C. F. 2005.Antibacterial activity of N-alkylated disaccharide chitosan derivatives.Int. J. Food Microbiol., 97: 237-245.

Yao, K. D., Liu, J., Cheng, G. X., Lu, X .D., Tu, H. L. and Lopes, D. and Silva, J. A. 1996. Swelling behavior of pectin/chitosan complex films.J. Appl. Polym. Sci., 60: 279.

Yep and Terence. 2016. Application of chitosan in the treatment of wastewater from agricultural sources. Elec. Thesis. Dissert., 5774pp.

Zamani, A., Edebo, L., Sjostrom, B. and Taherzadeh, M. J. 2007. "Extraction and precipitation of chitosan from cell wall of zygomycetes fungi by dilute sulfuric acid," Biomacromolecules, 8(12): 3786-3790.

DESCRIPTION OF SOME PLANT GALLS FOUND IN NANDED DISTRICT OF MAHARASHTRA STATE

M. S. Siddiqui

Department Of Zoology

Sharadchandra College Naigaon, Dist. Nanded 431709

Corresponding author E-mail: dr.mssiddiqui50@yahoo.com

Plant galls or tumors are abnormal growths are found on different plant parts. Almost all plant parts i.e. leaf, shoots, stems, roots and inflorescences develop plant galls. Plant galls develop as a interaction between organisms and plant. The organisms that cause plant galls are popularly known as Zoocecidia or Cecidozoa. The example of Zoocecidia is Protozoas, Nematodes, Mites and Insects. Plant galls are also caused by Phytocecidia like bacteria and fungi. Over 106 plant galls have been reported from Maharashtra which includes 37 plant galls from Marathwada region (Sharma 2003, 2009). More than 25 plant galls previously reported from Nanded district so far.

Plant Galls Found In Family Rhamnaceae:

Presently twelve galls are known from this family, of which four leaf, one bud, one fruit and other shoot galls are described. Five midge galls, four mite galls, one each by thrips, psyllid and fungal galls are described so far. Most of these galls have strictly localized distribution either in south India or at outer ranges of Himalaya. One gall of Ethiopian affinity is distributed widely in south India.

One shoot axis gall and one new cecidomyiidae galls are reported from the study area.

Shoot axis gall on Zizyphus jujuba Lamarck. caused by *Eriophis cernns, Masses (Acarina)*

Zizyphus jujube is common wild tree found in both wild and in forests of the study area. It is medicinally important tree. Leaves boiled with milk is used in treating virulent gonorrhea whereas decoction of bark is used in treating diarrhea, colic, and dysentery troubles. The fruit is a blood purifier and helps in digestion and also act as mild laxative.

Description of gall:

Shoot axis gall; irregularly globose, lobed, conspicuously rugs or tuberculate, hard, yellow to reddish brown, solitary or often crowded on branches, representing auxiliary branches, growing continuously, attending size from 25 mm to 50 mm in diameter. The mass of gall

consist wholly of parenchyma with scattered vascular elements basally. Galls may become brittle when grow old and readily crumbled in to a black dust. Mites are seen feeding externally on surface of gall or in the cervices the galls.

Ecological notes:

The galls were found throughout the year on both young and old branches of the host plants.

Distribution:

This is most common gall found throughout India. Mani M.S. (1948,) reported this gall from different parts of India. This known gall is reported for first time from forests of Barul (Dist. Nanded); forests of Bhokar (Dist. Nanded). This known gall was collected throughout the year during 2020 and 2021.

Leaf gall on *Zizyphus nummularia* (L) wild caused by *Lasioptera nummulariae* male female sp. nov. (Cocidomyiidae: Diptera)

Zizyphus nummularia (L) wild is a tree is of common occurrence in this region of Maharashtra, India. It is medicinally importantly plant.

A new cecidomyiidae leaf gall found extensively on this host plant in the study area is reported herewith.

Description of gall:

leaf gall, sub lenticular, solitary, free, globose, on midrib or lateral veins, visible equally on both side of leaf blade; indehiscent, persistent; shiny red from upper surface while pale yellow or yellow on lower surface; unilocular, gall cavity with a single maggots; sometimes two to three galls also found; galls 6-12 mm in diameter; pupation inside the gall.

Ecological notes:

The gall formation was observed during October-November months during 2019, 2020 in the area. The gall formation was noticed initially on tender leaflets.

Distribution:

Earlier this gall was reported on unknown species of Zizyphus by Mani M.S. (1948) from south India. He could not rear midge fly from this gall (gall no 300).

Present gall collected from Vishnupuri and forest of Kandhar (Dist. Nanded) is the first record of gall midge reared from any species of Zizyphus. New gall midge fly Lasioptera nummulariae sp. nov. is reared from this new host plant species (Siddiqui M.S. 2010).

References:

Mani M. S. (1948): Cecidozoa and Zoocecidia from India, J.Roy.Asiatic Soc.Bengal Sci. 14(2)-27.

Siddiqui M. S. (2010): Description of a new Indian gall-midge and a gall associated with *Zizyphus nummularia*", (L), (Family Rhamnaceae) Geobios, 37(4): 113-116.

Sharma R. M. (2003): Checklist of Plant Galls From Maharashtra State. Annals Of Forestry 2(1), 101-112.

Sharma R. M. (2009): Checklist of Indian Gall midges (Diptera: Cecidomyiidae). http://www.zsi.gov.in/zoological survey of India /zsidata/checklist (Accessed on 15.07.2009)

AN OPTIMISTIC UTILIZATION OF POLLEN AS A POLLUTION BIOINDICATOR

Vivek R. Narkhedkar

Mahatma Jyotiba Fule Commerce, Science and

Vitthalrao Raut Arts College, Bhatkuli, Amravati (M.S.)

Corresponding author E-mail: viveknarkhedkar@rediffmail.com

Abstract:

Industrial development has altered the course of natural changes in the biological world. The living system is always under the influence of its surroundings which includes the edaphic factors and the environmental contaminants contributed by the anthropogenic activities. The continuous rise in pollutants has created an alarming situation. Many organisms which are susceptible to environmental changes act as bioindicators. Likewise in this review, based on the previously published scientific literature related to the effects of pollution on the male gametophyte, a pollen grain, optimistically the possibility is explored to utilize it as an air pollution bioindicator. This review contains scientific evidence validating the negative effects of air pollutants on pollen morphology, physiology and biochemistry. It had been concluded that the level of pollution has direct proportionate effects on pollen biology. So, this review endorses the plausible application of pollen as a bioindicator.

Keywords: Pollen, Pollution, Bioindicator, Physiology

Introduction:

The Anthropocene is witnessing the enhanced level of various environmental contaminants, such as nitrogen oxides, ozone, sulfur dioxide and particulate matter in the atmosphere from traffic or combustion-related activities. These environmental contaminants could enhance the biochemical modifications of allergens and oxidative stress in the organisms.

Air pollution is harmful and brings discomfort to humans or other living organisms. Air pollutants are the substance in the air that damages the environment by altering its chemical, physical and biological properties. These pollutants could be in the form of gases, solid particles, or liquid droplets and they may be man-made or natural. The adverse effect of pollution, in particular air pollution, on health has been known for many centuries.

At the present time, industrial air pollution is a major concern. Generally, the industrial area is among the vastly contaminated region in any country. To date, approximately 300 substances are identified which acts as potent air pollutants. Along with that lesser-known substances frequently enhance this magnitude due to the advances and commencement of new technologies and industrialized processes (Emberson *et al*., 2003).

In the present scenario, climate change and air pollution are significant drivers for the enhanced load of allergic diseases. The mechanisms by which environmental contaminants and atmospheric parameters may influence allergic diseases are complicated and indefinable.

Pollen: A Male Gametophyte:

In the life cycle of an organism, reproductive development is a crucial phase. Pollen being a gametophyte carries a male gamete for fertilization with the egg. The pollen grains are dispersed in the air or transferred by the pollination agencies to the female receptacle i.e. stigma. On compatible stigma, pollen germinates to produce a pollen tube. Later, the pollen nucleus divides to produce the male gametes or sperm nucleus which is then carried to the egg apparatus in the growing pollen tube. Soon after the dispersion of pollen grains and subsequently landing on the stigma, pollen grains are continuously exposed to the atmosphere.

In the current scenario, the atmosphere is witnessing a rise in the level of various contaminants. Such contaminants have a direct or indirect influence on the biological world. The impediment in any step in the reproductive phase, involving gametogenesis, pollination, fertilization and seed development, causes severe repercussions on seed quality and quantity in crops. This will also impact the species diversity and competition in semi-natural and natural populations, principally if germination or seed set were to be likely affected (Kabir *et al*., 2012).

Bioindicators of Pollution:

Naturally, some life forms are very susceptible to the changing environmental conditions, either positive changes or negative changes. Such life forms are termed Bioindicators; which could be employed to evaluate the fitness of the atmosphere and its succeeding effects on the biological world. The presence of Bioindicators in the environment is influenced by many factors. Bioindicators are plausible tools to envisage the natural health of a region or the degree of pollution (Khatri and Tyagi, 2015). The utilization of Bioindicators has certain advantages, such as determines the biological impact, monitor the antagonistic effects of pollutants, being easy to observe because of their prevalence and economically more suitable than related advanced alternatives (Parmar *et al*., 2016).

Effect of Air Pollution:

Environmental contaminants, such as air pollutants, have a significant undesirable effect on the biochemical and physiological properties of plants, which leads to a decline in overall development and growth. The adverse effect of different air contaminants in terms of biochemistry and physiology has been under assessment for many years (Rai, 2016). As plants are persistently exposed to polluted air, they are the chief recipient of particulate and gaseous pollutants from the atmosphere (Rai, 2016). The decreased in the germination of pollen and tube growth had been reported earlier after being exposed to O_3 and SO_2 in some plant species (Masaru *et al*., 1976; Feder *et al*., 1982 and Dubay and Murday, 1983).

The male gametophytic phase of the plant is identified with the pollen grains. They are liberated into the airflow for subsequent fertilization in angiosperms as well as gymnosperms. Meanwhile, there is a maximum chance that air pollutants can get deposited onto the pollen. Such events decrease the rate of fertility by direct or indirect effects on the reproductive apparatus. Air pollution can cause indirect effects on pollen via the soil (Newson *et al*., 1997). Air pollutants influence the discharge of allergens from pollen and enhance the allergenic potential which may lead to the increase in hay fever (Rezanejad, 2008). Also, air pollution showed an inflammatory effect in the mucous membranes due to pollen allergens. This causes easier interaction with the immune system and hence, some constituents of air pollution have an elevated immune response by synthesizing IgE (Damato *et al*., 2007). Therefore, altered efficiency leads to the production of lesser, small size pollen and an enhanced number of malformed pollen grains as compared to plants of the same species grown in non-polluted or relatively less polluted areas (Rezanejad, 2008). Behrendt and Becker (2001) also reported the changes in pollen morphology, germination and tube growth among the samples collected from control areas and polluted.

1. Effect on Pollen Physiology:

Ahmadi *et al*. (2019) reported the influence of air pollution on the reproductive system of plants and, therefore, their fertility and yield. The report stated that air pollution directly influences the anther development and microsporogenesis as well. Furthermore, after the anthesis and the dispersion of pollen to the surroundings till they reach the target, the detrimental influence of air pollution is noted on the structure, the pollen tube growth, and biological vigor (Emberline, 1998). Additionally, Emberlin (1998) reported the effects of air pollutants on vegetative parts affecting the development of generative cells and consequently plant fertility. Similarly, Behrendt and Becker (2001) reported the effect of pollutants on pollen physiology after dehiscence of anthers until reaching stigma. Also, the accumulation of pollutants on the

stigma directly affects pollen and/or obstructs pollen-pistil interactions. As a result, it is suggested that air pollution causes pollen sterility. Thus, this reduces the fruit set and enhances the number of deformed fruits as compared to plants of the same species grown in areas with reduced pollution (Rezanejad, 2009 and 2012).

The study conducted by Ahmadi *et al*. (2019) revealed the relationship between the direction of the polluted wind and the rate of negative effect on pollen physiology. A decrease in rates of pollen tube growth and pollen germination was reported. Out of the studied aspects, pollen germination was more sensitive phase leading to fertilization (Dubay, 1981), as it is directly in contact with the pollutant (Wolters and Martens, 1987). Many studies are reported on the harmful effects of air pollutants on pollen germination (Majd *et al*., 2004). Pfahler (1981) reported the defect in the tapetum and thus developing pollen due to the air pollutants. The structural defect in the tapetum causes pollen abnormality which leads to sterility, and as a result malfunctions of fertilization.

In Tuscany, Bellani *et al*. (1997) investigated the negative effects on apple production caused by the level of pollutants in the rain and reported a great reduction in pollen germination and viability. Also, expected the likeliness of enhanced negative effects if acid rain continue.

2. Effect on Pollen Morphology

The pollen developmental has very severe damages due to wrinkle and deformation of pollen grains that are being caused by air contamination (Rezanejad, 2009). Likewise, the reports on ‘Golab’ cultivar’s pollen grains deformation and wrinkles were quite evident at the balloon stage in the polluted region which was relatively polluted than the other two sites of the study (Ahmadi *et al.,* 2019). Many researchers had reported similar results (Rezanejad, 2009).

Ahmadi *et al.* (2019) utilized an electron microscope to show that the pollen shape in apple reflects an effect on pollen morphology due to air pollution oval and of three-furrow type; these three furrows extend throughout the pollen grains. Similar findings were reported by Currie *et al*. (1997). The distortion in pollen structure is also reported in *Platycladus* due to the effect of air pollution (Rezanejad, 2009, 2012). The researchers also commented that air contamination affects a great number of pollen grains and leads to defects in shape, surface structure and size. In addition, Behrendt and Becker (2001) noted modification in the morphology of pollen grains collected from non-polluted and polluted sites.

The pollution effects on pollen morphology observed under scanning electron microscopy and light microscopy revealed that pollen exposed to pollution became abnormal in structure and showed deposition of pollutants. The data collected in the study suggest that

extended plant exposures to air pollution lead to various biological effects on the cell, tissue and organ (Amjad and Shafighi, 2012).

3. Effect on Pollen Biochemistry:

The phenolic substances, such as flavonoids and phenols are among the most valued secondary metabolites that have a large significance in the environment-plant relationship (Rhodes, 1994). The flavonoids not only provide pigmentation in leaf, seed, fruit and flower but also play varied roles in development and growth of plants, including defense mechanisms, pollen development, symbiosis, male fertility, etc. It is also significant as pigments that attract pollinators, protect against UV rays and regulates the cell cycle. A mutant of *Petunia* Juss deficient in flavonoid is male sterile as pollen grains fail to germinate (Woo *et al*., 2005). In contrast to that Rezanejad (2009) reported the high-level accumulation of flavonoids in plants in response to air pollution. Also, the elevated quercetin in plants in response to ozone was consistent with the report of Dixon and Paiva (1995). They suggested that the enhanced content could be linked with the protective role of flavonoids under circumstances of environmental stress.

The pollen grains contain various types of proteins located in the cytoplasm and on the intine and exine surfaces. Such molecules are significant as they participate in the intercellular recognition reactions between male pollen and the female stigma (Dickinson *et al*., 2000). An enhancement in the Betv1, birch pollen allergen, was noted in regions with high nitrogen loads (Jilek *et al*. 1993), whereas a reduction in Betv1 contents was reported because of air pollution (Hjelmroos *et al*., 1994 and Parui *et al*., 1998). Likewise, Helander *et al*. (1997) no considerable distinction was observed in the protein bands of pollen from non-polluted and polluted regions.

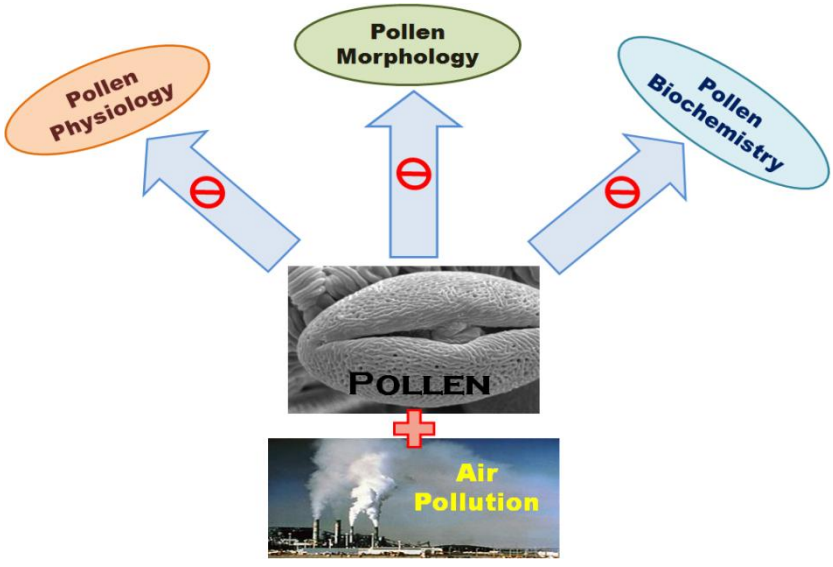


Figure 1: Representation of harmful effects of air pollution on pollen grains

The established relationship between allergenicity and air pollution was explored in pollen genes of *Lolium perenne* by RNAseq. Two wild populations with varied pollution were compared for differential expression of pollen genes. In this study, Lucas *et al*. (2020) observed that increase in NO_2 and SO_2 is the probable reason for the enhanced allergenic capacity of pollen grains. The report also emphasized that the knowledge of the established relationship between *Lucas perenne* pollen allergens and air pollution could be utilized for the development of vaccines.

The comparative biochemical investigation on pollen grains of *Lagerstroemia indica* from two sites (polluted and less polluted) was reported from Tehran city, Iran (Rezanejad *et al*., 2003). The pollen protein contents were analyzed by SDS-PAGE and Bradford method. The adverse effect of air pollution indicates the decrease in protein band staining intensity and the total amount of protein in *Lagerstroemia indica* L.

Pollen: A Prospective Tool:

In conclusion, pollen is a cosmopolitan biological entity that is susceptible to changing environmental conditions. Pollen in its gametophytic stages of development witnessed many physiological events. These events are promising steps that lead to the successful fertilization and generation of new progeny. Such pollen physiological parameters have the potential to be utilized as a monitoring tool for assessing the level of air pollution in the area. The comparative studies reported on the pollen collected from polluted and less or non-polluted/control sites emphasizing the parameters of pollen morphology, physiology and biochemistry promise its status as a biological indicator.

References

Ahmadi, F., ValizadehKaji, B., and Abbasifar, A. (2019). The Effect of Air Pollution on Developmental Stages and Pollen Germination, Pollen Tube Growth and Fruit Set of the Apple Cultivars ‘Golden Delicious’ and ‘Golab’. Gesunde Pflanzen, 71(4), 237-247.

Amjad, L., and Shafighi, M. (2012). The effect of air pollution on *Chenopodium album* L. pollen structure. Journal of Agricultural Science and Technology. A2 (1A), 143-148.

Behrendt, H., and Becker, W. M. (2001). Localization, release and bioavailability of pollen allergens: the influence of environmental factors. Current opinion in immunology, 13(6), 709-715.

Bellani, L. M., Rinallo, C., Muccifora, S., and Gori, P. (1997). Effects of simulated acid rain on pollen physiology and ultrastructure in the apple. Environmental Pollution, 95(3), 357-362.

Currie, A. J., Noiton, D. A., Lawes, G. S., and Bailey, D. (1997). Preliminary results of differentiating apple sports by pollen ultrastructure. Euphytica, 98(3), 155-161.

Dickinson, H. G., Elleman, C. J., and Doughty, J. (2000). Pollen coatings–chimaeric genetics and new functions. Sexual Plant Reproduction, 12(5), 302-309.

Dixon, R. A., and Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. The plant cell, 7(7), 1085-1097.

Dubay, D. T. (1981). Interspecific Differences in the Effects of Sulfur-Dioxide on Angiosperm Sexual Reproduction. Emory University, Atlanta, Georgia.

DuBay, D. T., and Murdy, W. H. (1983). The impact of sulfur dioxide on plant sexual reproduction: in vivo and in vitro effects compared. J. Environ. Quality, 12 (1), 147-149.

Emberlin, J. (1998). The effects of air pollution on allergenic pollen. European Respiratory Review, 8(53), 164-167.

Emberson, L., Ashmore, M., and Murray, F. (2003). Air pollution impacts on crops and forests: a global assessment. Imperial College Press, London.

Rezanejad, F. (2008). The structure and ultra structure of anther epidermis and pollen in *Lagerstroemia indica* L. (Lythraceae) in response to air pollution. Turkish Journal of Botany, 32(1), 35-42.

Feder, W.A., G.M. Krause, B.H. Harrison and W.D. Riley. (1982). O_3 effects on pollen tube growth in vitro and in vivo. In: Effects of gaseous air pollution in agricultural and horticulture (Eds.): M.H. Unsworth and D. Ormrod. London, Butterworth Pub. 482-489.

D'amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., (2007). Allergenic pollen and pollen allergy in Europe. Allergy, 62(9), 976-990.

Helander, M. L., Savolainen, J., and Ahlholm, J. (1997). Effects of air pollution and other environmental factors on birch pollen allergens. Allergy, 52(12), 1207-1214.

Hjelmroos, M., Schumacher, M. and Hage-Hamsten, M. van (1994). Variations in birch pollen (*Betula verrucosa*) allergens between different trees in view of both their provenance and air pollution. In Abstracts. 5th International Conference on Aerobiology. Bangalore, India.

Jilek, A., Swoboda, I., Breiteneder, H., Fogy, I., Ferreira, F., Schmid, E., Heberle-Bors, E., Scheiner, O., Rumpold, H., Kraft, D., Koller, H. T. and Breitenbach, M. (1993). Biological Functions, Isoforms, and Environmental Control in the Bet v i Gene Family. Molecular Biology and Immunology of Allergens, 39, 245-271.

Kabir, M., Iqbal, M. Z., and Shafiq, M. (2012). Traffic density, climatic conditions and seasonal growth of *Samanea saman* (Jacq.) Merr. on different polluted roads of Karachi city. Pak. J. Bot, 44(6), 1881-1890.

Khatri, N., and Tyagi, S. (2015). Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. Frontiers in life science, 8(1), 23-39.

Lucas, J. A., Gutierrez-Albanchez, E., Alfaya, T., Feo Brito, F., and Gutierrez-Mañero, F. J. (2020). Search for New Allergens in Lolium perenne Pollen Growing under Different Air Pollution Conditions by Comparative Transcriptome Study. Plants, 9(11), 1507.

Masaru, N., Syozo, F., and Saburo, K. (1976). Effects of exposure to various injurious gases on germination of lily pollen. Environmental Pollution, 11(3), 181-187.

Parmar, T. K., Rawtani, D., and Agrawal, Y. K. (2016). Bioindicators: the natural indicator of environmental pollution. Frontiers in life science, 9(2), 110-118.

Parui, S., Mondal, A. K., and Mandal, S. (1998). Protein content and patient skin test sensitivity of the pollen of Argemone mexicana on exposure to SO2. Grana, 37, 121–124.

Pfahler, P. L. (1981). In vitro germination characteristics of maize pollen to detect the biological activity of environmental pollutants. Environmental Health Perspectives, 37, 125-132.

Newson, R., Strachan, D., Archibald, E., Emberlin, J., Hardaker, P., and Collier, C. (1997). Effect of thunderstorms and airborne grass pollen on the incidence of acute asthma in England, 1990-94. Thorax, 52(8), 680-685.

Rai, P. K. (2016). Biodiversity of roadside plants and their response to air pollution in an Indo-Burma hotspot region: implications for urban ecosystem restoration. Journal of Asia-Pacific Biodiversity, 9(1), 47-55.

Rezanejad, F. (2009). Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). Grana, 48(3), 205-213.

Rezanejad, F. (2012). Air pollution effects on flavonoids in pollen grains of some ornamental plants. Turkish Journal of Botany, 36(1), 49-54.

Rezanejad, F., Majd, A., Shariatzadeh, S. M. A., Moein, M., Aminzadeh, M., and Mirzaeian, M. (2003). Effect of air pollution on soluble proteins, structure and cellular material release in pollen of *Lagerstroemia indica* L. (Lythraceae). Acta Biologica Cracoviensia Botanica, 45(1), 129-132.

Rhodes, M. J. C. (1994). Physiological roles for secondary metabolites in plants: some progress, many outstanding problems. Plant molecular biology, 24(1), 1-20.

Wolters, J. H. B., and Martens, M. J. M. (1987). Effects of air pollutants on pollen Bot. Rev. 53,372-414.

Woo, H. H., Jeong, B. R., and Hawes, M. C. (2005). Flavonoids: from cell cycle regulation to biotechnology. Biotechnology Letters, 27(6), 365-374.

CO-EVOLUTION OF PATHOGENS AND PLANTS

Vinda Manjramkar

Department of Zoology

B. N. Bandodkar College of Science (Autonomous), Thane

Corresponding author E-mail: vindamanjramkar@yahoo.com

The term coevolution (the influence of closely associated species on each other in their evolution) is used to describe cases where two (or more) species reciprocally affect each other's evolution. Coevolution is likely to happen when different species have close ecological interactions with one another. These ecological relationships include: Predator/prey and parasite/host, Competitive species, Mutualistic species.Pathogens are able to infect a host and, as a result of infection, they causedamage to this host. Woolhouse *et al.* (2002) pointed to three conditions that are required forhost-pathogen coevolution: (1) reciprocal effects of the relevant traits ofthe interaction (e.g., defense and pathogenicity) on the fitness of the twospecies (Le., pathogens and hosts), (2) dependence of the outcome of the host-pathogen interaction on the combinations of host and pathogengenotypes involved, and (3) genetic variation in the relevant host andpathogen traits.Terrestrial plants evolution occurred in the presence of various pathogens.Coevolution is defined as the process of reciprocal adaptation and counter-adaptation between ecologically interacting species (Janzen, 1980). The sophisticated and complex association between plants and pathogens, including bacteria and fungi, have existed since the early stages of life on Earth.The evolution of terrestrial plants from the aquatic environment brought a plethora of challenges.

The evolution of plants has been accompanied by the evolution of beneficial and non-beneficialpathogenic microbes and others, all of which play critical roles in modern plant physiology and developmentAn expanding body of fossil evidence shows the interactions among early terrestrial communities included Virus, bacteria, fungi, algae, lichens, and bryophytes—the ecosystem servicesprovided by these organisms include the weathering of parent rock material, soil formation, stabilization of sediments, and the productivity of ecosystems (Elbert *et al.*, 2012; Edwards *et al.*, 2015)

Pathogenic microbes establish complex and diverse intimate relationships with plant hosts to obtain nutrients required for microbial growth and development, thus causing plant infection and disease (Mendes *et al.,* 2013) Pathogens that cause plant diseases are mainly

microorganisms divided into biotrophs and necrotrophs, which rely on living or dead plant tissue.Over the course of evolution, plants have developed robust immune systemswhich confer the ability to resist pathogen infection. These systems include harnessing beneficial bacteria that can contribute to plant defenses against pathogenic microbes. Plants face a wide variety of organisms that range from being extremely pathogenic to being highly beneficial. Major pathogenic organisms include viruses, bacteria, fungi, nematodes and insect pests that have evolved quite distinct and with specialized strategies for attacking plants.

The virus infection in plant can be symbiotic, increasing survival chances, virus infection may be beneficialfor plants, as shown by an increase of tolerance to abiotic stress in virusinfected plants as compared with uninfected controls (Xu *et al.,* 2008), or by a decreased herbivory on tymovirus-infected Kennedia rubicunda (commonly known as the dusky coral pea, is a species of flowering plant in the family Fabaceae, endemic to Australia) in Australia (Gibbs, 1980). Thus, it is obvious that the effects of virus infection on plant fitness in natural ecosystems may vary largely according tothe specific virus-host interaction, or on other hand viral infection can be destructive it may reduce the competitive ability of the infected plants, a phenomenon (apparent competition) that may also occuramong genotypes of the same species (Pagan *et al.,* 2009). Virus infectionhas also been shown to increase mortality and to reduce fecundity in wildcabbage in southern England (Maskell *et al.,* 1999), and to reduce lifespanof wild pepper in its natural habitat in México (unpublished results)

Bacterial pathogens infect a wide variety of evolutionary distinct hosts, including both lower and higher eukaryotes. In all of these cases, the pathogen must have the ability to recognize, become associated with, exploit the nutrient reserves of, and combat the defense responses of its specific host. To accomplish these tasks, pathogens use an extensive arsenal of virulence-related factors.Plant pathogenic bacteria cause many different kinds of symptoms that include galls and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers. In contrast to viruses, which are inside host cells, walled bacteria grow in the spaces between cells and do not invade them. Pathogenic bacteria cause many serious diseases of vegetables. They do not penetrate directly into plant tissue but need to enter through wounds or natural plant openings. Wounds can result from damage by insects, other pathogens, and tools during operations such as pruning and picking. Bacteria and there spores can survive in the soil and crop debris, and in seeds and other plant parts. Weeds can act as reservoirs for bacterial diseases. Bacteria spread in infected seed, propagating material and crop residues, through water splash and wind-driven rain, and on contaminated equipment and workers' hands. Overhead

irrigation favors the spread of bacterial diseases.Bacterial canker (*Clavibacter michiganensis pv. michiganensis*). Seedlings may die and older plants may wilt and die eventually. Older plants have leaves that turn yellow and wilt only on one side Cankers on stems and fruit. Tissue inside stems becomes discolored bacterial soft rot (*Pseudomonas spp.*, Erwinia spp.). Wet, slimy, soft rot that affects any part of vegetable crops including heads, curds, edible roots, stems and leavesand may have a disagreeable odor.Bacterial blight (*Pseudomonas syringae* - various strains) Beet – irregular, round leaf spots with a grey center surrounded by a purple margin. Spring onions/shallots – pale yellow to light-brown lesions with a water-soaked appearance around the margins; outer leaves wither and die and youngest leaf turns lemon to light-green. Leeks – brown streaking on the shank (*Pseudomonas syringae pv*. tomato). Small dark spots surrounded by a yellow halo on leaves; dark raised specks on fruit.Epidemiological studies carried out in the 1970s suggested that clinical isolates of *Pseudomonas aeruginosa* might be capable of causing disease in plants.Bacteriophages, the viruses of bacteria, have received increased research interest in recent years as a realistic environmentally friendly means of controlling bacterial diseases. Their use presents a viable control measure for a number of destructive bacterial crop diseases, with some phage-based products already becoming available on the market.

The other positive side of this coevolution is some plants engage in symbiosis with bacteria called rhizobia that "fix" nitrogen from the atmosphere, making it available to the plant. Rhizobia enable legumes like soybeans and alfalfa to grow without nitrogen fertilizer.Some legume seeds, such as soybeans and peanuts, contain high levels of protein and are among the most important agricultural sources of protein in the world, also acacia species of savannah is using bacteria for nitrogen fixation, no wonder they are surviving in harsh climate of savannah.Soil bacteria, collectively called rhizobia, symbiotically interact with legume roots to form specialized structures called nodules in which nitrogen fixation takes place. This process entails the reduction of atmospheric nitrogen to ammonia by means of the enzyme nitrogenase. Therefore, using rhizobia is a natural and environmentally-friendly way to fertilize plants.

A nutrient depletion zone can develop when there is rapid soil solution uptake, low nutrient concentration, low diffusion rate, or low soil moisture. These conditions are very common; therefore, most plants rely on fungi to facilitate the uptake of minerals from the soil. Mycorrhizae, known as root fungi, form symbiotic associations with plant roots. In these associations, the fungi are actually integrated into the physical structure of the root. The fungi colonize the living root tissue during active plant growth. Mycorrhization, the plant obtains phosphate and other minerals, such as zinc and copper, from the soil. The fungus obtains

nutrients, such as sugars, from the plant root. Mycorrhizae help increase the surface area of the plant root system because hyphae, which are narrow, can spread beyond the nutrient depletion zone. Hyphae are long extensions of the fungus, which can grow into small soil pores that allow access to phosphorus otherwise unavailable to the plant. Mycorrhizae function as a physical barrier to pathogens. They also provide an induction of generalized host defense mechanisms, which sometimes involves the production of antibiotic compounds by the fungi. Fungi have also been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils.There are two types of mycorrhizae: ectomycorrhiza and endomycorrhiza. Ectomycorrhiza form an extensive dense sheath around the roots, called a mantle. Hyphae from the fungi extend from the mantle into the soil, which increases the surface area for water and mineral absorption. This type of mycorrhizae is found in forest trees, especially conifers, birches, and oaks. Endomycorrhiza, also called arbuscular mycorrhizae, do not form a dense sheath over the root. Instead, the fungal mycelium is embedded within the root tissue. Endomycorrhiza are found in the roots of more than 80 percent of terrestrial plants.

Pests are programmed to recognize and rapidly respond to patterns of host cues.The phytophagous insects that exist today and the plants they feed on are the product of a coevolutionary process that has been ongoing for 400 million years (Labandeira, 2013). Studies of fossil plant–insect associations suggest that insects have been feeding on plants for 400 million years.Phytophagous insects due to their role as pests in agricultural ecosystem have at large and negative impact on food security for humanity and related species (Bruce, 2010). They not only damage agricultural crops but also medicinal and aromatic plants. The grubs of the three species of scarab beetles that damaged rose plants in Assam attacked the root system of the plant. The rose plant has high economic importance in making rose water, aromatic compounds, cosmetics, medicines. The adult beetles were positively phototactic which came out at night and fed on the leaves of rose plant by making some holes and the severe infestation led to complete plant defoliation. Even the plant like Neem was attacked by *Parasa hilaris* (Suresh, 1992). There are 13 pests that attacked neem in southern Tamil Nadu. Tulsi, *Ocimum sanctum* were seriously attacked by sucking insect pests like lace wing *Cochlochila bullita* (Stal.) (Verma, 2006) and Aphids which are very common and kill the plants. Twelve species of aphids were found to cause considerable damage to medicinal and aromatic plants in Chikkamagaluru district, Karnataka. 26 species phytophagous arthropods were reported on ashwagandha. This is major threat to Ayurveda medicine manufacturer, learning from the above literature, the coevolution so strong that, they are going along together for ages.

Coevolution entails the rise of new alleles, by mutation or migration, and the fixing in the population (Woolhouse *et al.,* 2002). Two models have described the dynamics of the coevolution process. The first, the Red Queen hypothesis, is synthetized as 'running as fast as you can to stay in the same place'. It posits that for a given species adaptation increases the fitness against another interacting species, but at the same time such adaptation of the first necessarily causes a decline in fitness of the second species (Rausher 2001; Woolhouse *et al.,* 2002; Paterson *et al.,* 2010). Red Queen metaphor became central in the description of continuous race in the process of evolutionary adaptation to prevent extinction (Antonovics *et al.,* 2011; Jensen *et al.,* 2012; Nemri *et al.,* 2012). Such coevolutionary interactions give rise to continual natural selection for adaptation and counteradaptation in interacting species.The second evolutionary hypothesis of coevolution is known as the 'Arms Race model', here the Coevolutionary dynamics are described as a continuous escalation of defenses and counter-defenses gained with new genetic traits that can be fixed in the population through a slow process. In such a model, genetic improvements are accumulated in both populations. The natural selection of new genetic traits is a biological phenomenon.

Plants also have had to defend themselves against insect attack. Being rooted to the ground they are unable to run away from attacking herbivores. They have evolved a wide range of sophisticated defense systems to protect their tissues (De Moraes *et al.,* 2001; Kessler and Baldwin, 2001; Ballare, 2011). These include toxic or anti-feedant secondary metabolites that represent a major barrier to herbivory (Harborne, 1993; Mithoefer and Boland, 2012), and physical defenses such as lignin (Franceschi *et al.,* 2005). These provide direct defense via toxic, anti-nutritive or repellent effects on herbivores. The capsaicin from chills, caffeine from coffee, tannin from tea, gossypol of cotton plant, but human is clever pest like other animals for plant he thrives and relishes on these products and he knows to use them, thus started cultivation of this plants in large scale which is positive side because which the plant species flourishedthis can be marked as coevolution.

Plant defenses can be classified into resistance against herbivore, tolerance to herbivore, phenological escape from herbivore and overcompensation (Agrawal 2000). Classic example of coevolution is of *Acacia nigrescens* (nick name Devil thorn) and Giraffe, Giraffes have a fondness for the tree that is unrivalled by any other because it leaves (umbrella canopy) are at great height from ground, and this fondness results in a fascinating 'to and fro' relationship between fauna and flora. Over time, the acacia tree has developed several clever defense mechanisms to prevent giraffes from munching on them. The giraffe's tongue is about 45cm in length and highly prehensile. This allows the animal to successfully negotiate the bigger thorns

and pull the leaves from the branch. But the acacia trees have the developed a further defense – the release of tannins, thereby acacia trees within 50 yards react to the release of the tannin by their neighbour and start by emitting their own. Thus, making Giraffe to stay away from excessive eating, but at the some instances this plant needs Giraffe during flowering season, as they bring pollination of flowers, As giraffes move from treetop to treetop, pollen gets stuck on their heads and necks, and is transferred between trees, aiding in pollination, but they also eat lot of flowers, this sets classy example of coevolution.

Similar are insect pollinators having coevolution with plants, the grand example is honey bees despite human being using the pesticide they are stillsurviving, the reason is the phenomenon of haplodiploidy, queen mates with several drones who are sons of different queens who are healthy biotypes withstanding the chemical (pesticide) attacks on them. As the workers and queen as diploid developed by fertilization of ova of queen the sperm from drone , whereas the drone are haploid, with set of chromosome from mother. During her mating flights, early in life, she will mate with many drones. Since these drones have various genetic traits, they offer a genetic diversity that will serve the queen and her offspring well. Her mating flights, across a few days, will result in collecting sperm from 10-25 drones. She will store this sperm in her spermatheca for many years. This protects the population from undergoing devastation due to any calamity. If all the honey bees die the pollinating plants will perish in no time. Flowers too love the presence of honey bees, thus attract them by vibrant colors and the scent.

A case study of coevolution: squirrels, birds, and the pinecones, where squirrels are the main seed predator, trees should have stronger defenses against squirrel predation, and where birds are the main seed predator; trees should have stronger defenses against bird predation. This turns out to be true. Where there are squirrels, the pinecones are heavier with fewer seeds, but have thinner scales, like the pinecone on the left. Where there are only birdcrossbills, pinecones are lighter with more seeds, but have thick scales, If the crossbills have evolved in response to the pine trees, there is geographic differences in birds: where the pinecones have thick scales, birds should have deeper, less curved bills than where the pinecones have thin scales,So we have evidence that the trees have adapted to the birds (and the squirrels) and that the birds have adapted to the trees. It is easy to see why this is called a coevolutionary arms race: it seems possible for the evolutionary "one-upping" to go on and on even thicker-scaled pinecones are favored by natural selection, which causes deeper-billed birds to be favored, which causes even thicker-scaled pinecones to be favored (Benkman, 2010).

Many fruit-eating birds, especially in tropical rain forests are coevolving with the plants whose fruits they eat. The birds get nourishment, and in the process the plants get their digestion-resistant seeds dispersed by regurgitation or along with the birds' droppings. Many characteristics of the plants have evolved to facilitate dispersal and the behavior and diets of the birds have responded to those changes. In particular, the plants have evolved conspicuously colored, relatively odorless fleshy fruits to attract the avian dispersers of their seeds. They are coevolving in response to the finely honed visual systems of the birds; plant species coevolving with color-blind mammalian seed-dispersers have, in contrast, dull-colored but smelly fruits. The bird-dispersed plants often have evolved fruits with giant seeds covered by a thin, highly nutritious layer of flesh. This forces the bird to swallow the fruit whole, since it is difficult or impossible just to nip off the flesh. In response, birds that are specialized frugivores (that is, that do not take other kinds of food) have evolved both bills with wide gapes (so they can swallow the fruit whole) and digestive tracts that can rapidly dissolve the flesh from the large impervious seed, which then can be regurgitated (Ehrlich *et al.,* 1988.).

There many seeds from plant which require wash of animal digestive enzyme on them for germination process, the extinction of ‘Dodo’ bird, an endemic sapotaceous tree Calvaria major found on the island of Mauritius is nearly extinct because its seeds apparently required passage through the digestive tract of the now-extinct dodo (*Raphus cucullatus)* to overcome persistent seed coat dormancy caused by a specially thickened endocarp.Coevolution is complex web , yet beautiful creation of nature it has to sustain for benefitting of flora and fauna.

References:

Agrawal (2000) Agrawal AA. (2000): Overcompensation of plants in response to herbivory and the by-product benefits of mutualism . Trends Plant Sci . 5 : 309 – 313 . doi: 10.1016/S1360-1385(00)01679-4

Antonovics J , Thrall PH , Burdon JJ , Laine AL. (2011): Partial resistance in the Linum-Melampsora host-pathogen system: does partial resistance make the red queen run slower? Evolution . 65 : 512 – 522 . doi: 10.1111/j.1558-5646.2010.01146.x

Ballaré, Carlos L (2011) Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals:Trends in plant scienceVolume15,Issue5,pages249-257 Current Trends.

Benkman Craig W. (2010): Evolution: Education and Outreach volume 3, pages47–53 Original Scientific Article Open Access Published: 16 December 2009 Diversifying Coevolution between Crossbills and Conifers

Bruce, TJA CAO Midega, MA Birkett (2010): Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass- Biology- royalsocietypublishing.org

De Moraes, CM MC Mescher, JH Tumlinson (2001): Caterpillar-induced nocturnal plant volatiles repel conspecific females;- Nature,

Edwards, D.; Cherns, L.; Raven, J.A. (2015): Could land-based early photosynthesizing ecosystems have bioengineered the planet in mid-Palaeozoic times? Palaeontology, 58, 803–837

Ehrlich, Paul R. David S. Dobkin, and Darryl Wheye (1988): Birder's handbook- 1988 - books.google.com

Elbert, W.; Weber, B.; Burrows, S.; Steinkamp, J.; Büdel, B.; Andreae, M.O.; Pöschl, U. (2012): Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nat. Geosci., 5, 459–462.

Franceschi,VR P Krokene, E Christiansen (2005): Anatomical and chemical defenses of conifer bark against bark beetles and other pests- New 2005 - Wiley Online Library

Gibbs, A. (1980): A plant virus that partially protects its wild legume host against herbivores Intervirology, - karger.com

Harborne, J.B (1993); Advances in chemical ecology- Natural Product Reports, - pubs.rsc.org

Janzen, (1980): When is it coevolution? - copa.acguanacaste.ac.cr

Jensen H.R. Dreiseitl A , Sadiki M , Schoen DJ (2012): The red queen and the seed bank: pathogen resistance of ex situ and in situ conserved barley . Evol Appl . 5 : 353 – 367 . doi: 10.1111/j.1752-4571.2011.00227.x

Kessler and Baldwin, (2001): Defensive function of herbivore-induced plant volatile emissions in nature - science.sciencemag.org

Labandeira, (2013). A paleobiologic perspective on plant–insect interactions- Current Opinion in Plant Biology, - Elsevier

Maskell, LC AF Raybould, JI Cooper (1999): Effects of turnip mosaic virus and turnip yellow mosaic virus on the survival, growth and reproduction of wild cabbage (*Brassica oleracea*)- Annals of Applied 1999 - Wiley Online Library

Mendes, R.; Garbeva, P.; Raaijmakers, J.M. . (2013): The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev, 37, 634–663.

Nemri A , Barrett LG , Laine AL , Burdon JJ , Thrall PH . (2012): Population processes at multiple spatial scales maintain diversity and adaptation in the *Linum marginale–Melampsora* lini association. PLOS One 7: e41366 – e41366. doi: 10.1371/journal.pone.0041366

Pagán, I A Fraile (2010): Arabidopsis thaliana as a model for the study of plant–virus co-evolution of the Royal - royalsocietypublishing.org

Paterson S , Vogwill T , Buckling A , Benmayor R , Spiers AJ , Thomson NR , Quail M , Smith F , Walker D , Libberton B (2010): Antagonistic coevolution accelerates molecular evolution . Nature . 464 : 275 – 278 . doi: 10.1038/nature08798

Rausher MD. (2001):. Co-evolution and plant resistance to natural enemies . Nature . 411 : 857 – 864 . doi: 10.1038/35081193

Suresh S. (1992): Neem a new host record of Parosa hilaris(Cochilidiidae: Lepidoptera). Neem Newsletter, 9(3):42

Verma JS. (2006): Insect pest problem in medicinal plants- A review. Agricultural Reviews.; 27(3):130-136

Woolhouse MEJ , Webster JP , Domingo E , Charlesworth B , Levin BR. (2002):. Biological and biomedical implications of the co-evolution of pathogens and their hosts . Nat Genet . 32 : 569 – 577 .

Xu, P., F Chen, JP Mannas, T Feldman (2008): Virus infection improves drought tolerance - New 2008 - Wiley Online Library

Yilamujiang, A J Vadassery, W Boland (2012): Calmodulin-like proteins, CMLs: New players in plant defense regulation?- Endocytobiosis and cell - pure.mpg.de

DISEASE RESISTANCE IN PLANTS AND ITS FUTURE PERSPECTIVES

S. Sayen Merlin Sophia*[1], M. Muthu Selvi[2], M. SanjivKumar[1],

A. Parameswari[1] and K. Nagajothi[1]

[1]Department of Microbiology,

K. R. College of Arts and Science, K. R. Nagar, Kovipatti – 628 503, Tamil Nadu, India

[2]PG Department of Microbiology,

Sri Paramakalyani College, Alwarkurichi - 627 412, Tamil Nadu, India

*Corresponding author E-mail: sophia_micro@krcollege.net

Abstract:

Plants are efficient in defending themselves against pathogens but in some instances pathogens overcome the strategies and cause diseases. The world's growing population has made the modern agriculture even more strenuous because of the loss due to diseases. The melioration of plant resistance play a significant role in adjusting crop production to meet the increase in population. Disease resistance is more often the most dynamic component in the plant growth. New breeding techniques and genetic engineering modify the composition of plants and also boost resistance against microbial pathogens. New plant engineering techniques are been highlighted in plant research areas like developmental biology, abiotic stress tolerance or plant-pathogen resistance. Disease-resistant varieties of plants offer an effective, safe, and relatively inexpensive method of control for many crop diseases. This chapter entails the different approaches done to develop disease resistance in plants against various plant pathogens.

Key Words: Plant Disease, pathogen, resistance, genetic engineering

Introduction:

Disease involves a complex inter-play between a host plant and a pathogen, and the resistance/susceptibility response can involve several components. Crops are defenceless to disease causing pathogens such as fungi, bacteria, and viruses responsible for economic losses, (FAO, 2017). Some powerful new molecular techniques of the 21st century lead to the exploration of genes encoding regulators of disease resistance and susceptibleness (Llovera et *al*., 2012). Nowadays because of the high evolutionary potential of plant pathogens, the novel genotypes are not liable to resistance gene.

In recent times, chemicals like insecticides, pesticides or fertilizers are used to minimize the yield loss by improving the plant health as well as develop resistance against pathogens (Tyagi *et al.,* 2018; Vannier *et al.,* 2019). The usage of these chemical-based products contaminates soil, water bodies, and vegetation and also imposes risk to the host as well as to birds, fish, beneficial insects, and non-target plants.

New breeding techniques like powerful molecular approaches employ site-directed nucleases to introduce double stranded breaks at predetermined sites in DNA. Nevertheless, mutation breeding methods have been quite successful in improving disease resistance, and traditional plant breeding has been used to generate new crop varieties for decades. Numerous mutants have been developed through mutation induction, showing enhanced resistance to various diseases. Miklis *et al*. (2007) reported that the mutants induced at the mildew resistance locus (MLO) in barley for resistance to powdery mildew is the most commonly known mutant and Christopoulou *et al*. (2015) too reported the resistance conferred to several lettuce diseases by mutations. Genetic engineering is a valuable tool to develop disease-resistant plants by the interchange of the gene among species (Yin *et al.,* 2019)

In recent past CRISPR-Cas9 transformed agricultural science by exhibiting its capability to edit the genome of plant species and providing new possibilities (Dominguez *et al.,* 2016; Wang *et al.,* 2016; Shapiro *et al.,* 2018). CRISPR-Cas9 approach is cost effective and user-friendly and thus becomes a trendy technique when compared with other genetic engineering techniques.

Pathogen Derived Resistance:

Bacteria Disease Resistant Plants:

The development of plant varieties with inherent disease resistance through breeding provides an environmentally friendly approach to plant disease management (Sanchez-Martín and Keller, 2019). Nakajima *et al.* (1997) reported an effective approach to protect crops against bacterial diseases by introducing human lysozyme gene in *Nicotiana tabacum* cv `SR1'.The plant retarded the development of the pathogenic bacterium *Pseudomonas syringae* pv. *tabaci* and reduced the disease symptom to 17% of that observed in the wild-type tobacco. Mentag *et al*. (2003) enhanced the disease resistance by introducing the gene coding for D4E1, a synthetic antimicrobial peptide into poplar sps. via *Agrobacterium*-mediated transformation. The transgenic poplar plant reported resistance to *Agrobacterium tumefaciens*. Jaynes *et al.* (1993) reported the transgenic tobacco plants (RO) obtained via *Agrobacterium* transformation by introducing Cecropin B found in *Hyalophora cecropia* showed enhanced resistance to *Pseudomonas solanacearum*.

A synthetic gene encoding cecropin–melittin cationic peptide chimera (MsrA1) introduced into *Solanum tuberosum* L. by Osusky *et al*. (2000) enhanced the resistance of the plant to *Erwinia* sp. Huang *et al*. (1997) expressed a chimeric gene fusion cassette by joining sequence from barley a-amylase with modified cecropin (MB39) coding sequence and introduced into tobacco via *Agrobacterium*-mediated transformation. The transgenic tobacco exhibited enhanced disease resistance to the tobacco wildfire pathogen, *Pseudomonas syringae* pv. *tabaci.* In like manner De La Fuente- Martinez *et al*. (1992) introduced an ornithine carbamoyltransferase gene into plants to detoxify phaseolotoxin produced by *P. syringae* pv. *phaseolicola.*

Huang *et al.* (2007) expressed a foreign gene to enhance the plant disease resistance to bacterial pathogens. Sweet pepper ferredoxin-I protein (PFLP) expressed in tomato *Lycopersicon esculentum* cv. cherry Cln1558a safeguarded from the root-infecting pathogen, *Ralstonia solanacearum*. Hao *et al*. (2017) evaluated the citrus resistant to canker and Huanglongbing (HLB) by transgenically expressing D2A21 peptide . Disease resistant to *Pseudomonas syringae* pv. *tabaci* and *X. citri* was also seen in transgenic tobacco expressing D2A21 obtained by *Agrobacterium*-mediated transformation. The expression of cecropin B peptide in rice transformed it to confer resistance against *Xanthomonas oryzae pv. oryzae* (Arun Sharma *et al.,* 2000).

Fungal Disease Resistant Plants:

Nakajima *et al.* (1997) introduced human lysozyme gene into *Nicotiana tabacum* cv `SR1' by the *Agrobacterium*-mediated method. The transgenically modified tobacco plants exhibited increased resistance against the fungus *Erysiphe cichoracearum* in which both conidia formation and mycelia growth were reduced. Cary *et al*. (2000) in an in vivo study observed reduction in disease symptoms caused by *Colletotrichum destructivum* in transgenic tobacco expressing D4E1. Similarly, De Lucca *et al*. (1998) too reported that D4E1 inhibited the germination of conidia of *Aspergillus* spp. and *Fusarium oxysporum*. The activity of the phytopathogens *Fusarium* and *Phytophthora* sp was decreased by expressing MsrA1 gene in *Solanum tuberosum* L. making it an extremely promising tool in plant antimicrobial warfare.

The constitutive overexpression of thionin enhances the resistance of the susceptible ecotype Columbia (Col-2) against attack by *Fusarium oxysporum* (Epple *et al.,* 1997). Lorito *et al.* (1998) demonstrated the transfer of a antifungal endochitinase gene from Trichoderma *harzianum* to tobacco and potato. The transgenic plants showed resistance to the pathogens *Alternaria alternata, A. solani, Botrytis cinerea,* and *Rhizoctonia solani*. The improvement of

groundnut disease resistance against fungal pathogens by genetic engineering was reported by Prasad *et al*. (2011). The disease resistance of *Rosa hybrida* cv was enhanced by introducing a gene Ace-AMP1 via *Agrobacterium*-mediated transformation against *Sphaerotheca pannosa*, a pathogen causing powdery mildew (Xiangqian Li *et al*., 2003).

Wang *et al.* (2017) isolated MoHrip1 and MoHrip2 from the pathogenic fungus *Magnaporthe oryzae* and transformed rice with these genes separately. The transgenic rice plants displayed higher protection to rice blast and stronger tolerance to drought stress than wild-type (WT) rice and the vector-control *pCXUN* rice. Transgenic rice plants expressing the puroindoline genes *pinA* and/or *pinB* throughout the plants were produced by Krishnamurthy *et al*. (2001). These transgenic rice expressing *pinA* and/or *pinB* showed significantly increased tolerance to *M. grisea* (rice blast), with a 29 to 54% reduction in symptoms, and *R. solani* (sheath blight), with an 11 to 22% reduction in symptoms. Nishizawa *et al.* (1999) introduced a rice class-I chitinase gene, *Cht-2* or *Cht-3* into the Japonica rice varieties Nipponbare and Koshihikari. The transgenic rice plants expressing either chitinase gene exhibited increased resistance against the rice blast pathogen *Magnaporthe grisea* races 007.0 and 333.

Viral Disease Resistant Plants:

Viral plant diseases account for serious problem in agricultural production, causing insufficient production of food crops. Different approaches are used in controlling viral plant infections. The tobacco plants cloned with N gene confers resistance to tobacco mosaic virus. Whitham *et al*. (1996) generated transgenic tomato plants bearing the N gene which showed resistance to tobacco mosaic virus in tomato and also demonstrated the use of isolated resistance genes to protect crop plants from diseases. Gaber Attia Abo-Zaid *et al.* (2020) used *Streptomyces cellulosae* isolate as a biocontrol agent for the management of tobacco mosaic virus (TMV) and for inducing tomato plant systemic resistance under greenhouse conditions. Abdelkhalek *et al.*(2020) assessed the antiviral activity of *Bacillus velezensis* strain PEA1 on *Datura stramonium* plants and showed it as a novel antiviral agent against plant viruses.

Li *et al.* (2016) reported *Enterobacter asburiae* BQ9 induced a priming of the plant defense responses to Tomato yellow leaf curl virus (TYLCV) by increasing the expression of defense response genes. *Nicotiana benthamiana* ABA biosynthesis mutant was developed by Li *et al.*(2021) and they studied the role of the ABA pathway in PDMP-induced callose deposition during resistance to TMV infection. Their results indicated that callose priming was induced by *Penicillium chrysogenum* (PDMP) to defend against TMV. A genome-editing approach, known as CRISPR/Cas9 was used to produce virus-resistant cultivars. CRISPR/Cas9 considered as a highly promising genome-editing method in crops due to its unique features, such as reliable

precision, multiple-gene editing, limited off-target impact, greater output, and simplicity, Kumar and Jain (2015). Kumar *et al*. (2016) investigated *Paenibacillus lentimorbus* as a biocontrol agent against Cucumber mosaic virus (CMV) in *Nicotiana tabacum* cv.

Raupach *et al.* (1996) investigated the protection against CMV infection in cucumber and tomato plants by employing *Pseudomonas fluorescens* 89B-27 strain as PGPR. Yang *et al*. (2013) tried to achieve plant resistance against viral disease through pyramiding of virus genes via crossing or backcrossing .This include glycine max-soybean mosaic virus, (Shi *et al*., 2008), *Capsicum annuum*- pepper veinal mottle virus (Caranta *et al*., 1996), barley yellow mosaic virus, *Hordeum vulgare*-barley mild mosaic virus (Werner *et al*., 2005) and tomato leaf curl disease (Kadirvel *et al*., 2013).

Insect Resistant Plants:

Advances in genetic transformation and gene expression have created a rapid progress in using genetic engineering for crop improvement and for protection of crops against the insects. Cao *et al.* (1999) introduced a synthetic *Bacillus thuringiensis* (Bt) *cry1C* gene into broccoli by *Agrobacterium*-mediated transformation. Plants with high levels of Cry1C protein showed complete mortality of diamondback moth (DBM) larvae with no defoliation. Cotton (*Gossypium hirsutum*) plants targeting a cotton bollworm (*Helicoverpa armigera*) P450 gene, CYP6AE14 was developed by YB Mao *et al*. (2011) by expressing double-stranded RNA (dsRNA).The transgenic cotton plants reported improved resistance to cotton bollworms.

The expression of introduced d–endotoxin genes in tobacco and tomato provided the example of genetically modified plants with resistance to insects (Barton *et al*., 1987; Vaeck *et al*., 1987). Perlak *et al*. (1993) expressed synthetic Cry III in tobacco and potato plants for the control of Colorado potato beetle (*Leptinotarsa decemlineata*). Chen *et al*.(2005) developed transgenic rice plants by introducing a synthetic cry2A* gene by *agrobacterium*-mediated transformation to develop resistance to lepidopteran rice pests. Barton *et al*. (1987) constructed a chimeric gene and introduced it to tobacco (*Nicotiana tabacum* cv Havana 425) cells via *Agrobacterium tumefaciens* system to provide resistance to Lepidopteran insects.

Nematode Resistant Plants:

Plant parasitic nematodes (PPNs) parasitize various crop plants and cause serious damage and reduction in crop yields. The three most economically damaging genera of PPNs on crops are the root-knot, root lesion, and cyst nematodes. Various management strategies have to be done against PPNs to protect the plants. Sayre (1986) reported nematode fungal parasites *Dactylella oviparasitica*, *Nematophthora gynophila*, and *Paecilomyces lilacinu*s along with a

bacterium *Pasteuria penetrans* as important candidates for management of nematodes. Marroquin *et al*. (2000) first used Bt toxin as an anti-nematode protein by exposing Caenorhabditis *elegans* to Cry5B and Cry6A resulting in the reduction in nematode fertility and viability.

Ali *et al*. (2013b) reported that transgenic plants resistant to nematodes could be achieved by using precise delivery of transgene into the feeding sites as the constitutive overexpression or suppression of a particular gene could have detrimental effects on the growth and development of the plants. Lilley *et al*. (1999) reported the overexpression of different protease inhibitors (PIs) such as cowpea trypsin inhibitor (CpTI), PIN2, cystatins, and serine proteases for producing nematode resistant plants. Priya *et al.* (2011) showed the tobacco transgenic lines expressing AtNPR1 show enhanced resistance to the sedentary endoparasitic root knot nematode, *Meloidogyne incognita.* Meur *et al.* (2008) reported that heterologous expression of AtNPR1 provides enhanced resistance to early larval populations of *Spodoptera litura* in transgenic tobacco plants. Parkhi *et al.* (2010) showed a significant resistance to reniform nematodes by transgenic cotton expressing the *Arabidopsis* (*Arabidopsis thaliana*) NPR1 gene.

Future Directions:

Efforts to alter crop genetics will continue to develop better resistance in plants. Nucleotide-binding domains and leucine-rich repeats (NLRs) can be used as a major tool of biotechnology to engineer resistance to any pathogen through the modified activity of the CRISPR/Cas9 system. Shao *et al.* (2003) and Qi *et al.* (2014) reported an approach utilizing the activation of Arabidopsis NLR RPS5 by *P. syringae* protease AvrPphB cleavage of PBS1. β-aminobutyric acid (BABA) can induce resistance in a wide range of plants against several types of pathogens, including potato infected with *Phytophthora infestans*. Bengtsson *et al.* (2014) elucidated the defence responses activated by BABA in potato using a genome-wide transcript microarray analysis in combination with label-free quantitative proteomics analysis of the apoplast secretome performed after two day treatment of the leaf canopy with BABA at two concentrations, 1 and 10 mM.

Plant resistance can also be induced through the application of plant resistance inducers (PRIs), which can be either chemical agents, extracts from plants or microbes (Alexandersson *et al.*, 2016). Gupta *et al.* (2021) reviewed the usage of different approaches, including conventional breeding and genome editing for developing disease-resistant cultivars using SWEET genes (as S genes).

Table 1: Genes expressed in plants that enhanced resistance to pathogens

Plant	Gene Encoding	Technique	Activity / Effect	Reference
Carrizo plants	Thionin gene	*Agrobacterium*-mediated transformation	Resistance to Citrus Canker	*Hao et al*. (2016)
Coffea arabica	S gene	CRISPR-Cas9	Resistance to Fungal diseases	Cui *et al.* (2020)
Rice	OsERF922 gene	CRISPR-Cas9	Resistance to Blast Disease	Wang *et al.* (2016)
Soybean	NLR gene	CRISPR/Cas9	Resistance to *Phakopsora pachyrhizi* and *P. sojae*	Nagy *et al.* (2021)
Arabidopsis thaliana	*AtERF019*	CRISPR/Cas9	Resistance to *Phytophthora parasitica*	Lu *et al.* (2020)
Maize	*Lr34*	-	Resistance to Leaf Blight Disease	Sucher *et al.* (2016)
Tobacco	*Avr9 or infl*	-	Resistance to potato virus X	Kamoun *et al*. (1999)
Tobacco	Bean Chitinase gene	-	Resistance to *Rhizoctonia solani*	Broglie *et al.*(1991)
Sorghum	Rice Chitinase gene	Biolistic Transformation	Resistance to Stalk Rot	Krishnaveni *et al.* (2001)
Sorghum	*Trichoderma harzianum* Chitinases and Chitosanases	Particle Bombardment	Resistance to Anthracnose	Kosambo-Ayoo *et al.* (2011)
Pigeon Pea	Rice Chitinase gene	*Agrobacterium*-mediated transformation	Resistance to Fungal diseases	Kumar *et al.* (2004)
Pearl Millet	*pin and bar* gene	Particle-Inflow-Gun (PIG)	Resistance to Downy Mildew Pathogen	Latha *et al.* (2006)

Lomonossoff, (1995) stated that researchers have observed transgenic plants displaying immunity to the pathogen and its related strains when expressing genes derived from viral pathogens. An effective approach of engineering resistance to viruses can be done by the activation of RNA interference (RNAi), Lindbo and Falk (2017) as viruses rely on the host cellular machinery to complete their life cycle.

Wright *et al*. (2016) reported CRISPR-associated (Cas) technology as a promising approach for engineering resistance against plant viruses. Chandrasekaran *et al*. (2016) and Pyott *et al.* (2016) showed the induction of resistance against multiple RNA viruses in *Arabidopsis* and cucumber (*Cucumis sativus*) by using CRISPR-Cas9 to introduce indels affecting eukaryotic translation initiation factor 4E proteins (eIF4Es). Kieu *et al.*(2021) reported the resistance conferred against late blight disease by mutation and screening of putative S-genes in potatoes, including two DMR6 potato homologues . Ahmad *et al*. (2020) reported the current work done by scientists on reducing off-target issues by using computational tools like Guide-seq, Diagenome-seq, DISCOVER, etc. and by re-editing the CRISPR components such as Cas proteins and gRNA.

Acknowledgement:

The authors gratefully acknowledge the Management and the Principal of K.R.College of Arts & Science for their moral support.

References:

Abdelkhalek.A, Behiry S.I and Al-Askar A.A (2020) : *Bacillus velezensis* PEA1 Inhibits *Fusarium oxysporum* Growth and Induces Systemic Resistance to Cucumber Mosaic Virus, Agronomy ,10, 1312.

Ahmad, S., Wei, X., Sheng, Z., Hu, P., and Tang, S. (2020): CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects, Brief. Funct. Genom,19, 26–39.

Alexandersson E, Mulugeta T, Lankinen Å, Liljeroth E, Andreasson E (2016): Plant resistance inducers against pathogens in Solanaceae species-from molecular mechanisms to field application,International Journal of Molecular Sciences;17(10):1673.

Ali, M. A., Plattner, S., Radakovic, Z., Wieczorek, K., Elashry, A., Grundler, F. M., et al. (2013b):An Arabidopsis ATPase gene involved in nematode-induced syncytium development and abiotic stress responses, Plant J,74, 852–866.

Antolín-Llovera, M., Ried, M.K., Binder, A., Parniske, M. (2012): Receptor kinase signaling pathways in plant-microbe interactions,Ann. Rev. Phytopathol, 50, 451–473.

Arun Sharma, Rashmi Sharma, Morikazu Imamura, Minoru Yamakawa and Hiroaki Machii, (2000): Transgenic expression of cecropin B, an antibacterial peptide from *Bombyx mori*, confers enhanced resistance to bacterial leaf blight in rice, FEBS Letters, 484 , 7-11.

Barton KA, Whiteley HR and Yang NS (1987): *Bacillus thuringiensis* 6-Endotoxin Expressed in Transgenic *Nicotiana tabacum* Provides Resistance to Lepidopteran Insects, Plant Physiol, 85, 1103-1109.

Barton, K., Whitely, H. and Yang, N.S. (1987): *Bacillus thuringiensis* d-endotoxin in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects, Plant Physiology, 85,1103-1109.

Bengtsson T, Weighill D, Wera E.P, Levander F, Resjo S, Burra D. D, Moushib L.I , Hedley P.E, Liljeroth E, Jacobson D, Alexandersson E and Andersson E (2014): Proteomics and transcriptomics of the BABA-induced resistance response in potato using a novel functional annotation approach, BMC Genomics,15:315.

Broglie, K., Chet, I., Holliday, M., Cressman, R., Biddle, P., Knowlton, S., Mauvais, C. J., and Broglie, R. (1991): Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*, Science,254,1194- 1197.

Cao.J, Tang J.D, Strizhov N, Shelton A.M and Earle E.D (1999): Transgenic broccoli with high levels of *Bacillus thuringiensis* Cry1C protein control diamond back moth larvae resistant to Cry1A or Cry1C, Molecular Breeding,5,131–141.

Caranta, C., Palloix A., Gebre-Selassie K., Lefebvre V and Moury B(1996):A complementation of two genes originating from susceptible *Capsicum annuum* lines confers a new and complete resistance to pepper veinal mottle virus,Phytopathol,86,739-743.

Cary, J.W, Rajasekaran K,. Jaynes J.M and Cleveland T.E (2000): Transgenic expression of a gene encoding a synthetic antimicrobial peptide results in inhibition of fungal growth in vitro and in planta, Plant Sci,154,171–181.

Chandrasekaran J, et al. (2016): Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology,Mol Plant Pathol,17(7):1140–53.

Chen.H, Tang.W, Xu .C, Li.X, Lin.Y and Zhang.Q (2005): Transgenic indica rice plants harboring a synthetic cry2A* gene of *Bacillus thuringiensis* exhibit enhanced resistance against lepidopteran rice pests, Theor Appl Genet,111,1330–1337.

Christopoulou, M., Reyes-Chin, W. S., Kozik, A., McHale, L. K., Truco, M. J., Wroblewski, T., et al. (2015): Genome-Wide architecture of disease resistance genes in lettuce,G3 (Bethesda) 5, 2655–2669.

Cui L, Hanika K, Visser R G F and Bai Y(2020): Improving Pathogen Resistance by Exploiting Plant Susceptibility Genes in Coffee (Coffea spp.), Agronomy , 10, 1928.

De Lucca, A.J., Bland J.M., Grimm C., Jacks T.J., Cary J.W., Jaynes J.M., Cleveland T.E. and Walsh T.J(1998): Fungicidal properties, sterol binding, and proteolytic resistance of the synthetic peptide D4E1,Can. J. Microbiol,44,514–520.

Destefano-Beltran, L., Nagpala, P., Cetiner, S., Dodds, J. H., and Jaynes, J. M (1990): Enhancing bacterial and fungal disease resistance in plants. Pages 205-221 in: The Molecular and Cellular Biology of the Potato. M. Vayda and W. Park, eds. CAB International, Wallingford, England.

Dominguez AA, Lim WA and Qi LS (2016): Beyond editing repurposing CRISPR-Cas9 for precision genome regulation and interrogation, Nat Rev Mol Cell Biol,17:5–15.

Epple, P., Apel, K. & Bohlmann, H (1997): Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*, Plant Cell, 9, 509–520.

FAO (2017): The Future of Food and Agriculture ,Trends and Challenges, Rome, FAO.

Gaber Attia Abo-Zaid , Saleh Mohamed Matar and Ahmed Abdelkhalek (2020) : Induction of Plant Resistance against Tobacco Mosaic Virus Using the Biocontrol Agent *Streptomyces cellulosae* Isolate Actino 48, Agronomy , 10, 1620.

Gupta P.K, Balyan H.S and Gautam T(2021): SWEET genes and TAL effectors for disease resistance in plants:Present status and future prospects, Mol Plant Pathol,22,1014–1026.

Hao G, Stover E and Gupta G (2016): Overexpression of a Modified Plant Thionin Enhances Disease Resistance to Citrus Canker and Huanglongbing (HLB),Front. Plant Sci,7,1078.

Hao G, Zhang S, Stover E (2017):Transgenic expression of antimicrobial peptide D2A21 confers resistance to diseases incited by *Pseudomonas syringae* pv. *tabaci* and *Xanthomonas citri*, but not *Candidatus Liberibacter asiaticus*, PLoS ONE,12(10): e0186810.

Huang, H. E., Liu, C.A., Lee, M.J., Kuo, C.G., Chen, H.M., Ger, M.J., Tsai, Y.C., Chen, Y.R., Lin, M.K and Feng, T.Y (2007):Resistance enhancement of transgenic tomato to bacterial pathogens by the heterologous expression of sweet pepper ferredoxin-I protein, Phytopathology,97,900-906.

Huang, Y., Nordeen R.O.,. Di M, Owens L.D and McBeath J.H (1997): Expression of an engineered cecropin gene cassette in transgenic tobacco plants confers disease resistance to *Pseudomonas syringae* pv. *tabaci*. Phytopathology,87,494–499.

Jaynes, J.M., P. Nagpala, L. Destefano Beltran, H. Huang Jian, J. Kim, T. Denny and S. Cetiner (1993):Expression of a cecropin B lytic peptide analog in transgenic tobacco confers enhanced resistance to bacterial wilt caused by *Pseudomonas solanacearum*, Plant Sci,89,43–53.

Kadirvel, P., De La Pana, R., Schaffeitner, R. (2013): Mapping of QTLs in tomato line FLA456 associated with resistance to a virus causing yellow leaf curl disease, Euphytica,190, 297-308.

Kamoun S., Honee G., Weide R., Lauge R., Kooman-Gersmann M., de Groot K., Govers F., de Wit P.J., (1999):The fungal gene *Avr9* and the oomycete gene *inf1* confer avirulence to potato virus X on tobacco, Molecular Plant-Microbe Interactions,12,459-462.

Kieu N P, Lenman M, Wang E S , Petersen B L & Andreasson E(2021): Mutations introduced in susceptibility genes through CRISPR/Cas9 genome editing confer increased late blight resistance in potatoes, Scientific Reports ,11:4487.

Kosambo-Ayoo L M, BaderM, Loerz H and Becker D,(2011): Transgenic sorghum (*Sorghum bicolor* L. *Moench*) developed by transformation with chitinase and chitosanase genes from *Trichoderma harzianum* expresses tolerance to anthracnose, African Journal of Biotechnology,10(19):3659-3670.

Krishnamurthy K, Balconi C, Sherwood JE, Giroux MJ (2001):Wheat puroindolines enhance fungal disease resistance in transgenic rice, Mol. Plant-Microbe Interact,14,1255-1260.

Krishnaveni S, Jeoung JM, Muthukrishnan S and Liang GH (2001):Transgenic sorghum plants constitutively expressing a rice chitinase gene show improved resistance to stalk rot, J Genetic Breeding, 55,151–158.

Kumar S M,Kumar B K,Sharma K K and Devi P,(2004): Genetic transformation of pigeonpea with rice chitinase gene, Plant Breeding,123, 485-489.

Kumar S, Chauhan PS, Agrawal L, Raj R, Srivastava A, Gupta S, et al. (2016): *Paenibacillus lentimorbus* Inoculation Enhances Tobacco Growth and Extenuates the Virulence of Cucumber mosaic virus,PLoS ONE,11(3): e0149980.

Kumar, V and Jain, M (2015): The CRISPR-Cas system for plant genome editing: Advances and opportunities., J. Exp. Bot, 66, 47–57.

Latha A M. Rao K V ,Reddy T P and Reddy V D,(2006): Development of transgenic pearl millet (*Pennisetum glaucu*m (L.) R. Br.) plants resistant to downy mildew, Plant Cell Rep,25,927–935.

Li .H, Ding.X, Wang.C, Ke.H, Wu.Z,Wang.Y, Liu.H And Gu.J (2016):Control of Tomato yellow leaf curl virus disease by *Enterobacter asburiae* BQ9 as a result of priming plant resistance in tomatoes, Turk J Biol,40,150-159.

Li.Y, Jiao M, Li.Y, Zhong.Y, Li.X, Zhuang Chen.Z, Chen.S, and Wang.J (2021): *Penicillium chrysogenum* polypeptide extract protects tobacco plants from tobacco mosaic virus infection through modulation of ABA biosynthesis and callose priming, Journal of Experimental Botany,72(10): 3526–3539.

Lilley, C. J., Devlin, F., Urwin, P. E., and Atkinson, H. J(1999):Parasitic nematodes, proteinases and transgenic plants, Parasitol Today,15, 414–417.

Lindbo JA, Falk BW(2017): The impact of "coat protein-mediated virus resistance" in applied plant pathology and basic research,Phytopathology,107,624–634.

Lomonossoff G P (1995): Pathogen-derived resistance to plant viruses. Annu Rev Phytopathol,33,323–343.

Lorito M, Sheridan L. Woo, Irene Garcia Fernandez, Gabriella Colucci, Gary E. Harman, Jose A. Pintor-Toro, Edgardo Filippone, Simona Muccifora, Christopher B. Lawrence, Astolfo Zoina, Sadik Tuzun And Felice Scala(1998): Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens, Proc. Natl. Acad. Sci. USA ,95, 7860–7865.

Lu, W., Deng, F., Jia, J., Chen, X., Li, J., Wen, Q., et al. (2020): The *Arabidopsis thaliana* gene *AtERF019* negatively regulates plant resistance to *Phytophthora parasitica* by suppressing PAMP-triggered immunity, Mol. Plant Pathol, 21, 1179–1193.

Mao YB, Tao XY, Xue XY, Wang LJ and Chen XY(2011):Cotton plants expressing CYP6AE14 double-stranded RNA show enhanced resistance to bollworms, Transgenic Res ,20,665–673.

Marroquin, L. D., Elyassnia, D., Griffitts, J. S., Feitelson, J. S., and Aroian, R. V(2000): *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans*,Genetics,155,1693–1699.

Mentag R, Luckevich M, Morency and Seguin A (2003) : Bacterial disease resistance of transgenic hybrid poplar expressing the synthetic antimicrobial peptide D4E1, Tree Physiology ,23, 405–411.

Meur G, Budatha M, Srinivasan T, Rajesh Kumar K.R, Gupta A.D, Kirti P.B (2008): Constitutive expression of *Arabidopsis* NPR1 confers enhanced resistance to the early instars of *Spodoptera litura* in transgenic tobacco, Physiol Plant, 133(4):765-75.

Miklis, M., Consonni, C., Riyaz, A. B., Volker, L., Schulze-Lefert, P and Panstruga, R. (2007): Barley MLO modulates actin-dependent and actin independent antifungal defense pathways at the cell periphery, Plant Physiol, 144, 1132–1143.

Nagy, E. D., Stevens, J. L., Yu, N., Hubmeier, C. S., LaFaver, N., Gillespie, M., et al. (2021): Novel disease resistance gene paralogs created by CRISPR/Cas9 in soy, Plant Cell Rep,40, 1047–1058.

Nakajima H, Muranaka T, Ishige F, Akutsu K and Oeda K (1997) : Fungal and bacterial disease resistance in transgenic plants expressing human lysozyme, Plant Cell Reports,16:674–679.

Nishizawa Y, Nishio Z, Nakazono K, Soma M, Nakajima E, Ugaki M, Hibi M (1999): Enhanced resistance to blast (*Magnaporthe grisea*) in transgenic japonica rice by constitutive expression of rice chitinase, Theor. Appl. Genet,99,383-390.

Osusky, M., Zhou G., Osuska L., Hancock R.E., Kay W.W. and Misra S (2000): Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens, Nat. Biotechnol,18,1162–1166.

Parkhi V, Vinod Kumar, Campbell L.M , Bell A.A , Shah J, Rathore K.S (2010): Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing Arabidopsis NPR1, Transgenic Res,19(6):959-75.

Perlak, F.J., Stone, T.B., Muskopf, Y.N., Petersen, Parker, G.B., McPherson, S.A., Wyman, J., Love, S., Reed, G., Biever, D. and Fischhoff, D.A. (1993):Genetically improved potatoes: protection from damage by Colorado potato beetles, Plant Molecular Biology,22,313-317.

Prasad, K and Bhatnagar-Mathur, P and Narasu, M L and Waliyar, F and Sharma, K K (2011):*Transgenic approaches for improving fungal disease resistance in groundnut,*Technology Spectrum, 5.(1):1-10.

Priya D.B, Somasekhar N, Prasad J.S and Kirti J.B (2011):Transgenic tobacco plants constitutively expressing *Arabidopsis* NPR1 show enhanced resistance to root-knot nematode, *Meloidogyne incognita*, BMC Research Notes,4,231.

Pyott DE, Sheehan E, Molnar A (2016): Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants, Mol Plant Pathol,17(8):1276–88.

Qi, D., Dubiella, U., Kim, S.H., Sloss, D.I., Dowen, R.H., Dixon, J.E., Innes, R.W (2014): Recognition of the protein kinase AVRPPHB SUSCEPTIBLE1 by the disease resistance protein resistance to *Pseudomonas Syringiae*5 is dependent on S-acylation and an exposed loop in AVRPPHB SUSCEPTIBLE1,Plant Physiol, 164, 340–351.

Raupach GS, Liu L, Murphy JF, Tuzun S, Kloepper JW (1996):Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR),Plant Disease,80,91–894.

Sanchez-Martin, J., and Keller, B. (2019): Contribution of recent technological advances to future resistance breeding, Theor. Appl. Genet, 132, 713–732.

Shao, F, Golstein, C, Ade, J,Stoutemyer, M, Dixon, J.E, Innes, R.W (2003): Cleavage of Arabidopsis PBS1 by a bacterial type III effector, Science , 301, 1230–1233.

Shapiro R, Chavez A, Porter CB, Hamblin M, Kaas CS, DiCarlo JE, Zeng G, Xu X, Revtovich AV, Kirienko NV, Wang Y. A (2018): CRISPR–Cas9-based gene drive platform for genetic interaction analysis in *Candida albicans*, Nat Microbiol,3:73–82.

Shi, A., Chen, P., Li, D.X., Zheng, C., Hou, A (2008): Genetic confirmation of 2 independent genes for resistance to soybean mosaic virus in J05 soybean using SSR markers, Journal of Heredity,99,598-603.

Sucher, J., Boni, R., Yang, P., Rogowsky, P., Büchner, H., Kastner, C., Kumlehn, J., Krattinger, S.G., Keller, B (2016):The durable wheat disease resistance geneLr34confers common rust and northern corn leaf blight resistance in maize, Plant Biotechnol. J, 15, 489–496.

Tyagi S, Mulla S I, Lee K-J, Chae J-C, Shukla P (2018):VOCs-mediated hormonal signaling and crosstalk with plant growth promoting microbes, Crit Rev Biotechnol,38(8):1277–96.

Vaeck, M., Reynaerts, A., Hoftey, H., Jansens, DeBeuckleer, M., Dean, C., Zabeau, M., Van Montagu, and Leemans, J. (1987): Transgenic plants protected insect attack,Nature,327, 33-37.

Vannier N, Agler M and Hacquard S (2019): Microbiota-mediated disease resistance in plants, PLoS Pathog,15(6): e1007740–e1007740.

Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Liu Y-G, Zhao K (2016): Enhanced rice blast resistance by CRISPR/ Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922,PLoS One,11(4): e0154027.

Wang Z, Han Q, Zi Q, Lv S, Qiu D, Zeng H (2017):Enhanced disease resistance and drought tolerance in transgenic rice plants overexpressing protein elicitors from *Magnaporthe oryzae*, PLoS ONE,12(4): e0175734.

Wang, F.;Wang, C.; Liu, P.; Lei, C.; Hao,W.; Gao, Y.; Liu, Y.-G.; Zhao, K 92016): Enhanced Rice Blast Resistance by CRISPR/Cas9-Targeted Mutagenesis of the ERF Transcription Factor Gene OsERF922, PLoS ONE, 11, e0154027.

Werner, K., Friedt, W., Ordon, F (2005),Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2),Mol. Breed,16,45-55.

Whitham, S., McCormick, S., and Baker, B. (1996):The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato, Proc. Natl. Acad. Sci. U. S. A,93,8776–8781.

Wright AV, Nuñez JK, Doudna JA (2016):Biology and applications of CRISPR systems: Harnessing nature's toolbox for genome engineering, Cell, 164, 29–44.

Xiangqian Li, Ksenjia Gasic, Bruno Cammue, Willem Broekaert and Schuyler S. Korban (2003) : Transgenic rose lines harboring an antimicrobial protein gene, Ace-AMP1, demonstrate enhanced resistance to powdery mildew (*Sphaerotheca pannosa*), Planta,218,226–232

Yang, L., Wang, W., Yang, W., Wang, M. (2013): Marker- assisted selection for pyramiding the waxy and opaque-16 genes in maize using cross and backcross schemes, Mol.Breed,31, 767- 75.

Yin K and Qiu J L (2019): Genome editing for plant disease resistance, applications and perspectives, Philos Trans R Soc Lond B Biol Sci,374(1767):20180322.

PHYTOREMEDIATION AND PHYTOMINING - AN OVERVIEW

Dhanya G.*, Vinod Gopal V. and Radhamany P. M.

Department of Botany,

University of Kerala, Kariavattom,

Thiruvananthapuram – 695 581, Kerala, India

Corresponding Author E-mail: gdhanyakrish@gmail.com, gopalvinod85@gmail.com, radhamany_m@rediffmail.com

Abstract:

Pollution of land and water by heavy metals is an important anthropogenic hazard inviting worldwide concern. The non-biodegradability of heavy metalsresult ingravemenace toall life forms even at extraminute concentrations. Therefore clean-up of these soils and water is unavoidable though it is very expensive and very hard to carry out. Chemical treatments and electro-kinetics have been result in soil degradation and become unsuitable for plant growth. Therefore vegetative methods (phytoremediation using hyper-accumulator plants) are thought to be most practical and economical. Yet, there are obstacles and drawbacks in plant-based remediation technologies; after harvesting, disposal of contaminated plant material has been one of the hurdles for commercial implementation of phytoremediation. To overcome these hitches, now researchers are focused to upsurge the economic possibility of phytoremediation techniques, and to diminish disposal risks via the application of metal enriched plant biomass in energy and metal recovery with the burnt process (Pyrolysis and Phytomining).

Keywords: heavy metal, phytoremediation, pyrolysis and phytomining.

Introduction:

Heavy metal (HM) pollution designates a grave menace for both the environment and human health. Heavy metals are those metallic elements, which have their atomic weight more than 23 or in other words with a density higher than 5 g cm-3 (Sharma and Dietz, 2006; Weast, 1984). There are 90 naturally occurring heavy metals, but not all of them are biologically significant and all the heavy metals do not have toxic effects. As per its solubility and absorptivity, seventeen metals are considered asmicronutrients (Fe, Mo, and Mn etc.)and are important for organisms and ecosystems and are very indispensable for satisfying many vital functions in plant metabolism (Weast, 1984; Raven *et al*., 1999).

Naturally all these elements are found in very low concentrations. But due to various anthropogenic activities the concentration exceeds limits and threatens the environment. Due to

itsimmutability, non-degradability and persistency, uptake of these heavy metals in extra low quantities (1 or 2 μg) resulting into severeimpacts on the health of humans, animals, and plants.Hence the clean-up of these soils is inevitable however it is very expensive, overheads time and it is very hard to carry out (Ansari *et al*., 2016). Their detoxification occurs either by stabilization or by elimination from the medium, e.g., soil/water. There are various advanced methods (physical, chemical, and biological) for the removal of heavy metals from the environment. But over dependency towards vitrification, landfilling, chemical treatment and electro-kinetics have been result in soil degradation (by removing all fauna, flora, and micro-organisms including nitrogen fixing bacteria and phosphorous-enhancing mycorrhizal fungi) and leaves the soil unfit for plant growth. Therefore vegetative methods (phytoremediation) are thought to be most practical and economical than that of other expensive treatments including the use of microbes for bioremediation because of the ease, availability and viability (Lasat, 2000). Recently, many morestudies has been reported on the use of hyper-accumulator plants for the treatment of heavy metal contaminated sites (Pilon-Smits and Freeman, 2006). Hyper-accumulators are plants capable of absorbing and translocating high concentrations (100-1000-fold higher than non-hyper accumulators)of heavy metals in their tissues without much apparent symptoms of toxicity are well demanded in phytoremediation (Rascio *et al*., 2011).

Phytoremediation is the clean-up of pollutedsediments, soils, or water by the use of photosynthetic hyper-accumulator plants and their rhizospheric micro-organisms by various mechanisms (phytostabilization, phytoextraction, or rhizofiltration and phytovolatalization) in which green plants modify the chemical structure of soil matrix into a harmless form. It includes isolation, uptake, accumulation, degradation, and metabolism of contaminants by plants or related rhizospheric free-living micro-organisms inducing biochemical and biophysical processes to ensure waste remediation. Phytoremediation can be executed through diverse methods based on the pollutant characteristics, nature of environment (aqueous or soil) and on the plant species preferred. Phytoremediation can be considered as biological, solar-driven, pump-and-treat systems with abroad networking of root systemto enhance the uptake and transformation of heavy metals from the polluted below-ground ecosystem for successive productive use (Negri *et al*., 1996). It has several advantages over other techniques, as it is cost effective, possibility forsimultaneous removal of many heavy metals, excavation is not needed, acceptability, and the opportunity for phytomining etc. (Suman *et al*., 2018).

Phytoremediation encompasses different methods such as degradation, removal (through accumulation or dissipation), or immobilization of various contaminants:

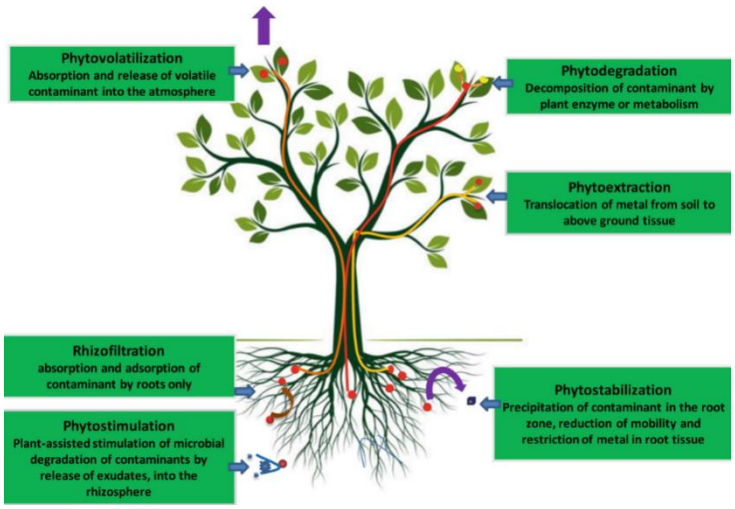


Figure 1: Phytoremediation- different types (Source: Kumar *et al*., 2021)

1. **Degradation for destruction or alteration of organic contaminants).**
   a. Rhizodegradation: improvement of biodegradation in the rhyzospheric zone by microbes.
   b. Phytodegradation: degradation of heavy metals by plants uptake breakdown and absorption in the plant cells(root, stem, or leaves).
2. **Accumulation of contaminants (organic and/ or metal)for removal from the medium.**
   a. Phytoextraction: absorption of pollutants and buildupin the cells for removal.
   b. Rhizofiltration: adsorption of pollutants on roots for immobilization and/or removal.
3. **Dissipationof the pollutants for removal of organic and/or inorganic contaminants into the atmosphere.**
   a. Phytovolatilization: pollutant uptake and volatilization.
4. **Immobilization for containment of organic and/or inorganic contaminants).**
   a. Hydraulic Control: control of ground-water flow by plant uptake of water.
   b. Phytostabilization: pollutantstabilization by immobilizing them in the soil (Pivetz, 2001).

Plants have advanced highly precise and very effectual mechanisms to acquire essential micro-nutrients from the soil/ water, even if their presence is at very low concentrations. Root exudates of plants acts as chelating agents and reduced the soil pH by redox reactions, thereby increasing the solubility and availability of micronutrients from the soil. Plants also possesses exact mechanisms to translocate and store micronutrients and these mechanisms are also responsiblefor the uptake, translocation, and storage of heavy metals or other toxic pollutants,

whose chemical properties mimic those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation [Antoniadis *et al*. 2014].

Therefore, phytoremediation is ideally suited for the protection of the environment. The advantages of this technique are economical, energy efficient and environmental friendly; plants can be easily monitored to ensure proper growth; valuable metals can be reclaimed and reused through phytoremediation; the least destructive method (Lasat, 2000). However, there are obstacles and drawbacks in plant-based remediation technologies; after harvesting, disposal of contaminated plant material has been one of the hurdles for commercial implementation of phytoremediation. Accumulation of massive amounts of dangerous and heavy metal polluted biomass is the main problem of phyto-extraction process. It biomass should be properly stored and disposed to avoidfurther environmental pollution.To overcome these disadvantages, now researchers are concentrating to increase the financial possibility of phytoremediation programs, and to reduce disposal risks by the utilizingthe metal enriched plant biomass in energy and metal recovery with the burnt process (Pyrolysis and Phytomining).

Phytomining:

Phytomining is considered as a phytoextraction technology and it is the in-situ removal of heavy metals from metal polluted soils, polluted mining sites, sub-economic ore bodieswith anintentionto acquirecommercialbenefitfrom the hyper accumulating plants (Sheoran *et al*., 2009). Hyper accumulating plants absorb metals from thesubstrate, hold them in root tissues, and then transport them to the aboveground part (Timofeeva *et al*., 2017). Subsequent satisfactory growth, plant is reaped and left for drying and is condensed to an ash with or without energy recovery, and metal ore from the ash is recovered through conventional metal refining methods such as acid dissolution and electro-winning, after treating the ash by roasting, sintering, or smelting methods (Robinson *et al*., 2009).

Factors Influencing Phytomining:

Even if hyper-accumulators are very effective in phytoextarction and phytomining, the process is controlled by several factors. These factors can be mainly categorized as internal factors and external factors. The metal bioavailability can be increased by bringing modulation in both internal (plant associated) factors and external (soil associated) factors.

Internal factors (plant associated factors)

1. Plant species (hyper accumulator- biomass yield),
2. Planting characteristics (e.g. plant density, seeding, cropping and harvesting methods),
3. Plant exudates (metal chelating compounds-phytochelators/phytosiderophores) to enhance accumulation by changing metal speciation) and
4. Fungal symbiotic associations (to enhance root absorption area and stimulate the acquisition of plant nutrients along with metal ions).

External factors (soil associated factors)

1. Contaminant concentrations,
2. Fertilizer application,
3. Chelating agent addition,
4. Soil properties (e.g. salinity, moisture, texture and pH),
5. Rhizobiologicalactivity,
6. Exudates release,
7. Prevailing temperature,
8. Competing ions affecting plant growth and
9. Solubility and availability of the metals in the soil-water system (Mohanty, 2016; Wang *et al*., 2019)

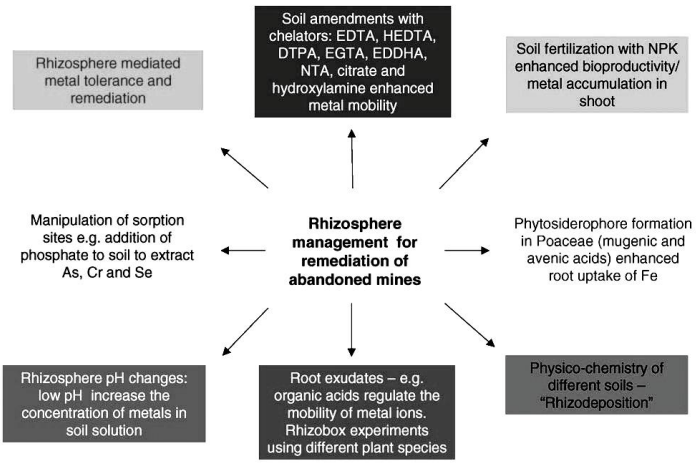


Figure 2: Factors affecting Phytomining (Source: Mohanty, 2016)

Advantages and Limitations of Phytomining:

Advantages:

1. Plants are renewable resources and source of energy used for the purpose is mostly solar. Hence this is environment friendly, aesthetically pleasing; visual unobstructive, non-invasive, non-destructive technology that has high probability of public acceptance and has approaches in mining fields (Sheoran*et al*., 2009).
2. It can restore the soil to health.
3. Cheaper alternative to excavation
4. Combustion of biomass for the release energy and bio-ore production
5. Reduces the need to obtain new ore by mining
6. Conserves limited supplies of high-grade ores
7. Reduces the amount of rock waste that must be disposed of after traditional mining

8. Bio-ore processing contribute less SOx emission than conventional mining
9. Helps in soil remediation, recovery and reuse of the metal with restoration of mined degraded land.
10. Re-vegetation in degraded mines minimizes soil erosion and surface run off and thus prevents metal spread.
11. Re-vegetation turns the degraded environment to a suitable habitat for diverse range of species.
12. It hasanopportunityto recover metals from over-burdens,low-grade ores, mill tailings, or metalliferrous soil that is highly expensive byordinary mining techniques (Nedelkoska and Doran, 2000).
13. Smelting of bio-ore requires less energy than sulphide ores as it is free from sulphur.
14. Metal content in the bio-ore is higher than that in the conventional ore and needs less space for storage.
15. It can be considered as a phytoremediation technique of heavy metal pollutedsites.
16. Phytomining produce nano-particles of metals inside the plant cells, which are of high economic importance.
17. Long term cultivation of plants with use of fertilizers may result in a permanent increase in soil organic matter levels and sequestration at atmospheric carbon dioxide
18. In large scale applications the potential energy stored can be exploited to generate thermal energy.
19. Restoration of vegetation cover can encounter the goals of stabilization and accelerate ecological succession (Sheoran*et al*., 2009).

Limitations:

1. Large-scale phytomining is currently more costly than extracting metals from mines.
2. Phytomining very slow and prolonged time for remediation process.
3. Phytomining depends on forces of nature (weather, soil quality etc.) (Patra and Mohanty, 2013)
4. Bioavailability of only target metal(s).
5. Proper storage of harvested biomass isvery essential to prevent potential risk pathways such as introduction to the food chain.
6. Metal collected in leaves can be released again to the environment during defoliation (Nevel*et al*., 2007; Sheoran*et al*., 2009).
7. Plants accumulate metals within above ground biomass, which is low.
8. Polluted site must be large enough to carry out phytomining.
9. Limited to low and medium metal contaminant concentrations.
10. Climate dependent/variable; seasonal effectiveness.

11. Introduction of non-native species (induced phytomining) may affect biodiversity (competition/allelopathy).
12. Tightly bound fraction of metals in soil clay requires higher chelate application rates, leading to ground water pollution.
13. The contaminants must be in the root zone (rhizosphere) to be drawn up by plants.
14. Most of the hyperaccumulators are not suitable for field applications due to low biomass and slow growth. (Ali *et al*., 2013; Mahar *et al*., 2016).

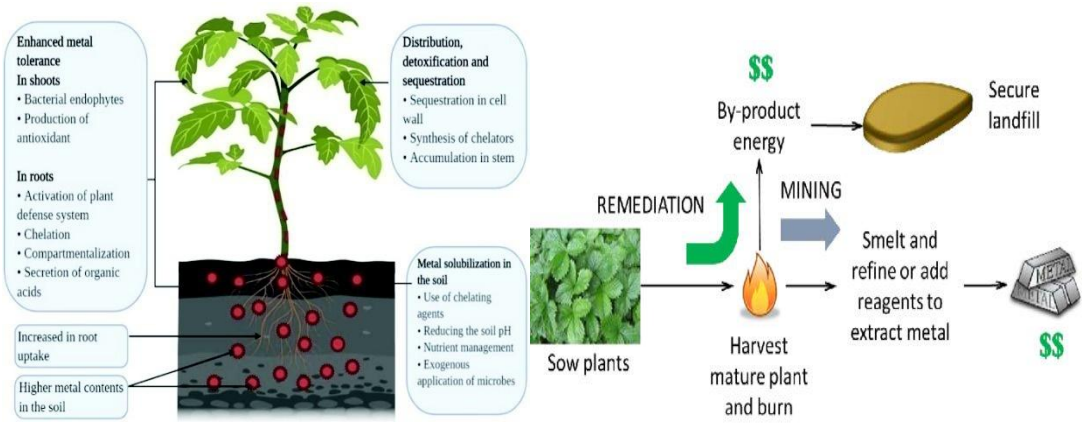


Figure 3 and 4: Phyto-mining and phyto-remediation processes

(Source: Yeboah *et al*., 2020)

Steps involved in Phytomining

In practical phytomining include the following steps:

1. Plant desired hyper-accumulator plant with high biomass and wide spread highly branched root system on an area of heavy-metal rich soil (Selection of plant);
2. After successful plant growth, irrigate with an appropriate chelating agent so that to increase the solubility and availability of the target metals to the plant roots (Induced hyper-accumulation).
3. Over the next days plants will absorb and translocate various toxic metals in the tissue (Hyper-accumulation).
4. Excessheavy metal build-up will kill the plants, so harvest the crop when they attain maximum growth (Harvesting).
5. The plants are harvested, and then burned to produce ash, which contains the metal compounds (ashing through pyrolysis).
6. Acid dissolution to produce a solution containing dissolved metal compounds (leachate).
7. Smelting and electrolysis (Wilson-Corral *et al*., 2012).

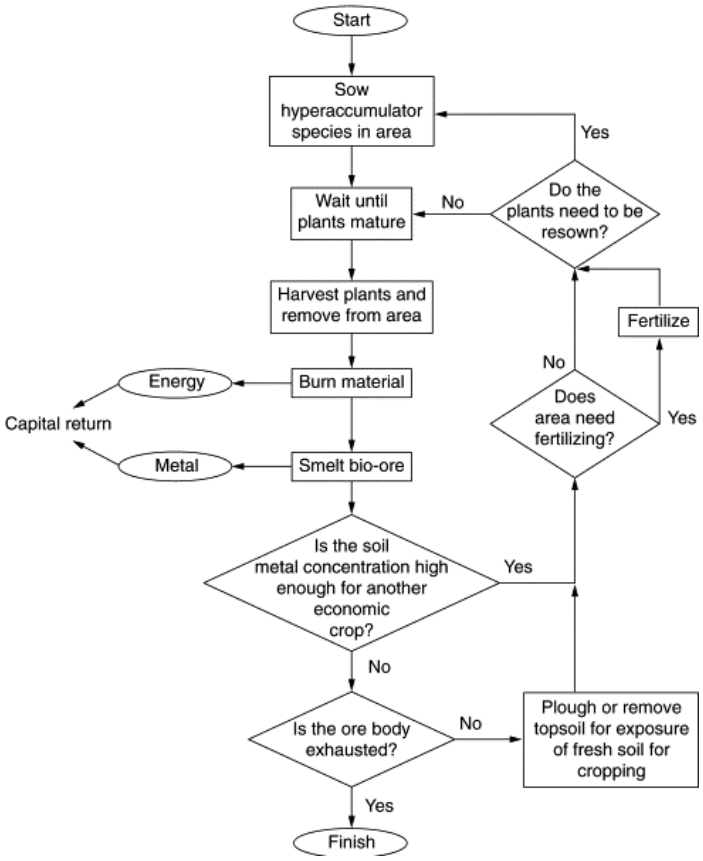


Figure 5: Phytomining- Steps involved (Source: Brooks *et al*., 1998)

1. Selection of plant:

Plant selection is a very important steps in phytomining (phytoextraction) process. Selected plant should have certain characteristics.

- They must have metal hyper-accumulating capacity preferably in the harvestable (above ground) parts,
- They must be tolerant to extreme weather conditions of the environment.
- They must be resistant to harsh environmental conditions,
- They must have fast growth and high biomass production,
- They must wide spread highly branched root system,
- They must be easily harvestable and non-consumable by humans or animals (Anderson *et al*., 1999, Arthur *et al*., 2005).

Plants are categorized into three main groups on the basis of their metal uptake. **Metal excluders** those are able to accumulate high quantities of heavy metals in their below ground parts (roots) and avoid transport of metals into the aerial tissues. **Metal indicators** will accumulate metals in theirareal parts (above ground) at levels similar to surrounding medium. **Metal hyper-accumulators** are species that can grow easily even in presence of relatively higher concentrations of heavy metals (toxic) in the growing medium by absorbing and

translocating appreciable quantities of metals in their tissuewithout much apparent symptoms of toxicity are well demanded in phytoremediation.

Concentration of heavy metals in the plant body serves as an indicator of concentration metals in the soil (Bhattacharjee, 2005) and metal absorption ability of the plant is proportional to amount of heavy metals, physico-chemical characteristics of soil, plant physiology etc. (Rascio *et al*., 2011). The atypical adaptive modulations of plants towards exasperating environmental stress can be hyper-tolerance. In traumatic environments the plants displays espouse modulations to maintain high metabolic activity – *tolerance* and permit plants to reduce autotrophic activity and become demand in the face of extreme stress - *avoidance*. Certain plants have thecapacity to grow in soils with lethal levels of heavy metals (Zn, Ni, Cu, Cd or Pb) and it is usually metal-specific and are only limited in number. These types of plants are referred as hyper tolerant plant species and they are hyper accumulators as well and these types of hyper accumulated, tolerant plants are known to be the spine of the phytoremediation (Verkleij and Schat, 1990).

2. Induced hyper-accumulation:

Natural capacity of plants to absorb contaminants including heavy metals present in the polluted soil *via* soil solutions is called natural or unassisted hyper accumulation. This natural hyper accumulating capacity of the plants can be improved by several techniques, so that the plants can absorb the heavy metals more easily.

- Amending the soil with chelating agents surfactants or helps to increase the contaminant mobility and bioavailability (Eg. EDTA, EGTA, SDS, ASA, EDDSetc.);
- Enhancing the uptake capacity of plants by improving their growth *via* nutrient amendments or management strategies (Eg. Fertilizers);
- Modification of microbial community in the plants rhizosphere (eg. Addition of bacterium *Ralstoniabasilensis*, microalga *Chlorellasorokiniana*, mycorrhizal fungi etc.);
- Genetic modification of plants (Eg. *B. juncea, Helianthus annuus, Liliodendrontulipifera and tobacco Nicotianaglaucum* (Eapen and D'Souza, 2005).
- Electro-kinetic enhancement: It is an effective technology to decontaminate heavy metal contaminated soil. This technology consists of theapplication of electricity at low level to the polluted soil where the plants grow and thereby enhancing the bioavailability of the toxic metals (Chirakkara*et al*., 2016; Li *et al.*, 2019).

Activating the hyper-accumulation capacity of the plants by adopting any of the above methods is known as induced hyper-accumulation or assisted hyper accumulation.

Table 1: Some specific hyper-accumulators with their metal concentration and definite biomass (kg/ha) (Sheoran *et al*., 2009)

Sr. No.	Elements	Plant species	Concentration mg/kg dry matter	Biomass kg/ ha
1	Cadmium	*Thlaspi caerulescens*	3000	4000
2	Cobalt	*Berkheya coddii*	10,200	4000
3	Copper	*HaumaniastrumKatangense* *Ipomea alpine*	8356	5000
4	Gold (induced-hyper-accumulation)	*Brassica Juncea, Berkheya coddii, Chicory, C. linearis*	10	20,000
5	Lead	*Thlaspi rotundifolium*	8200	4000
6	Manganese	*Macadamia neurophylla*	55,000	30,000
7	Nickel	*Alyssum bertolonii* *Berkheya coddii* *Streptanthus polygaloides*	13,400 17,000	9000 18,000
8	Thallium	*Iberis intermedia* *Biscutell alaevigata*	4055	8000
9	Uranium	*Atriplex confertifolia*	100	10,000
10	Zinc	*Thlaspi calaminare*	10,000	4000

NB: values in interpolations are mean concentrations usually found in non-accumulator plants.

3. Hyper-accumulation:

Over the next days selected plants starts to absorb metals from the rhizosphere across the root cell plasma membrane and a metallo-complex is formed with the aid of specific proteins, to neutralize the noxious effects of heavy metals.Then it willtranslocated from the roots to shoots *via*apoplast or symplast. Inside the tissues heavy metal sequestration and distribution takes place at cellular level (inside the root cell vacuoles). Finally these metal ions accumulated in metabolically fewer active cells (or unreactive cells) of the tissue (Milner and Kochian, 2008; Kotrba *et al*., 2009).

4. Harvesting:

Plants absorb heavy metals along with essential nutrient elements from the soil solution and have developed diverse strategies to handle with the accumulation of heavy metals. Continuous uptake of these heavy metals by the plants eventually results in death of the plant after reaching its maximum threshold level of accumulation. So subsequent satisfactory growth, plant biomass is reaped and left for drying (compaction). Compaction is an important phasein the post-harvest biomass treatments in phytomining. The harvested biomass is dried in the natural sunlight or by mechanical methods so that the volume of the contaminated biomass can be

reduced significantly. Compaction is very advantageous, as it will lessercharge of transportation and increases the easiness of handling. Compaction is found essential for processing metal rich phytoextraction residue (Mohanty, 2016).

5. Ashing:

Harvested, compacted biomass residue is then subjected to ashing through pyrolysis. Pyrolysis is the process by which burning of biomass at elevated temperatures in the absence of oxygen and thereby decomposition of organic materials takes place. The practice normally take place at a higher temperature (above 430 °C or 800 °F) and under pressure by changing the physical phase and chemical composition becausethis process is irreversible. Hence it can be considered as an important chemical reaction and precursor of gasification and combustion processes. The major goods of pyrolysis arebio-oil,bio-char, and gases such ascarbon monoxide, carbon dioxide,hydrogen and methane. Based on the final temperature pyrolysis will producechieflybiochar at a temperature below 450 ^{0}C, gases at an elevated temperature (800^{0}C) with a low and slowheating rate and bio-oil at an intermediate temperature with moderately high heating rates (Zaman *et al*., 2017)**.**

Mechanism of Pyrolysis:

There are mainly two mechanisms involved in pyrolysis - primary and secondary. In the primary mechanism, volatile compounds are released, though the chemical bonds within the polymers are broken during thermal heating of biomass. Besides, rearrangement reactions within the matrix of the residue take place. Char formation, depolymerisation, and fragmentation are occurring in primary mechanism. During char formation, primarily, benzene rings are formed, are combined with a solid residue and char is formed (aromatic polycyclic structure) with release of water or incondensable gas. The polymers are broken into monomer units in the de-polymerization process till the volatile molecules are produced, which lessen the rate of polymerisation. Finally, in fragmentation, incondensable gas and small chain organic compounds are formed through the linkage of many covalent bonds of the polymer, even within the monomer units.

In the secondary mechanismsome of the unstable volatile compounds undergo additional reactions includingcracking, recombination etc. In cracking, volatile compounds broken down into lower molecular weight molecules, while during recombination process, volatile compounds combine together to high molecular weight compounds, which in some cases may not be volatile. Secondary mechanism may also leads to the formation of secondary char (Uddin *et al*., 2018).

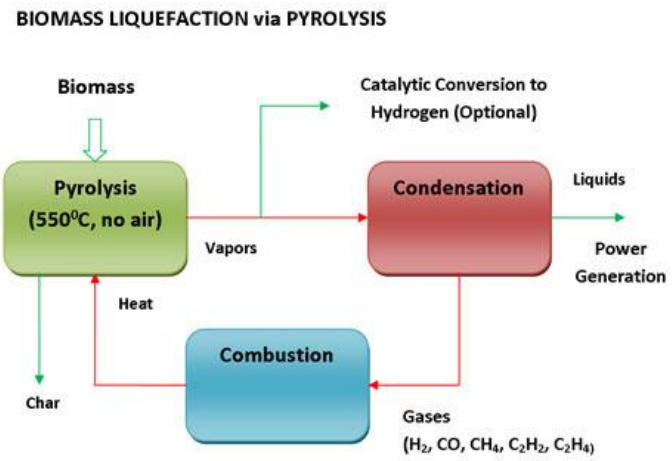


Figure 6: Process conditions for pyrolysis of biomass (Source: https://www.altenergymag.com/article/2009/02/biomass-pyrolysis/502/)

Types of Pyrolysis Reactions:

Depending on the operational conditions (processing time and temperature) pyrolysis are of three different types: slow pyrolysis, fast pyrolysis, and ultra-fast or flash pyrolysis.

Slow pyrolysis is the slow heating of biomass in the lack of oxygen. As a result the volatiles from the organic material evaporate partly, and charcoal remains contains 80% carbon and hence this process also called carbonization. Here, the temperatures varies from 0.1 to 2 °C (32.18 to 35.6 °F) /second and the prevalent temperatures are approximately 500°C (932°F). The gas thus produced may remain above five seconds (residence time) and the biomass may be from minutes to days.Biochar, a carbon-rich solid produced at low pyrolysis temperatures.

Fast pyrolysis is a veryquick decomposition of carbonaceous materials in the lack of oxygen in medium to high heating rates 400-650° C. The major product are bio-oil and gas. Bio-oil can be used as a standby for fossil fuels as it can generate heat, power and chemicals. The yields of the products are: liquid condensates – 30-60%; gases (CO, H_2, CH_4, CO_2, and light hydrocarbons) – 15-35%; and char – 10-15%.

Ultra-fast, or flash pyrolysis is atremendouslyfast thermal decomposition, with a temperature between100-10,000°C.The chief products are bio-oilandgases. The yields of the products are: liquid condensate ~10-20%; gases – 60-80%; and char – 10-15% (Czajczyńska et al., 2017;Chen et al., 2015; Demirbas, 2009).

6. Acid dissolution of biochar (leachate):

Ash (biochar) obtained as a result of pyrolysis is dissolved in appropriate acids to make leachate containing various heavy metals. The highest extraction of valuable metals in general was achieved with HCl and H_2SO_4. Organic acids did not leach metals as effectively as mineral acids. When bio-char is dissolved in 3 M H_2SO_4 and 7 M HCl, > 80% Zn, > 87% of Cu and

> 65% of Ni can be extracted. By using 3 and 5 M HCl as solvent >70% of Pb can be extracted (Elomaa*et al*., 2019).

7. Smelting and electrolysis:

The thermo-chemical treatment of bio-ore to yield specific metal requires heat input and a suitable chemical reductant. Metal is extracted from bio-ore mainly by two major methods: smelting or electrolysis.Smelting uses heat to separate the valuable metal from the ore. Smelting usually requires a reduction agent, or another chemical, to separate metal from its ore.Electrolysis separates metal from ore by using acid and electricity (Simonnot*et al*., 2018).

Ores are linked with countless impurities. Removal of these impurities to a maximum level can beattainedthrough concentration.After concentration ore is subjected to chemical treatment. Generally the metal compounds (e.g., oxides, sulphides) are reduced to the metal. Carbon, CO or even some metals are used as reducing agents. Reaction with metal oxides andthe reducing agent resulting in the decrease of metal oxide to metal at a raised temperature. Even though these metals still contain slight impurities. Refining is the ultra-purification of metals for getting pure metals.Depending upon the variances in properties of the metal and the impurityseveral techniques are used and are distillation, liquation, electrolysis, zone refining, vapour phase refining, chromatographic methods etc.Metals, in general, are very widely used and have contributed significantly in the development of a variety of industries.

Conclusion:

Plethora of evidence directs that some plants have inherent ability to store toxic (heavy) metals in the cells without any toxic symptoms. Phytoremediation and phytomining are making use of these genetic characteristics of plants for natural clean-up of contaminated land/water along with possible recovery and reuse of metals which are concentrated in plant cells. In spite of substantial research efforts, phytoremediation and phytomining are still emerging technologies. Phytoremediation as a low cost, green technology for environmental clean-up, however, completes only after proper post- harvest management- phytomining. Even if phytoremediation andphytomining offers a lot of advantages, economics of phytominingis a question for a developing country like India. Metal recovery from biomass requires a series of steps which are expensive, instrumental and need professionals to carry out. Themost important factor, however, is the revenue generation, world price of metalbeing phytomined (Brooks *et al*., 1999).The imminent of post-harvest management of phytoremediation products though phytomining practices are still in research and developmental phase. Finding out a low cost technology for metal recovery at house hold/industrial level is of great importance.

Acknowledgments:

Authors acknowledge the financial support granted for this work by the Kerala State Council for Science Technology and Environment (KSCSTE), Thiruvananthapuram, Kerala.

References:

Ali, H., Khan, E. and M. A. Sajad. 2013. Phytoremediation of heavy metals—concepts and applications. Chemosphere. 91(7):869-81.

Anderson, C.W., Brooks, R.R., Chiarucci, A., LaCoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R. and R. B. Stewart. 1999. Phytomining for nickel, thallium and gold. Journal of Geochemical Exploration. 1: 67(1-3):407-15.

Antoniadis, V., Levizou, E., Shaheen, S.M., Ok, Y.S., Sebastian, A., Baum, C., Prasad, M.N., Wenzel, W.W. and J. Rinklebe. 2017. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. Earth-Science Reviews. 1:171:621-45.

Ansari, A. A., Gill, S. S., Gill, R., Lanza, G. and L. Newman. 2016. Phytoremediation. Springer International Publishing.

Arthur, E.L., Rice, P.J., Rice, P.J., Anderson, T.A. Baladi, S. M., Henderson, K. L. and J. R. Coats. 2005. Phytoremediation—an overview. Critical Reviews in Plant Sciences. 1:24(2):109-22. Doi. 10.1080/07352680590952496.

Bhattacharjee S. 2005. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transducation in plants. Current Science. 10:1113-21.

Brooks, R.R., Chambers, M.F., Nicks, L.J. and B. H. Robinson. 1998. Phytomining. Trends in plant science. 3(9):359-62.https://doi.org/10.1016/S1360-1385(98)01283-7.

Chen, D., Yin, L., Wang, H. and P. He. 2015. Pyrolysis technologies for municipal solid waste: a review. Waste management. 1(37):116-36.

Chirakkara, R.A., Cameselle, C. and K. R. Reddy.2016. Assessing the applicability of phytoremediation of soils with mixed organic and heavy metal contaminants. Reviews in Environmental Science and Bio/Technology. 15(2):299-326.

Wilson-Corral, V., Anderson, C.W. and M. Rodriguez-Lopez. 2012. Gold phytomining. A review of the relevance of this technology to mineral extraction in the 21st century. Journal of Environmental Management. 111:249-57.

Czajczyńska, D., Krzyżyńska, R., Jouhara, H. and N. Spencer. 2017. Use of pyrolytic gas from waste tire as a fuel: A review. Energy. 134:1121-31.

Demirbas, M.F., Balat, M. and H. Balat. 2009. Potential contribution of biomass to the sustainable energy development. Energy Conversion and Management. 50(7):1746-60.

Eapen, S. and S. F. D'souza. 2005. Prospects of genetic engineering of plants for phytoremediation of toxic metals. Biotechnology advances. 23(2):97-114.

Elomaa, H., Seisko, S., Lehtola, J. and M. A. Lundström. Study on selective leaching of heavy metals Vs. Iron from fly ash. Journal of Material Cycles and Waste Management. 21(4):1004-13.

Kotrba, P., Najmanova, J., Macek, T., Ruml, T.and M. Mackova. 2009. Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. Biotechnology advances. 27(6):799-810.

Kumar, J., Malik, N.A. and N. S. Atri. 2021. Aromatic and Medicinal Plants for Phytoremediation: A Sustainable Approach. In: Aftab T., Hakeem K.R. (eds) Medicinal and Aromatic Plants. Springer, Cham. https://doi.org/10.1007/978-3-030-58975-2_20

Lasat, M.M. 2000. Phytoextraction of metals from contaminated sites – a critical review of plant/soil/metal interaction and assessment of pertinent agronomic issues. The Journal of Hazardous Substance Research. 2: 1-25.

Li, J., Zhang, J., Larson, S.L., Ballard, J.H., Guo, K., Arslan, Z., Ma, Y., Waggoner, C.A., White, J.R. and F. X. Han. 2019. Electrokinetic-enhanced phytoremediation of uranium-contaminated soil using sunflower and Indian mustard. International journal of phytoremediation. 21(12):1197-204. DOI: 10.1080/15226514.2019.1612847

Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R. and Z. Zhang. 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicology and environmental safety. 126:111-21.

Milner, M.J. and L. V. Kochian. 2008. Investigating heavy-metal hyperaccumulation using Thlaspicaerulescens as a model system. Annals of botany. 102(1):3-13. doi: 10.1093/aob/mcn063.

Mohanty, M. 2016. Post-harvest management of phytoremediation technology. Journal of Environmental Analysis and Toxicology. 6(5):398.doi: 10.4172/2161-0525.1000398

Nedelkoska, T.V. and P. M. Doran. 2000. Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Minerals engineering. 13(5):549-61. DOI: 10.1016/S0892-6875(00)00035-2

Negri, M.C., Hinchman, R.R.and G. F. Gatliff. 1996. Phytoremediation: using green plants to clean up contaminate soil, groundwater, and wastewater. Argonne National Lab., IL (United States); In Proc. International Topical Meeting on Nuclear and Hazardous Waste Management, Spectrum, 96: 1-13.

Van Nevel, L., Mertens, J., Oorts, K. and K. Verheyen. 2007. Phytoextraction of metals from soils: how far from practice? Environmental Pollution. 150(1):34-40.

Patra, H. K. and M. Mohanty. 2013. Phytomining: an innovative post phytoremediation management technology. Development. 25:27.

Pilon-Smits, E. A. and J. L. Freeman. 2006. Environmental cleanup using plants: biotechnological advances and ecological considerations. Frontiers in Ecology and the Environment. 4(4):203-10.

Pivetz, B. E. 2001. Phytoremediation of contaminated soil and ground water at hazardous waste sites. US Environmental Protection Agency, Office of Research and Development, Office of Solid Waste and Emergency Response.

Rascio, N. and F. Navari-Izzo.2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?.Plant science. 180(2):169-81.

Raven, P. H., Evert, R.F. and S. E. Eichhorn. 2005. Biology of plants. Macmillan, 6th ed. New York: W.H. Freeman and Company, 875p.

Robinson, B.H., Bañuelos, G., Conesa, H.M., Evangelou, M.W. and R. Schulin. 2009.Thephytomanagement of trace elements in soil. Critical Reviews in Plant Sciences. 28(4):240-66.

Timofeeva, S.S., Ulrikh, D.V. and S. S. Timofeev. 2017. Phytomining perspectives in rehabilitation of mining and industrial areas of South Ural. In: IOP Conference Series: Earth and Environmental Science. 66(1): 012030. doi:10.1088/1755-1315/66/1/012030.

Sharma, P.C. and C. P. Dietz. 2006. Chemistry of environment and pollution. APH publishing corporation, New Delhi, 261p.

Sheoran, V., Sheoran, A.S. and P. Poonia. 2009. Phytomining: A review: Minerals Engineering. 22:1007–1019. doi.org/10.1016/j.mineng.2009.04.001.

Simonnot, M.O., Vaughan, J. and B. Laubie. 2018. Processing of bio-ore to products. In: Agromining: farming for metals, 39-51. Springer, Cham.

Suman, J., Uhlik, O., Viktorova, J. and T. Macek. 2018. Phytoextraction of heavy metals: a promising tool for clean-up of polluted environment?.Frontiers in Plant Science. 9:1476.

Uddin, M.N., Techato, K., Taweekun, J., Rahman, M.M., Rasul, M.G., Mahlia, T.M. and S. M. Ashrafur. 2018. An overview of recent developments in biomass pyrolysis technologies. Energies. 11(11):3115.

Verkleij , J. A. and H. Schat. 1990. Mechanisms of metal tolerance in higher plants. Heavy metal tolerance in plants: evolutionary aspects. In: Shaw, A.J. (ed) Heavy metal tolerance in plants: Evolutionary aspects: 179–194, CRC Press.

https://www.altenergymag.com/article/2009/02/biomass-pyrolysis/502/

A REVIEW ON PROSPECTUS OF MUSHROOM CULTIVATION AS AGRO BASED INDUSTRY

V. A. Gagrepatil

Department of Botany,

K. K. W. Arts, Science and Commerce College, Pimpalgaon Baswant

Corresponding author E-mail: vrushaligagarepatil@gmail.com

Introduction:

Mushrooms are long been valued as high medicinal and dietary food by means of many societies around the arena. Mushrooms are eaten up as a remedy in Asian nations and plenty of researcher works have been accomplished on medicinal factors (Halpern and Miller, 2002). Mushrooms are utilized in Ayurveda and people medicine in India (Adhikari, 1981; Jitendra and Vaidya, 2000). India is an agricultural country and generating a large quantity of agro-wastes each year about 620 million lots (Sidhu, 2014). The existing agricultural state of affairs of India has to emerge as an economic strength within the world in terms of agricultural productiveness with the aid of adapting new technology main to the economic energy of the world. In India, any mushroom is used as a non-traditional coins crop and commonly cultivated species are white button mushroom, oyster, shiitake mushrooms and other mushrooms cultivated in small scale are paddy straw, milky and relish mushrooms. Button mushroom accounts for approximately 95 % of overall production and exports. Button mushroom is cultivated in temperate regions of Himachal Pradesh, Jammu and Kashmir, however the oyster, milky, paddy straw mushrooms are cultivated within the tropical and sub tropical regions. Mushroom cultivation has emerged as a business and export-orientated. The fundamental export locations for Indian mushrooms are Canada, US, Israel, and Mexico. The demand for processed mushrooms has improved drastically during the last few years. They may be canned, dried, packed in frozen paperwork, which include its utilization in food enterprise in mushroom pickle & sauces. India's FDI (foreign direct funding) policy targets to attract funding in technology to development and manufacturing of vegetables and mushrooms beneath managed situations (Annual file 2017-2018). Efforts are being made by using the Indian government to enhance mushroom research and development and to inspire mushroom growers to increase superior R&D technologies and guidelines. The R&D and biotechnology laboratories play a vital position inside the mushroom breeding system and within the optimization of the cultivation environment for the better yield. Such

technological advancements and favorable authorities' tasks promise ample boom opportunities for the industry individuals and the mushroom researchers.

Mushroom cultivation plays an important role to improve the strengths and livelihood of rural humans through monetary, nutritional and medicinal contributions. In India the first mushroom cultivation turned into publicized via Thomas inside the year 1943 in Agricultural University, Coimbatore, who cultivated paddy straw mushroom, which led to unfold the cultivation techniques in the course of the India (Prakasam, 2012). In 1961, Indian Council of Agricultural studies (ICAR), New Delhi, began first cultivation of the button mushroom (Agaricus bisporus) in Solan. The national Centre for Mushroom research and training (NCMRT) changed into hooked up in 1982 and upgraded to Directorate of Mushroom research in 2008. The success of early button mushroom cultivators to encourage other enterprising farmers to develop button mushrooms beneath natural climatic situations. Mushroom industry in India is mainly targeted on button mushroom cultivation and advertising and marketing. The recent production statistics found out that button mushroom manufacturing holds the maximum percentage of approximately 73% and observed by way of oyster mushroom with 16% (Sharma *et al.,* 2017). There are two varieties of mushroom growers in India, the seasonal growers and spherical the year growers. Each grower have centered on white button production (Agaricus bisporus) and harvest 2- 3 crops in a 12 months. Well qualified expert mycologist/ mushroom professional, knowledgeable and unemployed population area are contribution in the direction of the mushroom cultivation with empowered entrepreneurial abilities. Mushroom farming has end up a very essential cottage business pastime inside the incorporated rural improvement programme.

Bioresource availability for mushroom production in India:

India has an extensive range of various agro-climatic conditions with a cultivated vicinity of approximately four.37 % and 54.6 % of the population in which engaged in agriculture and allied sports (India census 2011). The land and water are two critical herbal resources, which influences manufacturing of sustainable food assets, in Indian overall geographical vicinity of 328.7 million hectares (Council of Agricultural research -ICAR- 2017- 2018). India has lead 2nd rank worldwide in farm output in agriculture and allied sectors displaying 13.7% of the GDP (Gross domestic manufacturing) in 2013. Agricultural residues are taken into consideration as agro wastes, in India as an approximation, the amount of crop residues produced exceeds 620 million lots yearly (Singh and Sidhu, 2014). Agro-waste in includes crop waste, animal waste (manure) and meals processing wastes. A total of 50% of agricultural residues are produced with the aid of rice, wheat and oilseed crops (Singh and Prabha, 2017). In the past, the whole paddy

and wheat straw was burned through the Indian farmers but these days it's miles being converted as a bio-renewable source. Different foremost agro wastes are maize, cotton, millets, pulse, sunflower and other stalks, bulrushes, groundnut shells, coconut trash, vegetable residue, coir dust, husk, dried leaves, pruning, espresso husk, tea waste. Overall of 39 residues from 26 crops, the ones agro wastes are the treasured substrate for mushroom production and organic manure forming. To provoke the farmers toward bio natural farming at a industrial scale which could worthwhile to the agricultural society (Singh and Prabha, 2017). The rural waste towards mushroom manufacturing, India can produce three million tonnes of mushroom and about 15 million tonnes of bio-compost. The extraordinary agro-weather and abundance of farm wastes, extraordinary types of temperate, tropical and subtropical climates favors for mushrooms cultivation in the course of the country.

Mushroom consumption trend worldwide and in India

Mushrooms are a good source of soluble protein and fiber which performs an important role in human fitness. Mushroom protein being easily digestible (70-90%) is taken into consideration advanced to vegetable proteins. Low protein intake by way of Indians leads to fatigue, slow metabolite, low synthesis of brain hormones and immunity. To finding the opportunity way to produce cheat and best food to overcome the malnutrition, microbes and the fit to be eaten mushrooms are maximum important. In India, attention about intake and fitness benefits of mushrooms available for cultivation is constrained, their call for is likewise much less. The cultivation of Pleurotus mushroom stands first in observed via Agaricus bisporus mushroom in India in terms of recognition and consumption (Khatun *et al.,* 2012). The normally grown safe to eat mushrooms in India are Pleurotus sajor-caju, Pleurotus ostreatus, Pleurotus florida, Pleurotus djamor, Pleurotus eryngii, Auricularia polytricha, Hypsizygus tessulatus, Lentinula edodes, Calocybe indica, Volvariella volvacea (Dhar *et al.,* 2011). Medicinal mushrooms cultivated in India are Ganoderma lucidum, Phellinus rimosus, Pleurotus spp. possessed profound medicinal residences (Ajith and Janardhanan, 2007; Jose *et al.,* 2002). Even though the intake of mushrooms has elevated, however there is increasing preference for fresh mushrooms. The intake of mushroom in India is currently approximately 30-40 g in comparison to 2-3 kg in USA and Europe. In India in step with capita intake is set 90 g, which very much less in comparison to other nations along with America 1.49 kg and China 1.16 kg (Directorate of Mushroom studies ICAR, Solan (India) 2011). To enhance nearby intake there may be a want to popularizing the beneficial effects of cultivated and wild amassed fit for human consumption mushrooms.

The worldwide mushroom production as in step with FAO statistics was predicted at approximately 2.18 to three.41 million tons over duration of 1997-2007 (Wakchaure, 2011). Mushroom marketplace fee is anticipated to exceed USD 50 billion in the next seven years due to growing mushroom call for in from the current beyond. The recent update indicates that the marketplace had a cost of $35 billion in 2015. Between 2016 and 2021, the marketplace is anticipated to develop by 9.2 percent. This will convey its length to almost $60 billion in 2021. China, USA, Netherlands, Poland, Spain, France, Italy, Irak, Canada and UK are the leading mushroom manufacturers. Key mushroom products include shiitake, button, oyster, and others which include paddy mushroom, milky mushroom and wintry weather mushrooms. Europe is the most important market place for cultivated mushrooms, accounting for more than 35 % of the global market. India exporting 105.4 tons of white button mushrooms in canned and frozen shape and button mushroom contribute total 15% of the percentage in the international production (Singh *et al.,* 2017). In line with the German alternate affiliation, the manufacturing of mushrooms general volume will exceed 70,000 tons, which is a growth of 3,000 tons in comparison to 2015. Apart from these classes processed mushroom additionally consists of pickled mushroom, powder mushroom, and mushroom sauces. There's increasing the commercial mushroom consumption in eating places, cafeterias, marriage receptions and birthday events is predicted to play an essential role in mushroom production and advertising in the near destiny. For the fitness subject purchasers are moving in the direction of vegetarian food and increasing demand for mushroom manufacturing. The global market has been segmented on the premise of main regions and its processing category.

Industrial contribution towards the mushroom production in India:

Mushroom industry globally has expanded each horizontally and vertically, which means that the progress has been in manufacturing and adapting all forms of human wellness mushrooms. Fresh mushrooms are perishable, so their export/import often has been restrained to transactions especially between neighboring countries. There had been increasing technologies in the direction of the boom of shelf existence are a number of the factors propelling the global market. The modern biotechnology laboratories and authorities research institutes play a vital position in the mushroom breeding procedure as they optimize the yield, nutritional and medicinal properties. Such technological improvements and favorable government guidelines promise enough increase opportunities for the industry participants. Rising economies consisting of China and India are anticipated to witness fast mushroom call for growth because of growing populace and growing fitness attention. In India mushroom production becomes 1.8% of the worldwide production, nonetheless India isn't always ranked a few of the leading mushroom

generating countries. The small scale mushroom farmers mainly producing the button mushroom and providing to home markets, this may effect on nearby marketplace development and cultivate the brand new verities. Indian government helps the entrepreneurs to set the high technology mushroom farms as industrial ventures. The Himalaya international Ltd is a pioneer mushroom industry in India yearly generating 10,000 metric tons of canned mushrooms. Zuari meals And Farms P Ltd (Dr. Kurade's mushrooms) was installed in 1994 with a manufacturing potential of 7000 kg/day. Kulkarni Farm fresh private confined is an established commercial enterprise entity engaged in cultivating, offering and exporting of Button Mushrooms, the everyday manufacturing ability 2 metric tons. The mushroom enterprise in India has gone through a prime transformation because of diversify mushrooms version and domestication. In India, hard work extensive industries have benefits in mushroom production and advertising. The FAO has been actively selling mushroom-forming for rural development and food security in developing countries (Marshall and Nair, 2009).

Research & development makes a specialty of conserve mushroom diversity, new sorts to enhance mushroom excellent, recycling spend compost for making manure and promote secondary agriculture for producing employment. The present day studies and improvement especially focusing on spawn production, caesing substances formula. Basically, the research has led to progressed production technology of edible and medicinal mushroom, cultivation technology of temperature tolerant, and incorporated pest management practices for manage of economically vital illnesses and insect pest and nematodes has been labored out and recommended to the mushroom growers (Karthick and Hamsalakshmi, 2017). The mushroom studies and development play an essential position in the flow of marketplace-based totally improvements via a complex system that affects the blended capabilities of researcher, scientists, marketers and industrialists. Sustainable success of mushroom-primarily based industries relies upon on each studies and development. Mushroom technological know-how and mushroom biotechnology are two legs of the mushroom industry (Chang, 1996). The huge effect on revenue and employment outcomes from innovation influences no longer best in excessive tech organizations but also new mushroom industries that benefit from extended skills and productiveness. The era development and current laboratories are enhancing the acceptability and technology development to increase mushroom shelf-existence.

Future scenario and opportunity of mushroom production in India:

Mushrooms can make a valuable dietary and might play a critical role in contributing to the livelihoods of rural and semi-urban dwellers, via meals protection and profits technology.

The modern situation of mushroom manufacturing in India is pretty encouraging with an ordinary boom in five to 6 folds and became predicted to pass 50,000 heaps (Verma, 2002). India has wealthy genetic sources of safe to eat mushrooms it's want for conservation and utilization for sustained manufacturing. In addition, India has numerous climatic conditions in special areas and possible to cultivate many varieties of mushrooms (Sharma, *et al.,* 2017). A success mushroom cultivation for trade requires to operating in joint natures or partnership with local agro-industries, universities or wholesalers can assist lessen vulnerability (Thakur, 2014). The improvement of R&D, infrastructure facilities and distribution community presents the extra scope for advertising of sparkling mushrooms. The advertising of sparkling mushrooms would determine the destiny of the mushroom industry in India. The awareness and know-how approximately dietary and medicinal values of mushrooms will growth the production and consumption of mushroom in India. From a dietary perspective, mushrooms are a selected food in vegetarian-essential in India. With a domestic population of more than one billion, India itself is a large marketplace for mushroom. The excellent mushroom spawn, cutting-edge cold storage facility and well-geared up processing gadgets are facilitated the mushroom manufacturing. The technology may be profitably considered in rural and concrete areas in which land is a limiting factor and chiefly to be had of agro-wastes. However, mushroom cultivation additionally gives opportunities for improving the sustainability of small farming systems via the recycling of natural matter and then again to the land as fertilizer. Currently, unemployment is increasing unexpectedly each in evolved and growing international locations. In this example, self-employment may be one vital manner to increase employment. Mushroom processing and storage can be another alternative of a business enterprise as it is employment and skill oriented. Mushroom cultivation and consumption trend in India, the government of India took numerous steps for its sustainable development. Additionally, encourages marketers and business houses to installation high generation farms as business ventures and the plan called a 100% export orientated devices. The coverage tasks brought about a good response from marketers/ investors and several corporations set up incorporated gadgets at exclusive locations by means of imported technology. However, India lags behind many European and Asian international locations in the generation of newer manufacturing technologies, their refinement, popularization and adoption with the aid of farmers.

Conclusion:

From above all we conclude that India's capacity as a chief mushroom producer is its strategic geographical region, making it more convenient to export mushrooms. In India button,

oyster, milky and paddy straw mushrooms are generally grown however button mushroom contributes the best percentage of manufacturing. The R&D, government schemes, policymakers and entrepreneur are contributing closer to the initiation and growth of the mushroom industry. Within the current scenario the best crop and excessive yield production these days are of propelling the mushroom farming as they lead to excessive-earnings margins that can be useful to each farmers and the economic system of the USA. There is a high quality dating among mushroom production and farm-size and the earnings of mushroom growers goes up with the growth in farm length. Also, the clients have recently proven a further liking for mushroom ingesting, which has greater the mushroom demand and deliver in India. Government and cultivators keeping in view the growing call for of mushroom because of globalization and compete to the global marketplace.

References:

Adhikari MK. (1981): Chyau: Ayurvediyavishleshanekvivechana (Mushrooms: An Ayurvedic concepts) J Nep Pharm Asso. 9: 17-21.

Ajith TA, Janardhanan KK. (2007): Indian Medicinal Mushrooms as a Source of Antioxidant and Antitumor Agents. J Clin Biochem Nutr. 40: 157-162.

Annual report 2016 – 2017: All India Coordinated Research Project on Mushroom, ICAR-Directorate of Mushroom Research, Kamal, S. Attri, BL. (ed.), Chambaghat, Solan - 173 213 (HP).

Annual report 2017- 2018: Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare. Government of India Krishi Bhawan, New Delhi-110 001.

Census of India. (2011): Report on post enumeration survey. Office of the Registrar General & Census Commissioner, India. Ministry of Home Affairs, Government of India. http://censusindia.gov.in/

Chang, ST. (1999): World production of cultivated and medicinal mushrooms in 1997 with emphasis on Lentinus edodes (Berk.) Sing, China. Int J Med Mush. 1: 291-300.

Directorate of Mushroom Research (ICAR), Solan (India). (2011): Mushrooms cultivation, marketing and consumption. Directorate of Mushroom Research (Indian Council of Agricultural Research). Food and Agriculture Organization of the United Nations (FAO).

Halpern GM, Miller AH. (2002): Medicinal mushrooms. Ancient remedies for modern ailments. New York: M. Evans and Company. p 172.

Jitendra, G, Vaidya. (2000): Traditional Medicinal Mushrooms and Fungi of India. Int J Med Mushrooms. 2: 209-214.

Karthick K, Hamsalakshmi. (2017): Current scenario of mushroom industry in India. Int J Commerce Manag Res. 3: 23-26.

Khatun S, Islam A, Cakilcioglu U, Chatterjee NC. (2012): Research on mushroom as a potential source of nutraceuticals: a review on Indian perspective. American J Exp Agric. 2: 47-73.

Manjit S, Kamal S. Sharma V.P. (2017): Status and trends in world mushroom production-I. Mushroom Research, 26: 1-20

Marshall E, Nair N. (2009): Make Money by Growing Mushrooms; Food and Agriculture Organization of the United Nations (FAO): Roma, Italy.

Pandey M. (2005): Mushroom Research and Development of Current prospects of mushroom production and industrial growth in India 249 India. http://www. mushworld.com/oversea/view.asp?_id= 5140&vid=7165.

Prakasam V. (2012): Current scenario of mushroom research in India. Indian Phytopath. 65: 1-11.

Sharma SR, Kumar S, Sharma VP. (2006): Physiological requirement for cultivation of Malaysian strain of shiitake, Lentinula edodes. J Mycol Pl Pathol. 36:149-152.

Sharma VP, Sudheer AK. Yogesh G, Manjit S, Shwet K. (2017): Status of mushroom production in India. Mushroom Research. 26: 111-120.

Sidhu HS. (2014): Management of cereal crop residues for sustainable rice-wheat production system in the Indo-gangetic plains of India. Proc Indian Natn Sci Acad. 80: 95-114.

Singh B, Shan YH, Johnson-beeebout SE, Singh Y, Buresh RJ. (2008): Cropresidue management for lowland rice-based cropping systems in Asia. Adv Agron. 98: 118-199.

Thakur MP. (2014): Present status and future prospects of tropical mushroom cultivation in India: A review. Indian Phytopath. 67: 113-125

Verma RN. (2002): Recent Advances in the cultivation Technology of Edible Mushrooms, NRCM Solan, 1-10.

Wakchaure GC. (2011): Production and Marketing of Mushrooms: Global and national scenario. Mushrooms cultivation, marketing and consumption global and National Scenario pp.11-2.

ARTS AND SCIENCE OF INTEGRATED PLANT DISEASE MANAGEMENT: A GENERAL OVERVIEW

S. G. Yadav

Department of Botany,

Shivaji Mahavidyalaya, Renaur, Dist. Latur 413 527 MS, INDIA

Corresponding author E-mail: shankar.yadav4@gmail.com

Preface:

Plant diseases are causing severe losses to humans and if we look into history we will come to know about the starvation and uprooting of families resulted from the Irish famine caused by Potato late blight disease. There are hundreds of Plant diseases which are causing economic losses throughout the world and some diseases are reducing the aesthetic values of landscape plants and home gardens. The purpose of editing the chapter "Arts and Science of Integrated Plant Disease Management: A General overview" is to disseminate the knowledge of plant disease management to farmers, students and even to researchers. The plant disease management is sole method which is trying to reduce the economic and aesthetic damage caused by plant diseases. Traditionally, this has been called Control of Plant diseases but because of current environmental values and multi-faced approaches I use the title of this chapter as "Arts and Science of Integrated Plant Disease Management". This chapter is a general overview of the methods used in integrated management of plant diseases.

Introduction:

Integrated Plant Disease Management (IPDM) came under focus in 1960's when chemicals especially, fungicides and insecticides came under the attack from environmentalists due to the overuse of chemicals that created the problems of environmental pollution, chemical residues in food stuff, land, water and air, and the associated health hazards. It focused on the other methods of disease control. It involved cultural, biological, epidemiological and alternative means to achieve the disease control. Nowadays, there is an emphasis on disease management‖ rather than on Control‖. Disease management system that in the context of associated environment and population dynamics of microorganisms utilizes all suitable techniques and methods in a manner as compatible as possible and maintains the disease below economic level. In general, it is the integration of all possible and suitable management techniques for the control of diseases. The practices which need to be avoided in IDM are indiscriminate use of fungicides,

monoculture and growing of susceptible cultivars. Integrated disease management ensures the proper management of soil health, use of healthy seeds and planting material, application of fungicides when required, field sanitation, cultural practices which suppress the disease , use of bio-control agents and growing resistant plant genotypes .

Different Approaches of Integrated Disease Management System:

1. The combined control approach: It is a combination of control methods like adjustment in sowing time, seed treatment, use of resistant variety, chemical spray schedule etc. This type of IDM is widely practiced as a package of practice where the occurrence of disease is certain and sure.

2. The surveillance based approach: It is an advanced IDM approach based on crop health monitoring and surveillance, and takes into account the economic threshold levels or economic damage levels.

3. Advanced integrated disease management system: It involves the high input technology like computer supported forecasting, remote sensing, scouting, multiple pathogen thresholds, information on life cycle of pathogens, epidemiology of diseases, environmental factor and knowledge based decision making.

MAIN COMPONENTS: Main components of integrated disease management (IDM)

1. Host resistance
2. Induced systemic resistance
3. Genetically improved plants
4. Cultural practices
5. Physical methods
6. Plant nutrition
7. Biological control
8. Use of pesticides of plant origin
9. Judicious use of chemicals

Host resistance:

Resistant varieties can be the simple, practical, effective and economical method of plant disease control. Apart from ensuring protection from diseases, they can also save time, money and energy spent on other methods of control and avoids environmental pollution with chemicals. They are the only practical method of controlling such diseases as wilts, rusts and others caused by viruses in which chemical control is very expensive and impractical. In low value crops, where other methods are often too expensive, development of varieties resistant to common and important diseases can be an acceptable recommendation for the farmers. Disease resistance in plants is also governed by their genetic constitution and can be monogenic, oligogenic or polygenic.

Advantages of host plant resistance:

No adverse effect on environment and man, rather the resistant cultivars put a constant and cumulative effect on pathogen. Host plant involves no extra cost to the farmers and does not require inputs and application skills.

Disadvantages of host plant resistance:

The development of pathogen resistant variety takes 5-10 years. Host plant resistance can put a selection pressure on pathogen to the extent that it may lead to the evolution of new biotypes of pathogen. Introduction of varieties with resistance to one pathogen leads to the emergence of new pathogen problem because of the absence of competition from the key pathogen.

Induction of host resistance:

Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Plants also respond to a variety of chemical stimuli produced by soil- and plantassociated microbes. Such stimuli can either induce or condition plant host defence through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. Induction of host defence can be local and/or systemic in nature depending on the type, source, and amount of stimuli. The systemic acquired resistance (SAR) is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes, some of which may act directly to lyse the invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death. Whereas, the induced systemic resistance (ISR) is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some non-pathogenic rhizobacteria.Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR.Pathogenic strains of Pseudomonas syringae produce coronatine, which is similar to JA, to overcome the SA-mediated pathway. Because various host-resistance pathways can be activated to varying degrees by different microbes and insect feeding, it is plausible that multiple stimuli are constantly being received and processed by the plant. Thus, the magnitude and duration of host defence induction will likely vary over time.

Genetically improved plants:

Genes from plants, microbes and animals can be combined and introduced in to the living cells of other organisms, and the organisms that have genes from other species inserted into their genome are called transgenics. Production of disease resistant transgenic plants has been achieved by this method; certain genes are inserted in to plant genome that confers resistance to

pathogens such as viruses, fungi and insects. These transgenic plants reduce the pesticide use and thereby provide environmental benefits while reducing farmers cost. Genetically modified plants are generally used to control the viral diseases, e.g., a transgenic papaya cultivar Rainbow'has been developed which is resistant to papaya ring spot virus in the US.

Integration of different cultural practices:

Different cultural practices like crop rotation, mulching, tillage, different soil amendments, soil solarization, soil sterilization, change in date of sowing, plant spacing etc. when applied alone are able to control diseases up to some extent; but when these cultural practices are combined with each other, they not only control the diseases but also increase the yield of crops. The inter-cropping of maize and sorghum with peppers serves as barriers against the aphid vectors of pepper venal mottle virus and reduces the virus spread. Soil solarization for 40 days along with the addition of cabbage, cauliflower, broccoli and sarson leaf residues controlled the gladiolus wilt (*Fusarium oxysporum f.sp. gladioli)* by 74.6% whereas soil solarization (for 40 days) alone reduced the gladiolus wilt by 67.3% compared to the un-solarized control.

Physical methods of disease control:

Solar heat treatment of the water soaked wheat seed in May-June for 5-6 hours provides good control of loose smut of wheat. Most of the post-harvest diseases can be avoided by irradiation, refrigeration, Controlled Atmosphere Storage etc. Soil solarization has been used to control soil borne diseases caused by otherwise difficult to control fungi, e.g., *Rhizoctonia solani, Fusarium spp., Sclerotium* etc. In this the soil beds are first irrigated and then covered with thin (20 µm) transparent mulch in the months of April, May and June. It raised the soil temperatures in some cases up to 500C, which is deleterious to many plant pathogens in the soil. It has been used in raising disease free nursery in tropical and subtropical climatic areas. It also provides excellent weed control. Hot water treatment of cabbage seed at 520C for 15-20 minutes controls black rot disease (caused by *Xanthomonas campestris pv. campestris*).

Plant nutrition:

The nutrition of crop plants has direct effect on the diseases, and is an important component of integrated disease management (IDM). Both deficient and over-nourished plants invite high incidence of diseases as well as loss in yield and quality of produce and products. The amount, proportion, time and method of application of fertilizers affect the metabolism of plants and thus occurrence and severity of diseases. Fertilization with both P and K significantly reduces the leaf rust damage and powdery mildew infection in wheat. The deficiency of macronutrients may also affect the incidence of many diseases. Potassium (K) plays an important role in survival of crop plants under environmental stress conditions. Potassium also affects the

reaction of plants to pests or diseases by having direct effect on the pathogen number, development, multiplication, survival, vigor and length of life cycle.

Biological control:

Biocontrol agents are used as a core component of integrated disease management system. The science and art of using living organisms as bio control agents is an important component of environment friendly disease management procedures. These bio control agents are of enormous value in integrated diseases management for sustainable agriculture where they often replace the need of fungicides. The biocontrol agents either suppress the pathogen growth either by the antibiotic production, hyperparasitism or by competition. Various biocontrol agents used in control of various diseases are *Bacillus subtilis, Pseudomonas fluorescens, Gliocladium* spp., *Trichoderma* spp., *Chaetomium globosum, Pseudomonas cepacia, Bacillus cereus, Agrobacterium radiobacter* etc. *Trichoderma viride* is the most important and versatile biocontrol agent used for the control of a number of plant pathogens like *Rhizoctonia solani* and *Sclerotium rolfsii* which are otherwise difficult to control by other methods. Similarly, *Fusarium lateriticum* has been used to cover primary wounds of apricot for avoiding the canker disease caused by *Eutypa armeniacae.* Application of *Peniophora gigantea oidia* paste on pine stumps provided effective control of *Heterobasidion annosus* root rot disease which spreads through unprotected stumps left over after felling. *Ampelomyces quisqualis* and Darluca spp. hyperparasitize powdery mildew and rust fungi, respectively, and therefore exploited for their biological control. Agrobacterium radiobacter K-84 strain has been used against crown gall disease world over.

Use of pesticides of plant origin:

Pesticides of plant origin are derived from plant parts and their genes are also used to transform crops to express resistance to insect, fungal and viral attack. The plant parts and their extracts with antifungal properties play an important role in plant disease management. Plants with pest killing properties have been recorded as early as Rig Veda in India. Garlic (*Allium sativum)* has a long history of reputed value and actual use for its medicinal, antimicrobial and pesticidal properties. The growth of *Rhizoctonia solani* can be reduced with ethanolic extracts of Eucalyptus sp., *Chenopodium ambrosioides, Lippia alba, Aegle marmelos and Cestrum diurnum*leaves. The seed extract of *Piper nigrum* was found to be effective against *R. bataticola.*

Judicious use of fungicides:

Chemicals have been used successfully to combat the ravages of these diseases for many years. Fungicides with different modes of action like protective (broad spectrum fungicides), post infection activity (EBI), pre- symptom and post symptom (benzimidazoles and triazoles) may be used for controlling a wide array of plant diseases ravaging various crops. The over-use

of these chemicals resulted in water pollution, residues on food and fruit crops, effect on non-target organisms and development of resistance in pathogens against the chemicals have drawn the attention toward the rational use of fungicides by including monitored control strategies and cultural practices.

Types of Integrated Disease Management:

i) Integration of cultural and chemical control:

The integration of chemicals and cultural practices (including improved cultivars) has resulted in a continuous supply of fresh watermelons, reduced diseases caused by *Colletotrichum lagenarium, Pseudomonas syringae pv. lachrymans* and *Pseudoperonospora cubensis*. The covering the tomato nursery seedlings with nylon net for 25-30 days plus 4 sprays of monocrotophos at 10-days intervals after transplanting, delayed the spread of Tomato leaf curl virus for 3-5 weeks and increased tomato yields.

ii) Integration of chemical and biological control:

Bio-control agents such as *Pseudomonas fluorescens, Trichoderma viride, T. harzianum, Bacillus subtilis, Pseudomonas putida, P. cepacia, Talaromyces flavus,* and *Agrobacterium radiobacter* strain K 84 etc. can be used with integration of chemicals for the effective control of certain diseases.

iii) Integration of resistance, cultural, biological and chemical control:

The integration of cultural practices (crop rotation, good farm hygiene procedures, quarantine), fertilizers, soil fumigation and solarization, pesticides (fungicide transplant dips, soil drench, soil incorporations, seed treatments, trace elements and surfactants), resistant cultivars and biocontrol agents are used for the control of club root (*Plasmodiophora brassicae*) of vegetables.

References:

Anonymous., (2001). Report of Task Force on Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India: pp. 76.

Chandrashekar., (H. M). 2010. Changing scenario of organic farming in India: An overview. Institute of Development Studies, University of Mysore, Manasagangotri, Mysore, Karnataka. International NGO Journal. 5 (1): pp. 034-039.

Gautam, H.R., and Bhardwaj, M.L. (2011). Better Practices for Sustainable Agricultural Production and Better Environment. Krukshetra- A Journal on Rural Development. 59 (9): 3.

Kohl. J. (2002). Integrated Disease Management. Plant Research International, Bio interactions & Plant Health, Wageningen, Netherlands: pp. 1-2.

Momol, T., Marois, J., Pernezny, K., and Olson, S. (2008). Integrated Disease Management for Vegetable Crops in Florida.U.S. Department of Agriculture, Cooperative Extension Service, University of Florida, IFAS, Florida: pp. 193.

Momol, T., Marois, J. Pernezny, K. and Olson, S. (2009). Integrated Disease Management for Vegetable Crops in Florida.U.S. Department of Agriculture, Cooperative Extension Service, University of Florida, IFAS, Florida: pp. 193(1).

Momol, T. Marois, J. Pernezny, K. and Olson, S. (2009). Integrated Disease Management for Vegetable Crops in Florida.U.S. Department of Agriculture, Cooperative Extension Service, University of Florida, IFAS, Florida: pp. 193(2).

Momol, T. Marois, J. Pernezny, K. and Olson, S. (2009). Integrated Disease Management for Vegetable Crops in Florida.U.S. Department of Agriculture, Cooperative Extension Service, University of Florida, IFAS, Florida: pp. 193(3).

Nagrajan, S. and Kumar, J. (2006). Diseases of Crops. Hand Book of Agriculture 6 ed: pp. 483-484.

Paull, J. (2010). The International Federation of Organic Agriculture Movements (IFOAM). Journal of Social Research & Policy. 1(2): pp. 93-102.

Rai, M. Pandey, S. Yadav, D.S. and Rai, A.B. (2007). Vegetable Production Technologies for Higher Productivity. Horticulture Summit Proceeding. Confederation of Indian Horticulture, Central Institute for Subtropical Horticulture, Lucknow: pp. 47-53.

Sharma, R.C., Govinda Krishnan, P.M., Singh, R.P. and Sharma, H.C. (1988). Effect of FYM and green manures on crop yields and nitrogen needs of potato based cropping systems in Punjab. Journal of Agriculture Science. Camb. 110: pp. 499-564.

Sharma, R.C. and Sharma, H.C. (1990). Fertilizer phosphorus and potassium equivalents of some green manures for potatoes in alluvial soils of Punjab. Tropical Agriculture. 67: pp. 74-76.

Shiva Shankar, K. (2002). Prospects of Organic Farming in Horticulture. Hi-Tech Horticulture. Horticultural Society of India, New Delhi: pp. 154.

Sud, K.C. and Grewal, J.S. (1990). Integrated use of FYM and Potassium in potato production in acidic hill soil of Shimla. Journal of Potassium Research. 6: pp. 83-95.

Thamburaj, S. (1994). Tomato responds to organic gardening, Kissan World, 21: pp. 10-49. 17. www.greenfiesta.com. (2009). Green Fiesta Official Website.

Agricultural Science: Research and Reviews (Volume II)

ISBN: 978-93-91768-12-6

About Editors

Dr. Parvinder Khanuja is working as Head Department of Pharmaceutical Chemistry, Govt. S. N. P. G. College, Khandwa (M.P.) INDIA. She received Ph.D. in 2014 and her topic for Ph.D. was "Study of Interaction Behaviour of Mono and Disaccharides in Aqueous Electrolyte and Zwitter -Ionic Ternary Solutions Through Thermodynamic and Transport Properties at Different Temperatures", from D.A.V.V. Indore (M.P.) India. She has 27 years of teaching experience at the U.G. level and P.G. level. Dr. Khanuja published 18 research papers in various National and International journals. She attended and presented 45 research papers at various national and international conferences.

Dr. Med Ram Verma is presently working as a Principal Scientist at ICAR-Indian Veterinary Research Institute Izatnagar, Bareilly, Uttar Pradesh. He passed M.Sc. in Agriculture Statistics in 1998 from Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. He obtained his Ph.D. degree in Statistics in 2005 from Dr. B. R. Ambedkar University, Agra, Uttar Pradesh. He joined Agricultural Research Service in 2003. Dr. Verma contributed significantly in the field of Sampling Theory, Applied Statistics and Biostatistics. He guided 4 Ph.D. and 10 M.V.Sc students in the discipline of Biostatistics as a Major Advisor at ICAR-IVRI Deemed University, Izatnagar, Uttar Pradesh. He published 212 research papers in various national and international journals. Dr. Verma was awarded with Best Teacher Award for the year 2014-15 by ICAR-IVRI Deemed University, Izatnagar, Bareilly, U.P. on 5th November 2016. Dr. Med Ram Verma was awarded with prestigious Bharat Ratna Dr. C. Subramaniam Award for Outstanding Teachers 2018 by Indian Council of Agricultural Research New Delhi on 16th July 2019. He is the referee of several national and international journals. He is the member of Editorial Board of several reputed national and international journals.

Dr. Satyendra Nath, currently works as Assistant Professor (Selection Grade) in Department of Environmental Sciences & NRM, College of Forestry, SHUATS, Prayagraj, Uttar Pradesh, India. He obtained B.Tech (Agriculture Engineering) degree from Allahabad Agriculture Institute, Allahabad Master of Engineering (M.E) and Ph.D in Environmental Engineering from Motilal Nehru National Institute of Technology (MNNIT-Allahabad), Prayagraj, Uttar Pradesh. He has been more than 16 years experiences in Consultancy, Research and Teaching in the field of Agriculture, Environmental sciences and Engineering. He has guided more than 40 P.G. Thesis and 05 Ph.D. Thesis. Dr. Nath published more than 40 publications in International/National Journals/Conferences. He has been attended more 30 International Conference/ seminar, training and workshop at International and National level. Dr. Nath delivered popular lecture and TV talk at national level. He has been awarded the Best Teacher Award- 2018 in the field of Environmental Sciences in International Conferences at Thailand.

Dr. (Mrs) M. S. Kadam is currently associated with the P. G Department of Zoology, Yeshwant Mahavidyalaya, Nanded (M.S) India as an Associate Professor. She completed her doctorate degree on the topic "Studies on Bio-diversity and Fisheries management of Masoli reservoir in Parbhani district, M.S (India)". She has completed one minor research project entitled with "Quantitative Assessment of Zooplankton and Morphometrics – meristrics of Rotifers from fresh water Reservoir in Nanded District (M.S) – As a study tool to rejuvenate the reservoir fishery". She has 15 years of teaching experience. Dr. Kadam has published more than 92 research papers in the field of Limnology and Fishery science in various national and international journals. She also attended and presented research papers in more than 81 conferences. She has achieved several recognitions in academic career, as a P.G Teacher, Research Guide in Zoology, S.R.T.M University, Nanded. She is a life Member of various national level research societies. She has Authored Two reference book and one U.G.C patterns book published by Educational Publisher and Distribution, Aurangabad.

Bhumi Publishing

ISBN 978-93-91768-12-6
9 789391 768126